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Histological and Histochemical Characterization of the Skin of Mud Eel, *Monopterus cuchia* (Hamilton, 1822) (Symbranchiformes, Pisces): A Light Microscopical Investigation

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Abstract: The skin of *Monopterus cuchia* is versatile producing different classes of GPs, proteins and lipids. The epithelial cells secreted GPs with oxidizable vicinal diols, general proteins, acidic lipids, unsaturated lipids and glycolipids. Both type I and type II mucous goblet cells produced increased amount of GPs with oxidizable vicinal diols and/or glycogen or GPs with carboxyl and/or with O-sulphate esters or mixer of all along with acidic lipids. The sacciform cells produced GPs with oxidizable vicinal diols in trace amounts revealing the dominants of proteinaceous secretions. Lipids in the epithelial cells and dermis formed a lipid bilayer protecting the skin from desquamation. Production of proteins, lipids and more than one type of GPs suggested a basis for functional discrimination in their role at the skin surface. This is considered to be an adaptation to environment inhaled by the fish and is discussed in relation to their role in lubrication, protection and inhibition of the invasion and proliferation of pathogenic micro-organisms.

Key words: Skin · GPs · Proteins · Lipids

INTRODUCTION

Fish skin is always in direct contact with their environment, so the epidermis and its secretion, the mucus act as a barrier between the fish and the environment [1-4]. The skin and mucus layer of fish are of great survival value as they provide the first line of defense against infection by potential environmental pathogens [5, 6]. The epidermis of skin is equipped with different types of unicellular glands which are involved in the secretion of surface mucus- a gel-like, slippery, gluey, viscous substance which is known to contain various biologically active macromolecules such as proteins, lipids and predominantly glycoproteins (GPs). Fish skin structure and its contents have been studied by many researchers till now [3, 7-15].

Gas metabolism through the skin in fishes plays a very important role in their respiration & can provide for 5 to 30 % of their required oxygen [11, 16, 17]. The integument morphology often widely vividly reflects the animal's ecological niches and its behavioral habitats. The skin is an ever-changing organ & many of its alterations are reflected, in its morphology. It is one of those important body organs that perform diverse functions. A good knowledge of its histology & particularly its chemical cytology should give greater significance to its adaptability, physiology & biochemistry. In view of these circumstances, attempts have been made in the present study to histologically & histochemically analyze GPs, proteins & lipids involved in the secretions of unicellular glands & other layers in the skin of mud eel, *Monopterus cuchia*.

MATERIALS AND METHODS

Live specimens of *Monopterus cuchia* (standard length, $L_{s,}$ 350±10 mm; weight 85.7±8.3g) were collected from the fish landing centre of Dibrugarh, Assam. The fishes were maintained at room temperature (23±2°C) in plastic aquaria containing water. The water in the aquaria was continuously aerated and the fishes were acclimatized with the laboratory conditions for a week prior to the commencement of the experiments. The fishes were fed regularly with dead small fishes.

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The fishes were cold anaesthetized following Mittal and Whitear [18] for the histological studies. The anaesthetized fishes were then taken out and skin pieces were excised from the dorsal side (above the lateral line) of the fish just behind the head for this study.

Histological and Histochemical Preparations: Excised pieces of the tissues were rinsed in physiological saline, fixed in desired fixatives [19] for mucopolysaccharide, protein and lipid histochemistry respectively. The tissues were then dehydrated in an ethanol series of ascending concentration, cleared in xylene and were embedded in paraffin wax (melting point 50-60°C)(E- Merck, India). Serial sections were cut at a thickness of 5 µm using a Leica Rotary Microtome and were mounted on dried glass slides. Sections were deparaffinized in xylene, hydrated in a descending ethanol series and were stained with routine histological stain, Hhrlich's haematoxylin and eosin (H/E) [19] to evaluate histological organization of the tissue. The stained sections were dehydrated, cleared and mounted in DPX. For lipid histochemistry sections were cut at a thickness of 8µm using a Cryostat. A series of histochemical methods were used to visualize, localize and identify different carbohydrate moieties, proteins and lipids in the cellular components of the tissues. The methods used in this investigation are summarized in Tables 2-4.

The stained sections of tissues were examined using Leica ATC 2000 microscope. The results were recorded using a digital camera system Sony or Nikon Coolpix-5400.

RESULTS

Histological Organization and Histochemistry: In *Monopterus cuchia* skin consists of multilayered cuticle, epidermis, dermis or corneum and subcutis or hypodermis and subcutaneous muscles (Fig. a). The average thickness of the skin is 710.25 ± 33.46 (in µm± SD). The dermis is 10-20 times thicker than the epidermis. The thickness of subcutis is less in comparison to epidermis and dermis (Table 1).

Cuticle: Cuticle the outermost layer of the skin is mainly composed of a complex mixture of glycoproteins, sulphated & non-sulphated acid mucopolysaccharides, proteins & lipids. It is approximately 1 µm thick (Fig. a). It is a complex mixture of cell cytoplasm, sloughed cells and any goblet cell mucus that has been secreted on to the surface. The cuticle layer gives positive reactions for almost each staining tests (Table 2, 3, 4).

Epidermis: Epidermis is pluristratified consisting of living cells. The epidermis of *Monopterus cuchia* is remarkable for the number and the size of the secretory cells it contains. The main structural component of epidermis is epithelial cells interspersed in between the large goblet mucous cells, basal cells and ionocytes and intrusive cells. Club cells are not present instead a special unicellular glandular cell namely sacciform cells are present in the epidermis.

Epithelial Cells: The surface layered epithelial cells are in direct contact with the immediate environment. These cells are typically small having basophilic staining cytoplasm. The epithelial cells in the basal layer are cuboidal and the surface layered epithelial cells are flattened. The shape of epithelial cells varies depending on their position in the epidermis. The middle layer epithelial cells are arranged less compactly in two to three layers, polygonal in shape with centrally placed rounded nuclei. They stained dark blue with Haematoxylin /Eosin (H/E) stain and the cytoplasm stained homogenously light pink in H/E (Fig. b). The epithelial cells in the superficial layer and the middle layer gave similar magenta staining as those in the basal layer but with darker staining. These cells are PAS positive indicating the presence of glycoproteins (GPs) with oxidizable vicinal diols and for glycogen. A gradual increase on the intensity of the reactions was observed towards the surface which indicates high level of mucogenicity. In Saliva/ PAS and Best Carmine tests the epithelial cells gave weak reactions indicating the presence of glycogen in trace amount. Epithelial cells showed very weak reaction with AB (pH 1.0 & 2.5) indicating the presence of small amount of GPs with carboxyl groups and O-sulphate esters. These cells gave deep blue reaction with mercury bromophenol blue test revealing the presence of general proteins & positive reactions for Sudan black B, 1% Nile blue, Nile blue technique and Performic acid Schiff confirmed the presence of general lipids, acidic lipids, phospholipids, unsaturated lipids & glycolipids respectively.

Mucous Goblet Cells: The density of mucous goblet cells (MGCs) in *Monopterus cuchia* is remarkably high. These cells are not renewed periodically but replaced when dead. They are the dominant cells in the epidermis. Two different types of mucous goblet cells are found i.e type I mucous goblet cells (MGC) and type II MGC. Type I MGCs are numerous in number occupying the whole depth of the epidermis (Fig. b). The nucleus of the cell is in the basal portion with a large reservoir of

Table 1: Average thickness of the skin in *Monopterus cuchia*. (Values are given as mean±SD)

Thickness (in µm±SD)			
Skin	Epidermis	Dermis	Subcutis
710.25 ± 33.46	218.75± 28.97	430 ± 8.14	62± 0.6

Table 2: Summary of the histochemical methods employed for the visualization of glycoproteins (GPs) in epidermal unicellular glands and sub-epidermal regions of the skin of *Monopterus cuchia.*

Histochemical methods	Reactions	Interpretation of reactions	References
1. WO/S	M	Free aldehydes	50
2. PAS	M	GPs with oxidizable vicinal diols and/or glycogen	51
3. Acetylation /PAS	0(M)	Same as '2'	52
4. Acetylation/deacetylation/PAS	M	Same as '2'	52
5. α-amylase/PAS	M 0(M)	GPs with oxidizable vicinal diols Glycogen	53
6. AB 2.5		T GPs with carboxyl groups and/or GPs with O-sulphate esters	54
7. AM/AB 2.5	0(T)	Same as '6'	55
8. AM/KOH/AB 2.5	T 0(T)	GPs with carboxyl groups GPs with O-sulphate esters	55
9. AB 1.0	T	GPs with O-sulphate esters	56
10. AM/AB 1.0	0(T)	Same as '9'	55
11. AM/KOH/AB 1.0	0(T)	Same as '9'	55
12. AB 2.5/PAS	M T P/Bl	GPs with oxidizable vicinal diols and/or glycogen	54
		Gps with carboxyl groups and/or with O-sulphate esters	
		Gps with oxidizable vicinal diols and/or glycogen and	
		Gps with carboxyl groups and/or with O-sulphate esters.	
13. AM/AB 2.5/PAS	M 0(T)	GPs with oxidizable vicinal diols and/or glycogen	55
		Gps with carboxyl groups and/or with O-sulphate esters	
14. AM/KOH/AB 2.5/PAS	M T 0(T)	GPs with oxidizable vicinal diols and/or glycogen	55
		Gps with carboxyl groups	
		Gps with O-sulphate esters.	
15. AB 1.0/PAS	M T P/Bl	GPs with oxidizable vicinal diols and/or glycogen	55
		Gps with O-sulphate esters	
		Gps with oxidizable vicinal diols and/or with O-sulphate esters	
16. AM/AB1.0/PAS	M 0(T)	GPs with oxidizable vicinal diols and/or glycogen	55
		Gps with O-sulphate esters	
17. AM/KOH/AB1.0/PAS	M 0(T)	GPs with oxidizable vicinal diols and/or glycogen	55
		Gps with O-sulphate esters	
18. Toluidine Blue	P R/Pk	β-metachromasia, acidic mucosubstances	
		γ-metachromasia, strongly acidic mucosubstances	57
19. Best Carmine	R	Glycogen	58
20. Tetrazonium	RV	Sulphate esters & sulphonic groups	50

Symbols:AB 2.5-alcian blue at pH 2.5;AB 1.0-alcian blue at pH 1.0; AM-active methylation; GPs-glycoprotiens; KOH- saponification; M-magenta; PAS-periodic acid/Schiff; S-schiff; T-turquoise; WO-without oxidation; P-purple;MP- magenta with purple tinge;Bl-blue; R-red, RV-reddish violet.

Table 3: Summary of the histochemical methods employed for the visualization of proteins in epidermal unicellular glands and sub-epidermal regions of the skin of *Monopterus cuchia*

Histochemical methods	Reactions	Interpretation of reactions	References
1. Mercury-Bromophenol blue	DB	General proteins	50
2. Ninhydrin/Schiff	PR		
	M	Protein bound NH2 group	59
3. Deamination/Ninhydrin/schiff	0(PR/M)	Same as '2'	50
4. Performic acid/ Schiff	P/M	Cysteine bound disulphide (SS) groups.	50
5. Masson's Trichrome stain	Bl	Collagen	50

Symbols: DB-deep blue; PR-purplish red; M-magenta; P-purple, Bl-blue

Table 4: Summary of the histochemical methods employed for the visualization of lipids in epidermal unicellular glands and sub-epidermal regions of the skin of *Monopterus cuchia*.

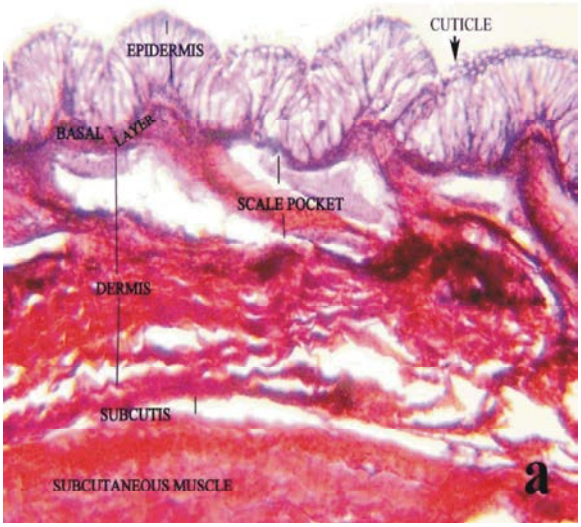
Histochemical methods	Reactions	Interpretation of reactions	References
1. Sudan Black B	B/Bl	General lipids	60
2. Pyridine /Sudan Black B	0(B/l)	General lipids	50
3. Oil red O	R	Neutral lipids	50
4. Nile blue test	Bl	Acidic lipids	
	R/P	Neutral lipids	61
5. Pyridine/Nile blue	0(Bl) 0(R/P)	Same as '4'	50
6. Acid haematin	DB	Phospholipids	
7. Pyridine/Acid haematin	0(DB)	Same as '6'	50
8. Performic acid/Schiff	P	Phospholipids with unsaturated bonds	50

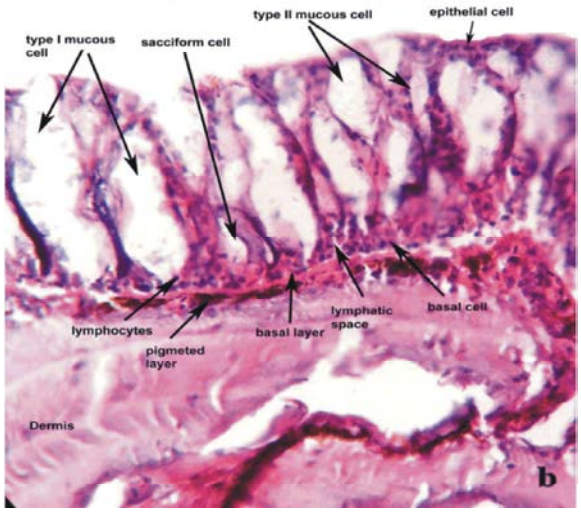
Symbols: B-black;Bl-blue; R-red; P-purple; DB-deep blue

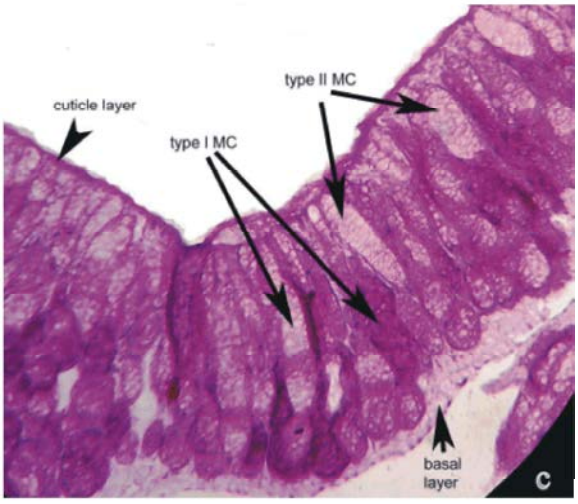
secreted material between it and the apex of the cell. The basal portion of the cell often penetrates between the basal layer epithelial cells in the form of reversed cone. The type II MGCs are less common than the type I MGCs. These cells occupy the superficial layer of the epidermis and are dilated near the apex. The secretory contents of MGCs do not stain with H/E stain. The type I MGC show weak to moderate reaction in their secretory contents and deep magenta staining in granular contents of their periphery and cone shaped basal portions. In type II MGCs the intensities of the reactions for PAS is moderate in comparison with type I mucous cells giving a light magenta staining (Fig.c). After Amylase digestion, the granular contents of type I MGCs disappear indicating the presence of glycogen contents. Moreover, the reaction in the secretory contents is rendered relatively weak by enzyme digestion showing the presence of low moieties of glycoproteins (GPs) with oxidizable vicinal diols and glycogen as well. In type II MGCs the intensities of the reaction for Saliva/PAS is moderate in comparison with type I mucous cell. Both type I and II MGCs gives weak reactions for Best Carmine stain (Fig. g).Type I &type II MGCs gives strong reaction for both AB pH 1.0 & 2.5 indicating the presence of GPs with both carboxyl groups and O sulphate esters. Tetrazonium & toluidine blue tests confirmed the presence of sulphated & acidic mucosubstances. In AB pH 1.0/PAS stain, the type I MGCs gives strong reactions with purple colour which indicates that these cells have GPs with oxidizable vicinal diols and/or glycogen and GPs with O-sulphate esters. At intervals, some of these cells stain magenta, turquoise or a combination of purple, magenta and turquoise depending upon different parts of the same cells. These cells dominants GPs with oxidizable vicinal diols and /or with o-sulphate esters or mixer of all (Fig. e). The type II MGCs contains periodate unreactive components, stains turquoise which indicates GPs with O-sulphate esters. The type I MGCs reacts deep purple indicating the presence of GPs with oxidizable vicinal diols and/ or glycogen or GPs with carboxyl and /or with O-sulphate esters with AB pH 2.5/PAS stain. They also give a mixer of reaction of magenta, purple or turquoise or blue. The secretory contents of type II MGCs are moderate to strong turquoise or blue revealing large amount of GPs with O-sulphate esters (Fig. f). Both type I & II MGCs gives weak to moderate reactions for bromophenol blue revealing the presence of general proteins (Fig. h). They showed strong positive reactions for general lipids, acidic lipids and giving positive reactions for Sudan black B, 1% Nile Blue tests and Nile blue technique (Fig. i).

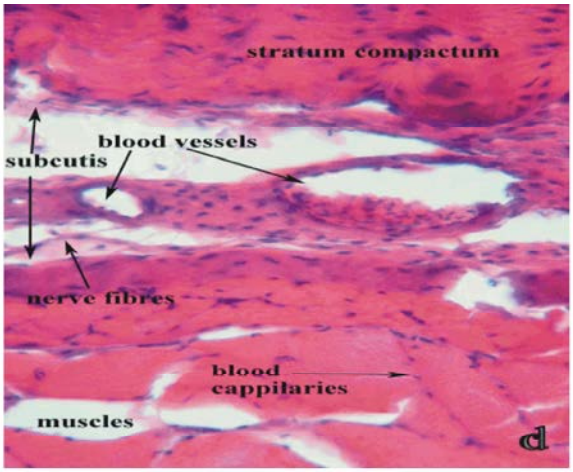
Sacciform Cells: A serous category unicellular sac like gland having mainly proteineous secretions was observed in the skin of the fish. These cells are the sacciform cells. Sacciform cell were present sporadically in the skin of *Monopterus cuchia* but in contrast to the granular secretions of MGCs these are homogenous, moderately alcianophilic and feebly PAS positive, staininig pink purple in H/E stain. Sacciform cells give positive reactions for Bromophenol blue test, Chloramine T/Schiff test and Ninhydrin T/Schiff test for proteins and protein bound NH2 group respectively (Figs. b, d). These cells are rich in glycolipids.

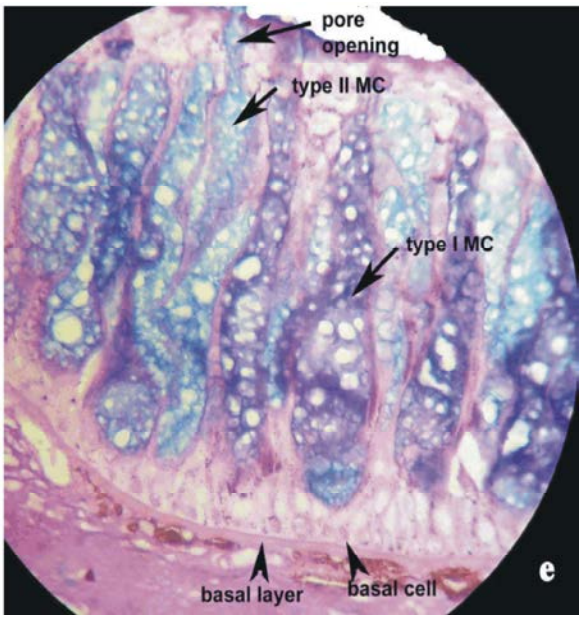
Basal Cells: The deeper regions of the epidermis composed of a single layer of cell i.e. the basal layer. The basal layer cells give moderate reaction for PAS test indicating the presence of GPs. After amylase digestion the cells give weaker reaction for PAS confirming the presence of glycogen. Basal cells give very weak reaction for AB pH 1.0 and AB pH 2.5 tests. Basal cells showed strong positive reactions for Bromophenol blue test, Sudan black B, 1% Nile blue tests and Nile blue technique for general proteins, general lipids, acidic lipids and phospholipids respectively.

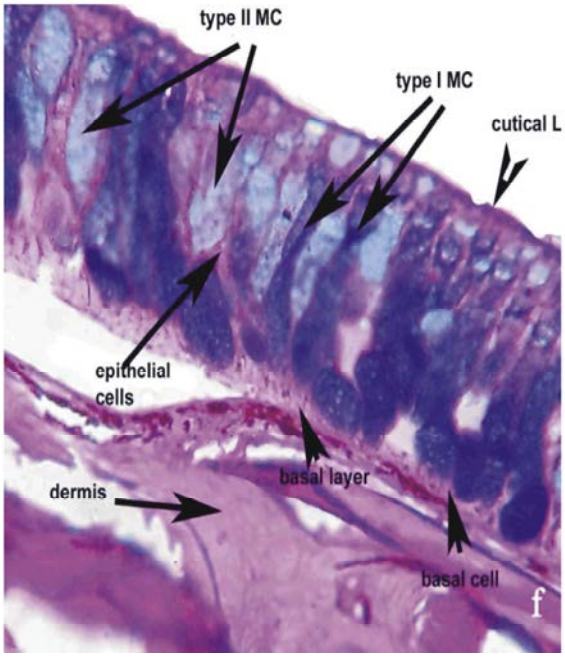

CUTICLE
EPIDERMIS
BASAL LAYER
SCALE POCKET
DERMIS
SUBCUTIS
SUBCUTANEOUS MUSCLE
a


type II mucous cell
epithelial cell
type I mucous cell
sacciform cell
lymphocytes
pigmeted layer
basal layer
lymphatic space
basal cell
Dermis
b


cuticle layer
type II MC
type I MC
basal layer
c


stratum compactum
subcutis
blood vessels
nerve fibres
blood cappilaries
muscles
d


pore opening
type II MC
type I MC
basal layer
basal cell
e


type II MC
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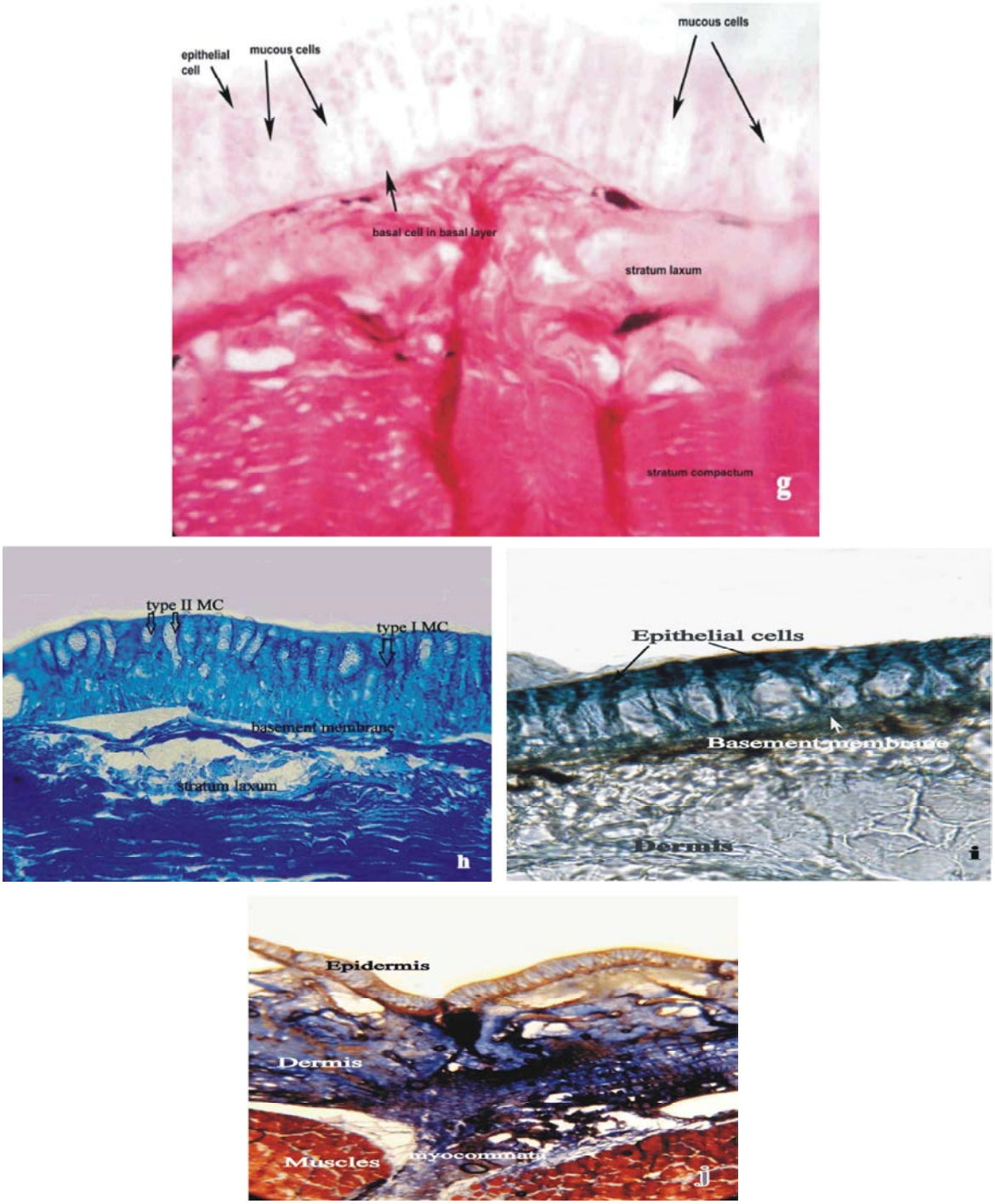

Figs. a-i: Photomicrographs of (a-c) cross section of the skin from the dorsal side (above the lateral line) of the fish *Monopterus cuchia* just behind the head. (a). skin divided into cuticle, epidermis, basal layer, dermis, subcutis and subcutaneous muscle. Sclae pockets are rudimentary (H/E x100). (b). epitheilial cells are dispersed in the superficial layer, middle layer and basal layer. The presence of mucous goblet cells, type I from basal layer occupying the whole epidermis and type II in the outer layer. Sacciform cells in the outer layer and middle layer and lymphocytes within lymphatic spaces in basal layer of epidermis.(H/E x400). (c). Numerous blood capillaries in the muscle region, in compact stratum compactum . Blood vessels and nerve fibres in the subcutis.(H/E x400)

Photomicrographs of (d-i) cross section of the skin from the dorsal side (above the lateral line) of the fish, *Monopterus cuchia* just behind the head, stained with histochemical methods for glycoproteins, proteins and lipids. (d). type I & type II mucous goblet cells (MGCs) stain strong magenta with type I stain strong at the base. Epithelial cells & basal cells gave moderate stain (PAS x400). (e&f). type I MGCstain strong purple to deep blue, type II MGCs stain torquise stain, epithelial cells, basal cells & sacciform cells stain moderate magenta stain (AB1.0/PAS & AB2.5/PAS x400).(g). type I & II MGCs stain very weak whereas epithelial cells & basal cells stain weak to moderate red, dermis, muscles & myocommata gave strong red (Best Carmine x100). (h). MGCs stain weak deep blue colour, epithelial cells & basal cells gave strong deep blue colour along with dermis & myocommata (Hg-Bromophenol blue x100). (i).epithelial cells & basal cells gave strong blue black stain. Weak stain in the MGCs, dermis & muscles. (Sudan Black B x100). (j). Stratum laxum and stratum compactum of dermis stain deep blue stain(Masson Trichrome stain x100).

Dermis: The dermis is very thick and hence it is the largest component of the dorsal skin of *Monopterus cuchia*. The dermis is provided intensively with collagen fibers that run at angle of 45 degree across the longitudinal axis of the fish. Dermis is divided into two distinct layers- the outer stratum laxum and the inner stratum compactum.

Stratum Laxum: The stratum laxum is composed of comparatively loosely arranged connective tissue fibers, mainly collagen and richly supplied with fine blood capillaries, nerves and pigment cells and it is adjacent to the basement membrane. This layer gives strong reaction for Masson Trichrome stain confirming the presence of collagen (Fig.j) These areas are stained pink in H/E stain, turquoise with AB at pH 1.0 and 2.5, magenta with PAS and purple with AB-PAS (Figs. a,b). These histochemical reactions indicate that GPs with oxidizable vicinal diols, carboxyl groups and with O-sulphate esters are present in low concentration. This region is rich in phospholipid & acidic lipids. It gives positive reactions for mercury bromophenol blue indicating the presence of general proteins (Fig. h)

Stratum Compactum: This layer is comparatively thin than stratum laxum. This layer is consists of layers of coarse collagen fiber bundles which are arranged parallel to the skin surface with a fine elastic fiber bundles. Stratum compactum also give positive reactions for Masson Trichrome stain (Fig.j). Fibrocytes are dominant and scattered among collagen fibers. Branches from the main blood vessels, pigment cells and nerves in the subcutis run through stratum compactum and supply the capillaries in the stratum laxum. This layer gave similar staining reactions to that of the stratum laxum.

Subcutis: It lies in between the stratum compactum and the muscles and it is the inner most and the thinnest layer of the skin composed of loose connective tissues. Numerous blood vessels and nerves are found in this region (Fig. d).

Subcutaneous Muscles: The muscle cells run in parallel direction and are connected to sheaths of connective tissue called myocommata. Myocommata is composed of collagen. These connective tissues which are anchored to the skeleton and the skin are stained red in H/E stain. These regions gives very strong reactions for PAS, saliva PAS, Best Carmine, Bromophenol blue and mild reaction for Nile blue test revealing the presence of glycoproteins, glycogen, general proteins and neutral lipids respectively.

DISCUSSION

The skin of mud eel, *Monopterus cuchia* is generally a 'mucous' dominant system. In the present study, the epithelial cells are in immediate contact with the outside environment; next to it are the large MGCs and the sacciform cells. The large MGCs have been found to occupy the major proportion of the epidermis. These cells were formed in the stratum germinativum and migrated to the surface of the skin, where they discharge their secretion [3]. Whitear [20] suggested that glycoprotein (GP) contents of epidermal secretions in fish may vary according to species & cell types. Increase in the secretion of mucus may be an adaptation to its peculiar bottom scooping habitats and frequently deals with organic debris in the bottom in search of food [3, 15]. In *Monopterus cuchia*, a high level of mucogenicity is very important to adapt them for the burrowing habit as well as for protection against abrasive injury during searching food at bottoms and to keep the surface clean. The mucus can precipitate mud held in suspension. The lubricant role of mucus reduces body friction in water, helping on swimming and also protecting the body from abrasion during burrowing and nest digging [21-26].

The detection of GPs with oxidizable vicinal diols and O-sulphate esters in epithelial cells and MGCs in the epidermis indicates its role on lubrication of skin surface in *Monopterus cuchia*. Mucus can act as barrier to various pathogens & prevent their colonization in the epidermis [27-30]. The present investigation clarified that the mucous cells reacted positively with AB pH 2.5/ PAS stain. This ensured that their contents are sialomucins and sulphomucins. The sulphomucins possibly protects fish from bacterial and fungal infections [31].

Richards [27] & Cinar *et al*. [28] suggested that carboxylated & slightly sulphated GPs in the mucus provides a respiratory film adsorbed to the surface of the cuticle by cuticular projections and the secretions rich in proteins would aid water retention in the acidic mucus film or modify the viscosity of the lubricating mucus. It ensures the protection of the fish against osmtic shocks. On the side, the presence of sulphated & carboxylated sialomucins in secretion of epidermal mucous may be significant because these substances have special importance in glycocalyx, essential component in most cell membranes [32]. Accordingly, we observed a variable degree of reaction in histochemical staining patterns of acidic & neutral GPs present in the mucous cells of the epidermis. These can be attributed to have a highly protective role of skin of *Monopterus cuchia* and its specific regulatory function during freshwater & semiterrestrial habitat. The present investigation revealed that as well as neutral & acidic GPs, sialic acid residues were also predominant in the epidermis of *Monopterus cuchia*. The presence of sialic acid residues together with sulphated groups is mainly responsible for negative charge of the GP residues in the skin of *Monopterus cuchia*. The presence of sialic acid residues together with sulphated groups is mainly responsible for negative charge of the GPs which may conceal receptor sites for viruses & mycoplasma species [33].

Amaral *et al*. [31], Cinar *et al*. [28], Sarasquate & Gutirrez [32] & Desantis [33] have performed metachromatic staining to evaluate distribution of GPs containing strongly ionized sulphated groups, which have negative charges but are also extremely hydrophilic, attracting large volumes of water & cations. Type I & type II MGCs of *Monopterus cuchia* have high water retention capacity and prevent the proliferation of pathogenic microorganisms on the surface of the skin. The mucous cells were highly alcianophilic, indicating more acid GPs than sulphated GPs. The presence of sulphate esters however deserves attention in the epidermis of boar [34] & fishes [35]. It seems likely that such high acidity of the secretory GPs is essential for the preservation of skin health. Thus, mucous cells contribute to epithelial barrier of many invertebrates providing a 1st line of defense mechanism. The acidic nature of the mucous cell was reported on other teleost fishes with cutaneous respiration [23, 24, 26, 36, 37, 38]. Mucus had greater ability to bind a large amount of water [39, 40] and the mucus secreted by the skin in air breathing fishes are used to keep the skin clear for respiration [26, 36, 41]. The main function of the basal cell layer is to anchor the epidermis to the underlying dermis.

Besides, the GPs in fish skin surface mucus, there is considerable variety of biological materials arising from the secretions. Many of these secretions contain not only neutral & acidic GPs & proteins but also lipids, acidic lipids & phospholipids. Mucus from several species of marine fishes contained upto 20 times more lipids per unit area than human sebum [25]. Lipids in mucous secretions, contribute to fiber-fiber interaction that markedly increase the viscoelasticity of the gel, which has been studied on evolved vertebrate's gastric mucus [25, 42, 43]. Lipids are essential building blocks found in skin's outermost layer. The lipids form layers around & in between skin cells, creating a barrier that keeps natural moisture intact & prevent it from escaping. The ultimate goal of epidermal differentiation is the production of a cohesive, relatively impermeable outersheeth, the epidermis. High levels of phospholipids in the basal cells are attributed to the metabolically active state, which are undergoing cell proliferation & differentiation [36]. This can be correlated with the epithelial cells & basal cells of *Monopterus cuchia* which have high contents of lipids & acidic lipids. The special role of intercellular lipids is correlated with the regulation of epidermis barrier function & desquamation [44, 45]. Lipids play an important role in energy production in carnivorous fish and playing major role in physiological activities [45].

In the present study, in addition to mucous cells another gland cell called sacciform cells are demonstrated in the epidermis. The two secretory unicellular glands (mucous cells & sacciform cells) showed a different tinctorial affinity when stained with H/E (eosinophilia for sacciform & unstained for mucous cells) thus suggesting acidic groups differences in the GPs contents as already observed in different fish species. This study revealed that the sacciform cells secrete basic proteins on fish skin surface. No sugar residues attached to it & so are distinct from those that form the mucus [32, 33]. These can be correlated with the present study in *Monopterus cuchia*. The secretions of these cells probably serve several

functions including those related to predation. The secretions of these cells may play an important role in local defense mechanism of fish skin which is very much prone to various kinds of infections [46, 47]. Both club cells & sacciform cells are source of alarm substances [48]. The sacciform cells compensate the presence of club cells in *Monopterus cuchia.*

The dermis have role in protection and hydrodynamics composed of loose collagenous matrix and it is strongly vascularized. The scaleless fishes have thick dermis in order to strengthen the thin skin to protect the fish against splitting under tensile force. The organization of the dermis renders the upper region of the fish skin transparent [47]. *Monopterus cuchia* is a scaleless fish and its dermis is richly supplied with vascularized collagenous matrix giving it a thick structure. The muscle cells of fish run in parallel & are connected to sheaths of connective tissue called myocommata which are anchored to the skeletal & the skin. The myocommata are formed of collagenous tissues & contruibutes to characteristic of flakiness. Fish myosystems can differ markedly in structure & chemical composition due to nutritional status, maturation & species. These differences may influence fish quality [48]. In *Monopterus cuchia* the myocommata are richly supplied with glycogen which contributes to the metabolic activities of the fish during swimming [49]. *Monopterus cuchia* has a versatile and highly metabolically active skin which protects, nourish and palys a vital role during its wriggling movements in aquatic and semi terrestrial habitats.

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