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Light Microscopical Study on the Skin of European Eel (*Anguilla anguilla*)

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Abstract: Skin was obtained from ten healthy adult male and female eels, a migrating teleost, they were caught from Manzalla Lake (Egyptian sea water). The specimens which resembled different body regions were processed for light microscopy. The results revealed that, the epidermis get formed of non keratinized stratisfied squamous epithelium In addition to the typical epithelial cells, the epidermis included mucous goblet like cells and club (alarm containing substance) cells in between its strata. Generally, the mucous cells occupied the superficial cell layers. The club cells occupied different epidermal cell layers. Their distributions varied greatly along the different body regions. The skin includes variety of sense organs which were represented by superficial neuromasts at the upper lip, taste buds in the dorsal region of snout and lateral line canal in the dorsal regions of snout, dorsum of the head and lateral region of trunk. It was concluded that, the skin of eel has its own specialized cellular structures that increased and condensed in some body regions to accommodate their habitat, such as digging, burrowing syndrome and travelling across land throughout wet grass.

Key words: Teleostean fish · Histology · Coetaneous structure

INTRODUCTION

Skin is the primary interface between the fish and its environment. It is a living metabolically active tissue, allowing normal internal physiological functions, so its condition is important to explain many disease processes [1,2]. It is a self active secretory organ that provides number of useful products. Mucous glands whose secretion plays a major role in protection against stress factors, imposed by changes in the nature and chemical composition of surrounding water [3]. It keeps the body surface moist, acts as antibacterial and antifungal agent [4]. Also, the skin houses the alerted content of club cells which initiates alarm reaction [5]. The skin contains melanocytes which provide fish with its specific colouration [6]. It is a vehicle for cutaneous sense organs adapted the fish for easily detection of predator and food. Notable among these are neuromasts, taste buds and lateral line canal [7-9].

Recently, eel aquaculture became important. It was of economical value. However, its digging habitat formed. its adaptation to artificial environment still difficult [10]. In fish medicine, skin scraping is an important diagnostic preparation. To date, information are lacking about skin structure of teleosts concerning possible species variations. The aim of this work was to focus on the important aspects of normal structure of eel skin to serve as reference for clinicians and pathologists.

MATERIAL AND METHODS

Skin samples from each of ten adult healthy male and female eels were used in this study. Fish body lengths ranged from 50-55 cm. The fishes were caught from Manzalla lake (Egyptian sea water) in March. The skin samples resembled different body regions were obtained (Fig.1). Paraffin sections (5-6 μ) were prepared and stained by haematoxylin and eosin (H and E), Masson's trichrome stain, periodic acid schiff reagent (PAS), alcian blue (AB) (PH 2.5) and combination of alcian blue and periodic acid Schiff reagent (AB/PAS). Procedures were adopted according to Bancroft and Stevens [11].

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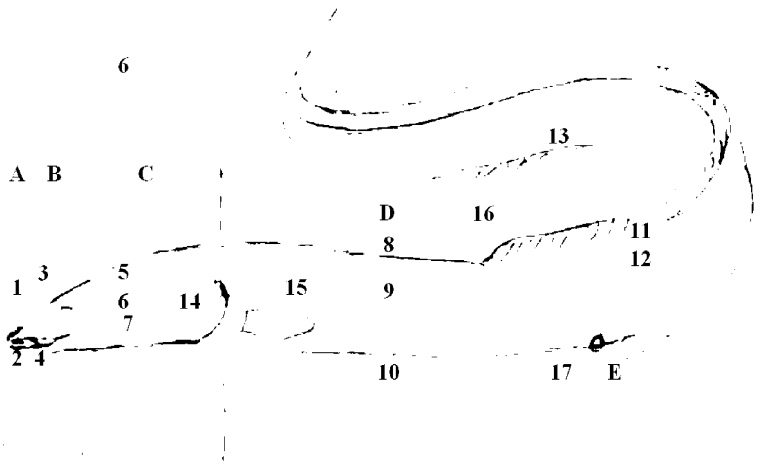

Fig. 1: A diagram illustrating the examined parts of the skin of eel:

-Lips (A):	upper lip (1)	-Lower lip (2).	
-Snout (B):	dorsal aspect of snout (3)	-Ventral aspect of snout (4)	
-Head (C):	dorsal aspect of head (5)	-Lateral aspect of head (6)	ventral aspect of head (7)
-Trunk (D):	dorsal aspect of trunk (8)	-Lateral aspect of trunk (9)	ventral aspect of trunk (10)
-Tail (E):	dorsal aspect of tail (11)	-Lateral aspect of tail (12)	ventral aspect of tail (13)
-Operculum (14)	-Pectoral fin (15)	-Dorsal fin (16)	-Urogenital orifice (17).

RESULTS

It was found that the skin of eel is composed of an epidermis which is formed of non-keratinised stratified squamous epithelium and underlying dermis which is formed of dense regular connective tissue, followed by hypodermis of loose connective tissue. These layers include morphological variations along the different body regions (Figs. 2 to 21).

At the upper lip region, the epidermis is formed of thickened stratified squamous epithelium having a well definite basal layer of columnar cells, stratum germinativum, which is situated on a thin basement membrane. On the top of stratum germinativum lies several layers of fusiform cells covered by a superficial squamous cells. The mucoid cells are distributed in the middle and superficial epidermal layers and they are varied from rounded to goblet-like cells. These cells directly open onto the surface. The sense organs were found to be numerously detected as superficial organized neuromasts, fusiform pale staining cells, rested on a fibrous dermal folds. The dermal layer is formed of dense compact parallel bundles of collagenic fibers. Aggregated melanocytes are located at the superficial dermal margin (Fig.2).

At the lower lip region, the epidermisis formed of less thickened stratified squmaous epithelium with high mitotically active stratum germinativum which is covered by several layers of large polyhedral cells and a superficial layer of squamous cells. The mucoid cells are aggregated in the superficial epidermal layers. They are large spherical in shape, showing strongly positive AB/PAS reaction at their foamy cytoplasm also, their cell membranes appear having intensive reaction. The dermal layer is more thicker than that of the upper lip. It is formed of compact parallel bundles of collagenic fibers. The hypodermis is clearly organized layer of loose connective tissue (Fig.3).

At the dorsal aspect of the snout. It was seen that the epidermis is formed of less thickened stratified squamous epithelium, having numerous unicellular mucous glands, condens at the superficial and middle layers. They are intensively reacted to AB/PAS (Fig.4). Sensory organs are represented by taste buds which distributed at this region. Taste buds are pear-shaped containing tall columnar sustentacular cells with ovoid darkly stained nuclei, ulternating with fusiform sensory cells having lightly stained nuclei. They are associated with small pyramidal basal cells. The sensory hairlets are long and showed surface protrusion. The taste buds are surrounded by large mantle layer of epidermal cells (Fig.5).

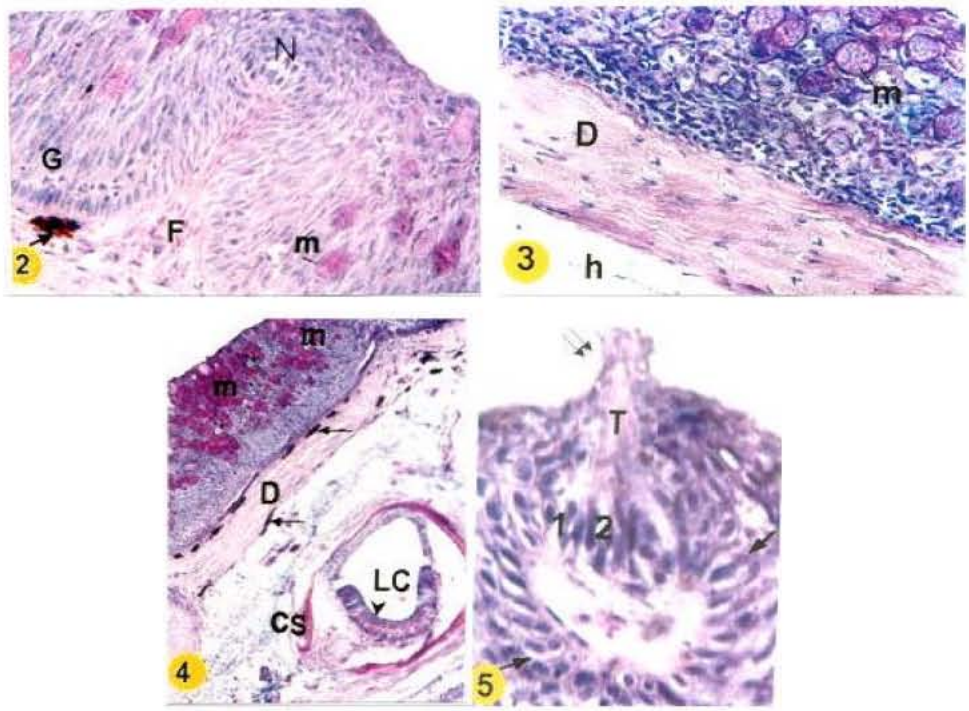

Fig. 2: A photomicrograph of a vertical section at the skin of upper lip of eel showing, thick epidermis of stratified squamous epithelium. Notice definite stratum germinativum (G) The mucoid cells (m) were distributed through the middle and superficial cell layers, neuromasts (N) of fusiform pale staining cells rested on a fibrous dermal fold (F). Aggregated melanocytes (arrow) were located at the superficial dermal margin. (AB/PAS ×400).

Fig. 3: A photomicrograph of a vertical section at the skin of lower lip of eel showing, the covering epidermal region of less thickened stratified squamous epithelium containing superficial aggregation of spherical mucous cells (m) have foamy cytoplasm and strong staining cell membrane. Notice dermal collagenic bundles (D) and hypodermal thick layer of loose connective tissue (h). (AB/PAS x400).

Fig. 4: A photomicrograph of a vertical section in the skin of the dorsal aspect of the snout of eel showing, condensation of mucous cells (m) at the middle strata of epidermis, dense collagenic dermis (D) containing melanocytes (arrows) at the superficial and deep margins. Lateral line canal (LC) surrounded by incomplete cartilagenous shell (CS) and lined by stratified cuboidal epithelium (arrow head) (AB/PAS ×100)

Fig. 5: A photomicrograph of a vertical section in the skin of dorsal aspect of the snout of eel showing, a well-organized pear-shaped taste bud (T). Notice the sustentacular (1) and sensory (2) cells, long protruded sensory hairlets (double arrows). It was surrounded by mantle layer of epidermal cells (arrows) (H and E ×400)

The dermal layer is less thickened and is formed of compact parallel bundles of collagenic bundles. Melanocytes are situated at the superficial and deep borders of the dermal layer. Large lateral line canal is located at the well-developed hypodermal region. It is supported by incomplete cartilagenous shell lined by stratified cuboidal epithelium containing a well differentiated sense organ (Fig.4).

At the ventral aspect of the snout, the epidermis was found to be formed of less thickened stratified squamous epithelium. The unicellular glands are numerous and occupied most of the epidermal thickness and condensed at the superficial layers. These cells are ovoid or goblet-like in shape, intensively reacted to AB/PAS and opened directly to the surface. The dermal layer is less thickened, it is formed of less compact parallel bundles of collagenic fibers, containing ill defined dermal spaces and few melanocytes arranged at its peripheral margins. The hypodermal layer isthick and formed of highly vascularized loose connective tissue (Fig.6).

At the dorsal aspect of the head, the epidermis was found to be formed of less thickened stratified squamous epithelium. The unicellular mucoid glands are distributed at the mid and superficial layers of the epidermis.

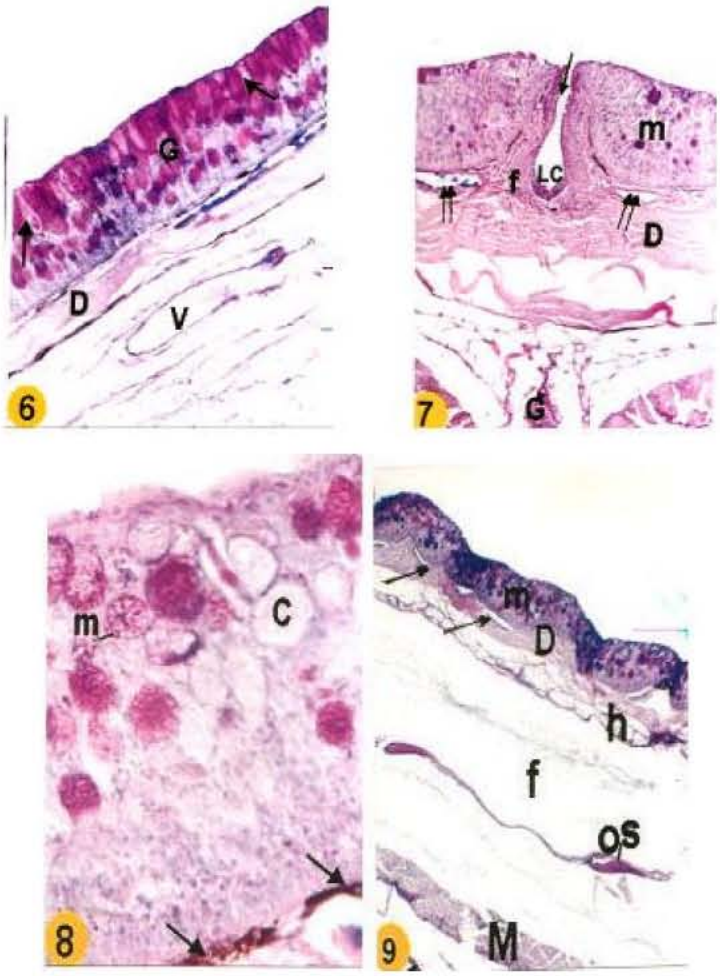

Fig. 6: A photomicrograph of a vertical section in the skin of ventral aspect of the snout of eel showing, aggregation of goblet mucous cells (G) through middle and superficial cell layers, some of them opened directly to the surface at the epidermal surface (arrows). Notice the parallel dermal collagenic bundles (D) showing melanocytes arranged at the peripheral margins (brown), thick layer of hypodermal loose connective tissue containing large vessels (V). (AB/PAS ×100)

Fig. 7: A photomicrograph of a vertical section at the skin of dorsal aspect of the head of eel showing, epidermis with scattered mucous cells (m), secondary lateral line canal (LC) which opened to the exterior by a pore (arrow), surrounded by a dense fibrous wall (f) continuous with the dermal stratum compactum (D). Notice large vessels arranged at the superficial dermal layer (double arrows). Thick layer of hypodermis containing ganglia (G) in advance to lateral line canal. (AB/PAS ×100)

Fig. 8: A photomicrograph of a vertical section in the skin of ventral aspect of the head of eel showing thick epidermis containing large spherical mucous cells (m) through the middle and superficial cell layers and club cells (C) in the middle cell layers of epidermis and a layer of melanocytes arrange at the superficial dermal region (arrows). (AB/PAS ×400).

Fig. 9: A photomicrograph of a vertical section at the operculum of eel showing, wavy-shaped epidermis formed of less thickened stratified squamous epithelium, distributed mucous cells (m) at different levels of the epidermis. Notice the subepidermal large vessels (arrows), undulated compact dermal layer (D), areolar hypodermis (h), opercular core formed of loose connective tissue (f) containing osteoid islets (os) and striated muscle bundles (M). (AB/PAS ×100)

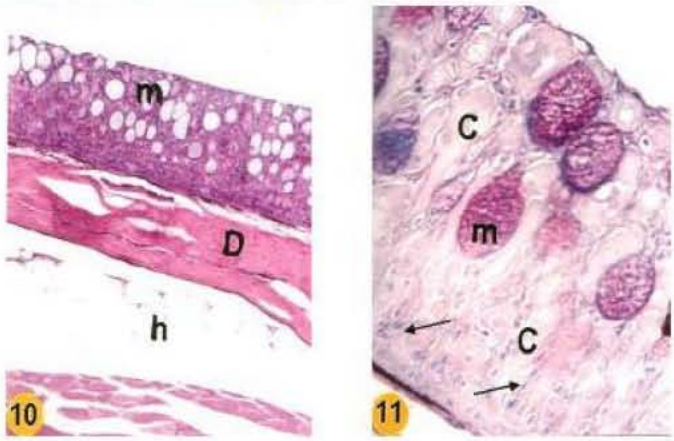

Fig. 10: A photomicrograph of a vertical section in the skin of the dorsal aspect of the trunk of eel showing, epidermal region. contained numerous large spherical mucous cells (m) through middle and superficial epidermal layers. Notice dermis (D) and hypodermis of areolar connective tissue (h). (H and E x100)

Fig. 11: A photomicrograph of a vertical section in the skin of the ventral aspect of the trunk showing, thick epidermis of stratified squamous epithelium containing mucous cells (m) and large club cells (c). Notice, squeezed elongated epithelial cells in between (arrows). (AB/PAS ×100)

They are intensively reacted to AB/PAS. The dermal layer showed less compact collagenic bundles and containing large vascular spaces at its superficial layer. Secondary lateral line canal was observed superficially in the dermis just beneath the epidermis and invest by a dense fibrous wall without any cortilagenous support. It is lined by stratified cuboidal cells and it is opened directly to the surface epithelium by a pore. The dermal layer became loosened at its deep layers containing lymph spaces, supported by hypodermis of loose connective tissue which contained sensory ganglia in advance to the secondary lateral line canal (Fig.7).

At the ventral aspect of the head, the epidermis is more thicker,it is formed of stratified squamous epithelium. The unicellular mucoid cells are large, spherical with foamy cytoplasm, they areintensively reacted to AB/PAS. They are observed at the superficial and middle layers of epidermis. The club cells are distributed at the mid epidermal level. They are large,they are varied from spherical to saccular in shape. The dermal layer is placed with parallel bundles of collagenic fibers. Melanocytes are condensed to form a continous layer at the superficial dermal layer (Fig.8).

The skin of the opercula is formed of wavy shaped epidermis of stratified squamous epithelium. Numerous spherical mucous cells are distributed through different epidermal layers. The dermal layer is formed of undulated compact collagenic bundles,it was found to be separated from the epidermal layer by numerous large vessels. The hypodermis is formed of continuous layer of areolar connective tissue. The opercular core is formed of loose connective tissue, it issupported by strands of osteoid tissue and connected with internal layer of longitudinally arranged striated muscle bundles. The opercula is lined internally by simple cuboidal epithelium (Fig.9).

At the dorsal aspect of the trunk, the epidermis is thickened. The mucoid unicellular glands are numerous, they are occupied different sites at middle and superficial cell layers. these glands are spherical or ovoid in shape, some cells are found to be opened directly to the surface. The dermal layer is formed of dense parallel collagenic bundles. It is differentiated into superficial stratum laxum and deep stratum compactum. The dermal layer is followed by a thick layer of areolar connective tissue hypodermis (Fig.10).

At the ventral aspect of the trunk region, the epidermal layer is thick stratified squamous epithelium. Large spherical and goblet like mucous unicellular glands are distributed through the superficial and middle epidermal layers. Large voluminous, vertically oriented oval shaped club cells are found to be occupied the epidermis. These cells included squeezed elongated epidermal cells in between (Fig.11). The dermis is formed of dense parallel collagenic bundles,they are separated from epidermal layer by large vessels.

At the lateral aspect of the trunk, in addition to the above structures, the hypodermis is thick. It is formed of loose connective tissue and contained a wide well organized lateral canal, it is lined by stratified cuboidal epithelia. It was found to be ensheathed by fibrous connective tissue layer. The lateral canal is capsulated by in complete cartilogenous ring (Fig.12).

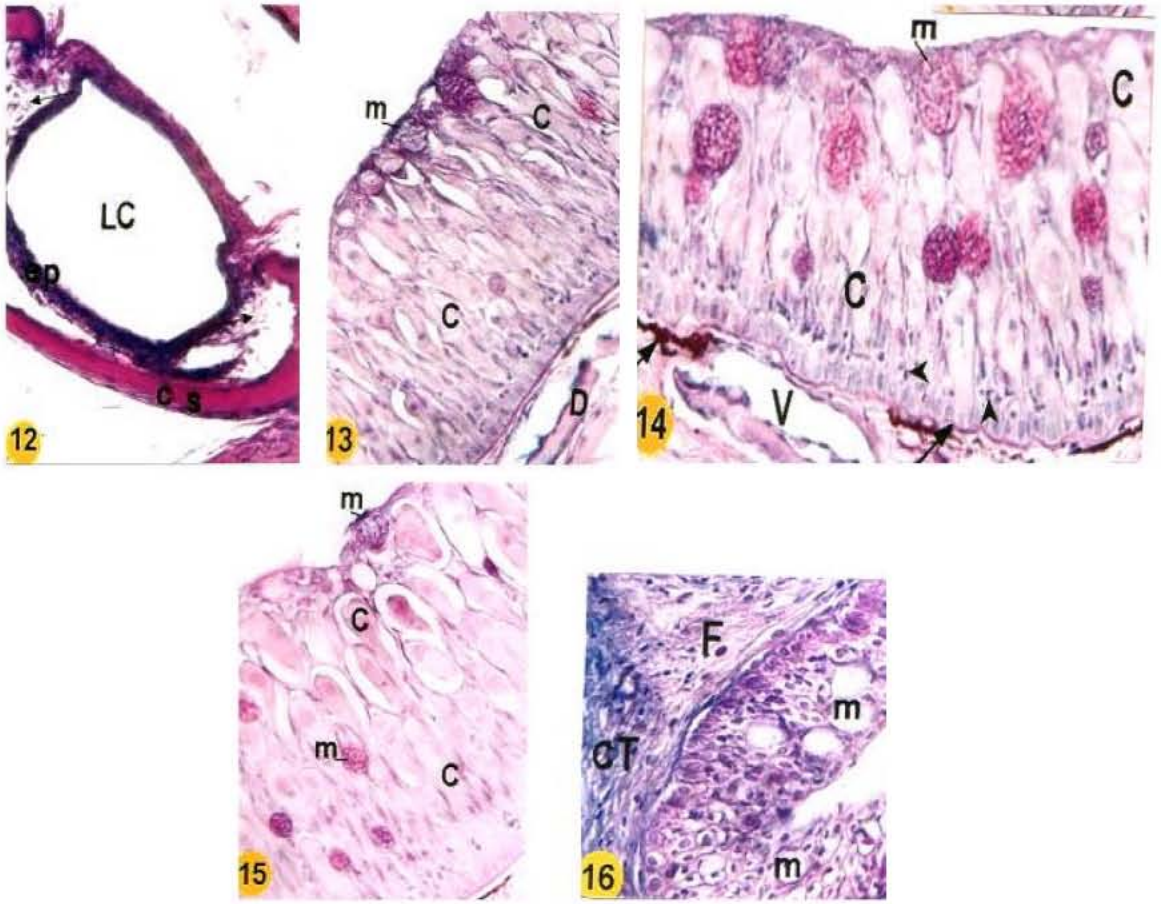

Fig. 12: A photomicrograph of a vertical section in the skin of the lateral aspect of the trunk of eel showing deep lateral canal (LC) present through the loose connective tissue of hypodermis, lined by stratified cuboidal epithelium (ep) and covered by connective tissue (arrows) and cartilagenous shell (cs). (H and E ×400)

Fig. 13: A photomicrograph of a vertical section in the skin of the dorsal aspect of the tail of eel showing, thick epidermis of stratified squamous epithelium containing superficial one cell layer of mucous cells (m) and highly condensed club cells (C) forming several layers and reduced dermis (D). (AB/PAS ×400)

Fig. 14: A photomicrograph of a vertical section in the skin of the lateral aspect of the tail of eel showing, thick epidermis containing several layers of club cells (C) squeezing epithelial cells in between (arrow heads), large scattered mucous cells (m) Notice large dermal vessels (V) and melanocytes arranged at the peripheral margin of the dermis (arrows). (AB/PAS ×400)

Fig. 15: A photomicrograph of a vertical section in the skin of the ventral aspect of the tail of eel showing, thick epidermis containing several layers of club cells (C) squeezing epithelial cells in between and few large spherical mucous cells (m) in the middle and superficial cell layers. (AB/PAS ×400)

Fig. 16: A photomicrograph of a vertical section in the skin of the urogenital orifice of eel showing thick folded epidermis containing spherical mucous cells (m) and club cells (C). The epidermis rests on a fibrous layer of connective tissue (CT) filling the core of the epidermal folds (F). (Masson's trichrome st. × 400)

At the dorsal aspect of the tail, the epidermis is formed of thick stratified squamous epithelium. Large spherical mucous cells are distributed through the superficial layers of epidermis, large elongated vertically arranged club cells are oriented through the different epidermal layers. They are numerous with condensed and squeezed epithelial cells in between. The dermis is formed of parallel bundles of dense fibrous connective tissue containing large lymph spaces (Fig.13).

At the lateral aspect of the tail, the epidermis is formed of thick stratified squamous epithelium, containing large spherical unicellular glands through the superficial

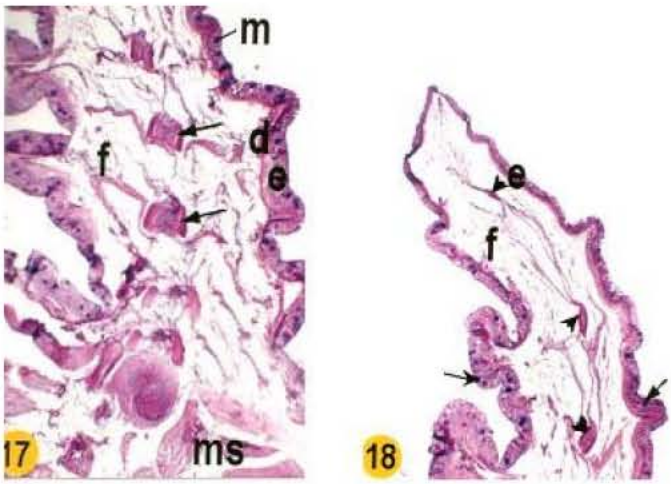

Fig. 17. A photomicrograph of a vertical section at the base of the dorsal fin of eel showing folded thin epidermis (e) containing few mucous cells (m). Notice, reduction of dermis (d), fin core as formed of loose connective tissue (f) containing partially cartilagenous lepidotrichia (arrows) associated with striated muscles (ms) (AB/PAS ×40)

Fig. 18. A photomicrograph of a vertical section at the distal region of the dorsal fin of eel showing, thin folded epidermis (e) with few small scattered spherical mucous cells (arrows). Notice the fin core (f) of loose connective tissue containing lepidotrichia (arrow heads). (AB/PAS ×40)

and mid-epidermal regions. Large, flask-shaped, elongated and vertically arranged club cells are aggregated through different layers of epidermis to squeeze the fusiform epithelial cells in between. The dermis is formed of dense fibrous connective tissue bundles containing large vessels and melanocytes at its peripheral layers (Fig.14).

At the ventral aspect of the tail. The epidermis is formed of very thick stratified squamous epithelium containing medium-sized and large sized spherical mucous cells which are distributed through the different epidermal cell layers. The club cells are large and elongated. They are vertically arranged in several layers. The club cells are condensed and squeezed the fusiform epithelial cells in between. The dermis resembled that of the dorsal aspect of the tail (Fig.15).

At the urogenital orifice, the covering epidermis is thick. It is highly folded,it is formed of stratified squamous epithelium that was found to be contained few, large spherical mucous cells and few club cells.These cells are located at the mid-epidermal layers. The dermis are contained compact collagenic bundles. They are extended to fill the core of the epidermal folds (Fig.16).

The dorsal, tail and pelvic fins are fused (Fig.1). They are formed a continuous circumscribed fin, there is no a significant difference in histological structure between its different regions. At the base of fins, the covering epidermis is thin convoluted , showing small spherical mucous cells. They are superficially distributed. The dermis is diminished to form a very thin layer of collagenic fibers. The core of the fins is formed of loose connective tissue contained central arrangement of ossified lepidotrichia which associated with striated muscle bundles. These muscle bundles showed a marked reduction toward the distal region (Fig.17). At the distal region of fins., the covering epidermis became more thin undulated layer, of stratified squamous epithelium. It showed reduction in number of mucous cells which disappeared toward its tapered end. The ossified lepidotrichia are centrally arranged and became reduced in size (Fig.18).

The pectoral fins are present at the lateral sides just posterior to the head region. At the proximal region of the pectoral fins, the epidermis is thick of stratified squamous epithelium. The mucous unicellular glands are spherical or goblet in shape. They are distributed through the superficial and mid-epidermis. The dermal region became very thin. It is formed of collagenic fibers and contained melanocytes. The fin core is formed of loose connective tissue, showed central arrangement of lepidotrichia, associated with striated muscle bundles (Fig.19). At the distal region of pectoral fin, the covering epidermis is thickened and contained spherical and goblet mucous cells. They are located through the superficial and mid-epidermal regions. Club cells are located at its middle layer. The dermis is reduced and formed thin layer of dense fibrous connective tissue containing melanocytes. The core of the fin is formed of loose connective tissue having central diminished lepidotrichia with enlarged striated muscles (Fig.20).

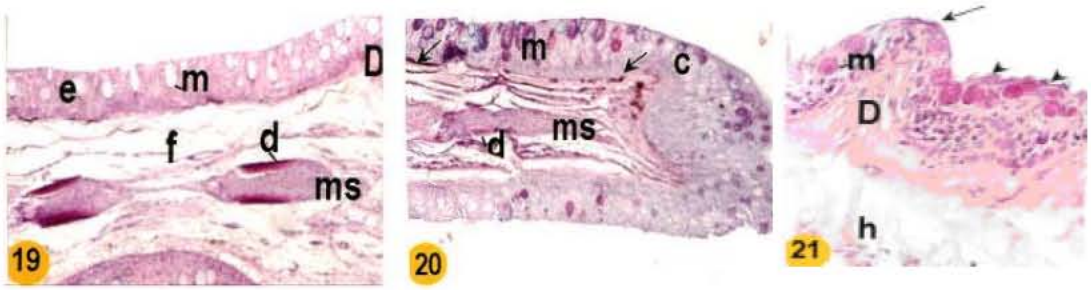

Fig. 19: Photomicrograph of a vertical section at the proximal pectoral fin of eel showing covering thick epidermis (e) with mucous cells (m), diminished dermis (D) containing melanocytes (brown) and fin core of loose connective tissue (f) containing lepidotrichia (d) and striated muscles (ms). (H and E ×100)

Fig. 20. A photomicrograph of a vertical section at the distal end of the pectoral fin of eel showing epidermis containing goblet like cells (m) at superficial and middle layers few ovoid club cells (C) at middle layers.Notice the reduction of the dermis which containing layer of melanocytes (arrows) and few lepidotrichia (d) associated with striated muscles (ms). (AB/PAS ×100)

Fig. 21. A photomicrograph of a vertical section in the skin of the pectoral axilla of eel showing, thick folded epidermis of stratified squamous epithelium (arrow), spherical mucous cells (m) distributed through the epidermal superficial layer Notice, the superficial cell exfoliation (arrow heads), dermis of dense collagenic bundles (D), filling the cores of the epidermal folds followed by thick layer of hypodermal loose connective tissue (h). (AB/PAS ×100)

At the pectoral axilla (skin region under pectoral fins), the covering epidermis is thickened and folded, it is formed of stratified squamous epithelium showing superficial cell exfoliation. The unicellular spherical mucous glands are distributed through the superficial layers. The dermis is formed of dense fibrous connective tissue extend to form the core of skin folds. The hypodermis is highly thick and formed of loose connective tissue (Fig.21).

DISCUSSION

The skin of eel in this study was analogous to that of terrestrial mammals. It is composed of an epidermis and dermis supported by hypodermis.

In the present study, the epidermis was found to be composed of non-keratinized stratified squamous epithelium. In addition to its typical epithelial cells, a variety of unique cell types have been described, mucous goblet like cells and club (alarm substance) cells. They were varied greatly in their location and distribution among different body regions.

The present investigation revealed. The mucous cells were located at the superficial epidermal layers. Previous studies reported that, the mucus covering the epidermis of teleosts was secreted from mucous cells throughout the epidermis. These cells were differentiated in the stratum germinativum and migrate to the surface of the skin, where they discharge their secretion [12]. Some authors demonstrated the mucous cells were pushed between the spindle-shaped epithelial cells discharged its entire contents and died [13, 14]. The present study clarified the mucous cells to be positively reacted with AB /PAS stain. This ensured their content of sialomucins and sulfomucins. The sulfomucins possibly protects fish from bacterial and fungal infections [4]

The distribution of the mucous cells in the epidermis varied greatly in this study. While they were condensed in general at the superficial epidermal layers mainly at the anterior body regions, they reduced in trunk regions and decreased in fins and tail. This is coincided with that observed in the epidermis of Brown trout and Char [15]. The condensation of mucoid glands in the skin of the crainal part of the fish was very important to adapt the fishes for the burrowing and mud dewelling habitation as well as for protection against abrasive injury during searching food at bottoms [16] in Poecilia reticulata. This phenomenon was the same happened in eel under investigation. Pickering and Macey [17] added that, the mucoid secretion of the goblet cells was huge when the fish became under stress factors.

In the current study, in addition to mucous cells, other cell types were demonstrated in the epidermis are club cells or (alarm containing substance) cells. The authors called these cells according to their shape as club cells [18,19].

On the bases of alarted content [20], the epidermal structural observations in this study revealed condensations of club cells in the all epidermal layers of the tail regions, ventral aspect of the trunk reduced in head region and not observed in lips, snout, opercula, dorsal aspect of the trunk and fins. This was contrary to that reported by Stoskope [21] who reported that club cells were found only in the middle of the epidermis. El-Morsy [22] demonstrated protein content in the club cells of some fresh water fishes. The function of club cells (containing alarm substance) cells was reported in carp [5]. The authors suggested that, the cells produced protein which initiates alarm reactions when perceived by olfactory organs of other fishes. Also, researchers believed that the content of these cells was released into the water when the skin of the fish was injured and this substance may serve as a warning of possible danger to the other fishes.

The dermal layer was formed of parallel bundles of collagenic fibers containing melanocytes at its peripheral margins. The melanocytes were increased in the upper lip, the dorsal aspect of snout, dorsal aspect of the trunk, tail and pectoral fins. These pigment cells give color to fishes these results are coinciding with that reported by Hybia [23].

The present study recognised sense organs of the skin, represented by neuromasts at the upper lip, taste buds in the dorsal aspect of the snout and lateral line canal in the dorsal aspect of the snout, dorsal aspect of the head and lateral aspect of the trunk. The basic structure of the superficial neuromasts and its association with the well developed dermal protuberance in the lip of eel was the same mentioned by El-Morsy [22] in *Clarias lazera*. The authors considered them as adaptation for providing a greater mechanoreceptive information for easily detection of predators and food [8].

Cutaneous taste buds in this research were nearly identical in structure to those of mammals. They were consists of sensory cells, basal cells and sustentacular cells. Their orientation in the dorsal aspect of the snout in the current study was contrary to those reported by Grizzle and Rogers [24] in catfish. They reported that taste buds were found all over the entire external surface of the epidermis.

The presence of the lateral canal system at the dorsal aspects of the snout and head as well as at the mid lateral line of the trunk in this study followed the basis of distribution pattern of the bottom dwelling fishes. It was an anatomical modification for receiving the stimuli coming from the surface water and its pores adapted for the direct contact of the canal fluid to the weak water vibrations at the bottom [7, 25]. The lateral line canal is surrounded by dense circular collagenic coat and get enclsoed by cartilagenous sheath. This was parallel to that reported by El-Morsy [22] in adult catfish, who considered this condition as adapative mean against the squeezing of canal by the high water pressure through the bottoms of watery dwellings.

It was concluded that skin structure of European eel in the egyptian sea water includes variety of sense organs, mucous goblet like cells, club (alarm containing substance) cells and melanocytes. Their distributions varied greatly along the different body regions. which accommodate their habitat.

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