

**HISTOLOGICAL AND ULTRASTRUCTURAL
OBSERVATIONS ON GILLS OF *TILAPIA NILOTICA* L.
(*OREOCHROMIS NILOTICUS*) IN LAKE QARUN, FAYOUM
PROVINCE, EGYPT**

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ABSTRACT

Fish productivity has been affected by a progressive increase of salinity and pollution in Lake Qarun. So, this work aimed to investigate the histology and ultrastructure of the gills of *Tilapia nilotica* to clarify the possible effect of water quality on the fish gills. The most observed changes include the occurrence of a prominent large number of chloride and mucous cells confined to gill filament, hyperplasia of primary lamellar epithelium which leads to obliteration of some interlamellar spaces between adjacent secondary gill lamellae, odema resulting in the separation of respiratory epithelium and inflammatory infiltration including eosinophils and macrophages. These findings demonstrate that *Tilapia nilotica* can adapt to poor conditions through cellular and histological changes.

INTRODUCTION

Lake Qarun is an inland water body in Fayoum province, Egypt. In the past, Lake Qarun was considered as reservoir of fresh water of the River Nile during the flooding season. Its salinity was always less than 1.0 gm/L. Later, dams have been constructed across the Nile stream where the water supply from the River Nile to this lake is being partially controlled and the water level in the lake started to decrease. With time, the salinity of the lake started to increase due to conveying dissolved salts into the lake and the excessive evaporation attained at the surface particularly during summer (Soliman, 1989). The average salinity of the lake increased gradually from 11 gm/L to about 81 gm/L in western side and 41 gm/L in the

eastern side (Lucas, 1906; Soliman, 1989 and Hassan, 2002). Actually the salinity due to salt content (gm/L) varies greatly in different areas of the lake, particularly near the outlets of the drains, where the lowest salinity can be observed, while the highest salinity often existed in the extreme western region (Soliman, 1989). Lake Qarun is bordered from its northern side by the desert, and by cultivated lands from its south and south eastern sides. The lake receives a continuous supply of drainage water which is loaded with salts, nutrients, wastes and pesticides (Abdel-Malek and Khalil, 1994). The annual increase of salinity in Lake Qarun and the decline of its fish production were the subject of several investigators. Although, increasing of lake salinity, fresh water species, *Tilapia zillii*, *Tilapia aurea* and *Oreochromis niloticus* are the most existing fishes in the lake. The physiology of salinity tolerance in tilapia has been reviewed by Prunet and Bornancin, (1989).

The adaptive capacity to different salinities in teleosts depends on the integrated osmoregulatory function of numerous organs, mainly gills, digestive tract and kidney (Evans, 1984, Cataldi *et al.*,1988 and Cionie *et al.*,1991). The gills are the largest proportion of the outer surface area of fish, having few micrometers separating the blood from water (Wood and Soivio, 1991), which facilitates gaseous exchange, but allows the branchial tissue to be exposed to variations of the medium. These induce morphological modifications or cellular adaptation related to the plasticity of the branchial epithelium (Laurent & Perry, 1990).

The present study aims to clarify how *T. nilotica* could be adapted to poor water conditions. through histological and ultrastructural examination of the gills of this species.

MATERIAL AND METHODS

Fish:

Tilapia nilotica were collected from western and eastern stations in lake Qarun. Fish had an average length 14 cm. \pm 2 and weight 54 gm \pm 2.

Light microscopy:

The freshly collected fish were immediately dissected and gill samples were fixed in Bouin's fluid for about 24 hrs. , then rinsed and dehydrated with a graded ethanol series, embedded in paraffin wax and sectioned at 5 μ m then stained with hematoxylin and eosin. The sections were examined and photographed on light microscope.

Transmission electron microscopy:-

According to the method of Mercer and Birbeck (1966), gill samples were immediately fixed overnight at 4°C in 5% glutaraldehyde in 0.1 M sodium cacodylate buffer. After post fixation in 1% osmium tetroxide in the same cacodylate buffer for 2 hours, specimens were rinsed twice in the same buffer, dehydrated through a graded ethanol series and processed through propylene oxide into Epon 812. Sections were cut on ultra microtome, at first 1 µm semi thin sections were stained with toluidine blue for light microscope, and then ultra thin sections were cut and picked up on mesh copper grids and stained with uranyl acetate, followed by lead acetate solution (Reynold, 1963). The ultra thin sections were examined by transmission electron microscope and electron micrographs were and photographed.

RESULTS

Gill structure:

Each gill arch of *Tilapia nilotica* bears a double row of primary gill filaments (non respiratory lamellae), and each filament carries two rows of secondary gill lamellae (respiratory lamellae). The gill filament lined by a thick stratified epithelium referred as primary epithelium. The epithelium contains several epithelial cell types; i.e. basal epithelial cells, mucous cells, mitochondria rich chloride cells and superficial pavement cells (Figs.1 & 2).

The secondary gill lamella (respiratory lamella) is lined on both opposite sides by an epithelium that is two squamous cells layer thick. The external layer is characterized by the flat elongated pavement cells. Their external plasma membrane sometimes forms a system of microridges which give a fuzzy appearance. They flattened nuclei characterized by dense peripheral heterochromatin. The cells forming the innermost layer are joined together with finger-like cytoplasmic projections.

Underlying the epithelium, there is a layer of basal lamina which separates the epithelium from the major part of the pillar system. The pillar system is a collective term for the pillar cells and blood capillaries. The pillar cells are the main body of this system which supports the lamella. The pillar cell has a large nucleus occupies the greater part of the main cell body with irregular outline and heterochromatic clumps. The cytoplasm has few mitochondria and poor endoplasmic reticulum. Four cytoplasmic projections known as pillar arms, two arms on each side of the cell extend to overlap

with the arms of the next pillar cell to form a space; (blood lacuna) in which blood cells move (Figs. 2 & 11).

Mucous cell:

Mucous cells are large modified columnar epithelial cells which synthesise, and secrete mucous, and may be stimulated by local irritation to release their entire mucigen contents. They are found at the surface between other epithelial cells of the primary gill epithelium and at the base of secondary gill lamellae (Fig.12). The mucous cells open to the external medium with a deep apical pit which is surrounded by extensions of adjacent pavement cells. Sparse microvilli forming the brush border are seen at the surface of the mucous cells. The base of the cell is occupied by a highly condensed basal nucleus. The cytoplasm adjacent to the nucleus is packed with rough endoplasmic reticulum (RER). Small vacuoles and few mitochondria are present. The distended apical cytoplasm contains a dense aggregation of mucigen granules packed into membrane bound secretory vesicles. The secretory vesicles are rounded and range widely in electron density. Due to the large number of vesicles, the nucleus is displaced to the basal margin of the cell. Small cytoplasmic processes from the mucous cells extend between adjacent epithelial cells (Figs. 5, 6, 13 & 14).

Chloride cell:

Chloride cells are large, polygonal, rounded, ovoid or columnar in shape, irregularly spaced and usually covered by superficial pavement cells. They are characterized by a rich population of mitochondria evenly distributed throughout the cytoplasm, which are variable in size and shape. The extensive smooth walled tubular membrane system ramifies throughout the cytoplasm. The cytoplasm contains also scattered ribosomes and glycogen particles. The euchromatic nucleus has a little heterochromatin. A conspicuous feature of many chloride cells is the presence of an apical pit opening to external environment. The pit is usually located between two superficial pavement cells. The pit may contain finely granulated material (Figs. 5, 7 & 15). The other chloride cells which were not in contact with the external medium had sheets of pavement cells covering their apical surfaces.

Eosinophil granular cells:

Eosinophils are phagocyte cells with a similar metabolism like neutrophils. The increased number of eosinophils in the tissue indicates some disorders. Eosinophils are characterized by large intensely basophilic granules of cytoplasm. The specific granules are

membrane bounded of uniform size. Cells are not readily identified in routine histological sections, due to the water solubility of their densely basophilic granules which tend to be lost during preparation. When stained with the basic dye, toluidine blue, the granules bind to the dye changes in color to red, a phenomenon described as metachromasia (Fig. 5, 6 & 7). With electron microscopy, the large specific granules are membrane bounded, round or oval in shape and filled with closely packed electron dense material. The cytoplasm contains free ribosomes, glycogen particles, few mitochondria and fragmented rough endoplasmic reticulum (RER) scattered out of the cytoplasm (Fig. 17).

Variation in gill structure:

Fish gill sections were examined and all alterations were recorded. The main marked change in fish specimens collected from the western region of the lake, where the highest salinity exists; is an oedematous change. The swelling of the inter-cellular spaces between the epithelial lining and the basal lamina of the respiratory gill lamellae led to the appearance of wide spaces from the base towards the tip of the gill lamellae (Fig. 3).

Extensive proliferation for both well developed mucous and chloride cells was obvious. Some of chloride cells have a large apical surface and became in direct contact with the external medium (Figs. 3 & 4).

An increase in the cellular layer of the primary epithelium led to partial fusion of some of the adjacent respiratory gill lamellae was also observed (Figs. 3 & 4). In addition, infiltration of large number of eosinophilic granular cells into the intercellular tissue was evident (Figs. 5, 6, 7, & 8).

Hyperplasia of the gill filament epithelium was the dominant change recorded in the gills collected from the eastern region of the lake, where high saline and polluted water. The increase in the cellular layers of the epithelium resulted in the obliteration of the interlamellar space between respiratory gill lamellae.

Hyperplasia was often accompanied by extensive proliferation of both mucous and chloride cells. Clusters of chloride cells were evident, some of them showing signs of degeneration. In addition inflammatory cells including lymphocytes and macrophages were widely accumulated (Figs. 9, 16 & 19).

DISCUSSION

In the present study, a large number of chloride cells located in the gill filament epithelium was observed in fish specimens, collected from west and east sides of Lake Qarun. The increase in number of gill chloride cells is known to occur in euryhaline teleosts transferred to salt water (Avella *et al.* 1993). This is also in agreement with Assem & Hanke (1983) and Dange (1985), where *Oreochromis mossambicus* may be acclimatized to 65‰ salinity.

Foskett *et al.* (1983) have suggested that the development of the ion transport mechanism in euryhaline teleosts involves activation and proliferation of immature chloride cells followed by differentiation of these cells. These cells may have a role in ion transport in both fresh and saltwater-adapted teleosts, there is evidence that chloride cells take up ions, particularly Na^+ Cl^- and Ca^{2+} (Laurent and Dunel 1980 and Flik *et al.* 1984).

According to previous studies, chloride cells located in lamellae may be involved in ion regulation in freshwater trout (Perry & Wood 1985; Avella *et al.*, 1987), whereas the increased number of chloride cells in filament in sea water reflects teleost adaptation to elevated external salinity (Karnaky *et al.*, 1976; Thomson & Sargent, 1977; Laurent and Dunel, 1980) and may be responsible for salt extrusion in hypertonic medium (Keys and Wilmer, 1932). This interpretation was strongly supported by the studies performed on opercular epithelium of *Fundulus* and *Sartherodon* (Foskett *et al.* 1981).

Karnaik *et al.* (1976) and Foskett *et al.*, (1981) reported that apical pit is often correlated with adaptation to high salinities. Many authors observed the apical pit in the chloride cells of some fresh water adapted fishes including tilapias (Fishelson, 1980 and Maina, 1990), guppies (Straus, 1963) and *Fundulus* sp. (Philpott & Copeland, 1963). Furthermore apical pit has been described in fresh water and sea water adapted *Rivulus marmoratus* at all tested salinities (King *et al.*, 1989).

Azab *et al.* (1999) studied the morphology and ultrastructure of the gill epithelial cells of euryhaline cyprinodont fish, *Aphainus dispar* in normal sea water fishes and the variation during acclimation to both hypersaline and fresh water. In sea water and hypersaline adapted fishes, chloride cells were confined to gill filament, whereas in freshwater adapted fishes, these cells were observed with some secondary lamellar epithelium in addition to the gill filament. In hypersaline adapted fishes a large contact area of chloride cell apical

membrane with the external medium was observed. Coleman *et al.* (1977) did not observe any difference in the number or distribution of chloride cells at different salinities, but the fish displayed apical pits and enlargement of chloride cells in sea water, suggesting salt excretion induced by saltwater adaptation.

During salinity challenge, an increase in the number of ridges on epithelial cells was observed in various *Tilapia* species (Fishelson, 1980). In the present study, microridges of the external epithelial layer lining the respiratory gill lamellae were observed. However, the physiological role of this specific change is still unclear, but according to Fishelson, the phenomenon is more pronounced in the more euryhaline species and may participate in osmoregulation.

Increased mucous cell production and enhanced mucous secretion, often in considerable amount, is considered to be the most distinctive feature of teleost epidermis (Harris & Hunt, 1975; Sibbing & Uribe, 1985). Increased mucous secretion under toxic conditions is known to bar the entry of toxicants into the fish, probably by interacting with toxic ions and then resulting in the formation of a film of coagulated mucus on the surface of the gill, or by providing a physical barrier for macromolecules. (Kumar and Pant, 1981). The proliferation and migration of the specialized mucous cells on to the interlamellar tissue between secondary gill lamellae appears to be a protective response to induced ionic disturbances at gill surface.

The present results are in agreement with Kantham & Richards (1995), where histological changes in the gills of carp exposed to phosphate buffered dilution water induced an extensive mucous cell proliferation on the lamellar and filamental epithelia, an increased infiltration of eosinophilic granular cells into the gill tissues, a moderate epithelial hyperplasia and an increase in the chloride cell densities on the gill epithelia.

Gill hyperplasia has been regarded as a common sign of chronic toxicity caused by various chemical pollutants. In the present study, mild to moderate gill hyperplasia was noted in west regions specimens, while severe hyperplasia was evident in east station specimens. It has been suggested that such a hyperplastic reaction may increase the epithelial thickness so as to retard or prevent the entry of toxic ions into the blood stream or to compensate for osmotic imbalance (Laurent, 1984).

The infiltration of eosinophilic granular cells and other granular leucocytes cells into the gill epithelium had been described as an

inflammatory reaction in carp by various workers (Hornich & Tomanek 1983; Kovacs-Gayer 1984) in response to different chemical toxic substances. As suggested by Al-Hussaini (1949), these granular cells might have an antitoxic effect although the exact mechanism involved is unknown.

The swelling of respiratory gill lamellae and lifting of their epithelial lining due to the subepithelial edema was a common and conspicuous change in the present study. The appearance of extended lamellar oedematous spaces seems to implicate a protective and osmoregulatory manifestation of the gills. Hughes and Wright (1970), Hughes and Gray (1972) and Hughes (1978) have described the intercellular spaces in the secondary lamellae as lymphoid spaces and as these spaces contained a variety of white blood cells, these authors suggested that they might perform a function analogous to alveolar macrophages, and that the circulating fluid may be important in the protective and osmoregulatory functions of the gills.

According to Azab *et al.*, (1999) the gills from hypersaline - adapted fresh water fishes showed swelling and fusion of the adjacent secondary lamellae, ballooning of the epithelial cells and separation of the epithelial layers of secondary lamellae by intercellular spaces. These intercellular spaces communicate with the corresponding intercellular spaces of filament epithelium, a feature that may be an important implication in consideration of trans-branchial fluid movement (Laurent & Dunel, 1980).

On the other hand, the swelling of the intercellular spaces and infiltration of leucocytes was parallel to those alterations seen by Skidmore and Tovell (1972) when fishes were exposed to pollutants.

The presence of an increased number of chloride cells in the gill tissue once again suggests an increased ionic activity at the gill surface caused by the prevailing water quality conditions. According to Laurent and Perry (1991) rapidly occurring changes in the morphometry of epithelial, mucous and chloride cells may be adaptive or pathological changes, depending on the nature of the ionic disturbance and the toxicant involved.

So, in the present study, it can be concluded that the histological and ultrastructure alterations in the gill of *Tilapia nilotica* indicate an accelerated adaptive response to the increasing salinity and poor conditions of lake Qarun water at present.

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LEGEND OF FIGURES

- Fig. (1): A light micrograph of sagittal section in gill of *Tilapia nilotica* specimens showing general gill structure. Each gill filament composed of primary (non-respiratory) gill lamellae (↑) carrying double rows of secondary (respiratory) gill lamellae (↑↑) the secondary gill lamellae are equally spaced separated from each other from the free side and connected by interlamellar tissue at the base. (H. & E. X 100).
- Fig. (2): A magnified part of gill filament showing, multilayered filament epithelium (E) and bilayered secondary gill lamella (S). Each secondary gill lamella composed of central pillar system (P.S.). The space between pillar cells formed lacunae through which red blood cells flow. The basillamellar region formed of different epithelial cells in between lay mucous cells (M) and chloride cells (C.C.). (H. & E. X 1000).
- Fig. (3): A lightmicrograph of sagittal section in gill (west Qarun Station) showing partial fusion some of secondary gill lamellae by proliferated interlamellar epithelial cells (↑↑) and swelling of others due to the subepithelial odema (↑) (H. & E. X 100).
- Fig. (4): A magnified part of gills showing partially fusion of adjacent secondary gill lamellae. Swelling of the lamellae, degeneration and desquamation of the epithelial layer covering secondary lamellae, deformed pillar system and aggregation of mucous cells packed with mucous granules. (H. & E., X 400).
- Fig. (5) A light micrograph of semithin section of gill showing bilayered secondary gill lamellar epithelium (↑) , pillar system (P.S.) composed of pillar cells (P.C.) , blood lacunae filled with red blood cells (r.b.c) and primary gill lamella covered by multicellular epithelium. Notice, the large well developed mucous cells (M) which have a darkly stained nucleus at the base and well developed mucigen granules (*). (Toluidine blue X 1000).
- Fig. (6) A light micrograph of semithin section of gill (west Qarun station) showing bilayered secondary gill lamellar epithelium & pillar system (pillar cells, red blood cells

and blood lacuna). Notice, the vacuolated space or the appearance of intercellular spaces (ICS) between pillar system and epithelial layer covering lamellae. Part of multicellular complex of gill filament epithelium which contains different epithelial cells, infiltrated eosinophilic cells (*) which characterized by large densely stained granules and mucous cells (M) which have densely stained basal nucleus and well developed mucigen particles. (Toluidine blue X 1000).

Fig.(7) A light micrograph of semithin section of gill filament (west Qarun station) showing, apex of gill filament covered multicellular epithelium. Notice, the rich mitochondria chloride cells (C.C.) and well developed mucous cells (M). The chloride cells are in direct contact with the external medium through their apical pits (↑) . The marginal blood vessel (B.V.) contains red blood cells and leucocytes. (Toluidine blue X1000).

Fig. (8) A light micrograph of semithin section through gill filament (west Qarun station) showing apex of gill filament. Notice, the complex multicellular epithelium covering including different types of epithelial cells (E), and the appearance of intercellular spaces (ICS), infiltrated eosinophile cells, corrugated borders (↓↓) covered by microridges which give a fuzzy appearance surface. Expanded congested blood vessel (B.V) filled with red blood cells and different leucocytes. The wall lining of the blood vessel became thinner. (Toluidine blue X 1000).

Fig. (9) A lightmicrograph of semithin section through gill filament (east Qarun station) showing, proliferation of interlamellar epithelial cells causing fusion of adjacent secondary gill lamellae, some of chloride cells (C.C.) have signs of degeneration, congesting blood lacunae, and separation of pillar system from lamellar epithelium. (Toludine blue X 1000).

Fig. (10) A light micrograph of semithin section through gill filament (east Qarun station) showing complex multicellular macrophage infiltration (↑), numerous well developed chloride cells (C-C). (Toludine blue X 1000).

Fig. (11) An electronmicrograph of a part of secondary gill lamella showing, the outer bilayered epithelium (↓) , the flat

elongated pavement cells have oval nuclei (N) characterized by dense peripheral heterochromatin, the cytoplasmic matrix contains fragmented rough endoplasmic reticulum, mitochondria and ribosomes. Pillar system (P.S.) which composed of pillar cell (P.C.), the lateral cytoplasmic processes (C.P.) of each pillar cell stretch out to touch those of adjacent pillar cells forming blood lacunae (B.L.) through which red blood cells (r.b.c.) flow. (X 3000).

Fig. (12) An electronmicrograph of gill (west Qarun station) showing the basal region of the secondary gill lamellae. Notice the well developed mucous cell (M) and rich mitochondria chloride cells (C.C.). (X 1800).

Fig. (13) An electronmicrograph of gill showing well developed mucous cell which open to the external medium with a deep apical pit (↓↓) which surrounded by extension of pavement cells. The mucous cell have large highly condensed nucleus (N) its cytoplasm is packed with a large number of electron dense mucigen vesicles (M.V.). (X 3600).

Fig. (14) An electronmicrograph showing a mucous cell (M). Notice the extensive rough endoplasmic reticulum (RER), small cytoplasmic processes (↑) from the mucous cell extend between adjacent epithelial cells (X 5400).

Fig. (15): An electronmicrograph of gill (west Qarun station) showing two of large chloride cells sharing in a wide apical pit (↑↑), surrounded by superficial pavement cells. Notice, the numerous rounded and oval mitochondria (m) distributed throughout the cytoplasm, the extensive smooth walled tubular membrane system, free ribosomes, scattered particles of glycogen, the euchromatic nucleus with small masses of heterochromatin. (X 7500).

Fig. (16) An electronmicrograph of gill (east Qarun station) showing degenerated chloride cells (C.C.) .Notice the disorganized mitochondria (m), reduction of both mitochondria and tubular system, nucleus (N) with more electron dense heterochromatin clumps and cytoplasmic vacuolated spaces (V). (X 7000).

Fig. (17) An electronmicrograph of gill (east Qarun station) showing eosinophile ranular cell. Notice the large electron dense material packed cytoplasmic granules which have uniform size and membrane-bound, free ribosomes and few rough endoplasmic reticulum scattered all over the cytoplasm. (X 5000).













