

LIGHT AND ELECTRON MICROSCOPIC STUDIES ON THE POSTERIOR KIDNEY OF OREOCHROMIS NILOTICUS

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ABSTRACT

The posterior kidney of Oreochromis niloticus was examined using both light and transmission electron microscope (TEM). This study showed that the posterior kidney could not be demarcated into a renal cortex or medulla, but instead numerous nephrons and ducts. Each nephron was formed of a renal corpuscle and a renal tubule. The renal corpuscle contained capillary glomerulus enclosed by Bowman's capsule. The renal tubule was subdivided into a short neck segment, first and second portion of proximal segment, intermediate segment, distal segment and collecting tubule. The latter was connected to the mesonephric duct.

INTRODUCTION

The posterior kidney is one of the most important excretory organs of teleost fishes. Together with the skin, intestinal mucosa, liver and gill epithelium, it maintains delicate osmotic balance between the fish and its environment (Bulger and Trump, 1969 and Harder, 1975). In fresh water fishes the trunk kidney and urinary bladder function in the excretion of dilute urine (Groman, 1982).

The structure of the kidney of the fresh water fish has been studied by many investigators (Edwards, 1935; Newstead and Ford, 1960; Ogawa, 1962; Bulger and Trump, 1969; Hendricks, 1971; Anderson and Lowen, 1975; Roberts, 1978; Endo and Kimura, 1982; Hendricks, 1983; Di and Yu, 1986, and Takashima and Hibiya, 1995). Other studies have been achieved on the sea lamprey (Youson and McMillan, 1971) and atlantie hagfish (Heath-Eves and McMillan, 1974). Moreover, ultrastructural features of the kidney were described in both marine species (Bulger and Trump, 1968) and freshwater trout (Anderson and Lowen, 1975).

The posterior kidney of the tilapia (*Oreochromis niloticus*), one of the teleosts species, needs

more investigation. The aim of this study was to clarify the histological and ultrastructural peculiarities of the **Oreochromis niloticus** posterior kidney. Such goal is an important prerequisite for elucidation of its possible functions.

MATERIAL AND METHODS

Nine live adult fish (*Oreochromis niloticus*) were obtained from commercial breeder. After severance of the spinal cord, the body cavity was opened along the ventral midline. Specimens of posterior kidney were removed and immediately fixed in Bouin's, Susa and 10% neutral buffered formalin. They were then dehydrated in ascending grades of ethyl alcohol and cleared in xylene, embedded in paraffin. Sections of 5-7 μ m were cut and stained by Harri's hematoxylin and eosin (H & E), PAS-technique, alcian blue (pH 0.1) and Crossmon's tircrome stains. The aforementioned methods were done as outlined by **Drury and wallington (1980) and Bahcroft and cook (1996)**.

For TEM, tissue samples were obtained from different regions of the posterior kidney and were fixed for 24 hr at room temperature in 2% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.4). The specimens were trimmed, rinsed in the same buffer, and postfixed for 1 hr at room temperature in 1% osmium tetroxide in the same buffer. Samples were dehydrated in ethanol, cleared in propylene oxide and embedded in Epon 812. "Thick" survey sections (1.0 - 1.5 μ m) were cut with a glass knife, and stained with toluidine blue and azure II for light microscopy. Ultrathin sections were obtained with a diamond knife, stained with uranyl acetate and lead citrate and examined at 60 kV with Jeol 100 CX TEM.

RESULTS

The two kidneys of **Oreochromis niloticus** were fused appearing as one organ. They occupied the dorsal retroperitoneal position along the entire length of the body cavity. They were bounded by the vertebrae dorsally, the ribs laterally and the swim bladder ventrally. The kidney was covered ventrally by a single layer of mesothelial cells.

Microscopically, the posterior kidney cannot be demarcated into a renal cortex and medulla. It was covered by thin fibrous connective tissue capsule formed of collagenous and reticular fibers (Fig. 1). Few smooth muscle cells were also detected in the renal capsule (Fig. 1). The parenchyma of the kidney was composed of numerous nephrons and ducts. Each nephron was formed of renal corpuscle and renal tubules (Fig. 2).

The renal corpuscles were nearly identical and each contained a vascular capillary glomeru-

lus that was enclosed by a Bowman's capsule (Fig.3). The Bowman's capsule was composed of an outer parietal layer of squamous cells with flattened nuclei (Fig. 4) and an inner visceral layer of differentiated epithelium (podocytes). The cytoplasm of podocytes contained PAS-positive materials. Their euchromatic nuclei were spherical or ovoid. At the ultrastructural level, the podocytes appeared as irregular cells with large spherical or ovoid centrally located electron-lucent nuclei with some electron-dense peripheral heterochromatin. The cytoplasm contained free ribosomes, mitochondria, well-developed Golgi apparatus, some strands of rER and vesicles of different size (Fig. 6). The cell has several large processes that gave rise to smaller ones (pedicels) which interdigitated with those of adjacent cells forming a layer of interdigitated pedicels adjacent to the basement membrane (Fig. 5). Filtration slits with occasionally slit membrane (diaphragm) were located among the interdigitating pedicels (Fig. 6). Juxtaglomerular cells were hypertrophic smooth muscle cells that were identified in the wall of the afferent arterioles (Fig. 4). They showed elongated nucleus. Their cytoplasm contained lightly basophilic fine secretory granules that stained by PAS-reagent (Fig. 4). At the ultrastructure level, the cytoplasm of this cell revealed sparse rER, mitochondria and was entirely occupied by an extensive number of electron dense granules (Fig. 7). The basement membrane appeared thin between the juxtaglomerular cells and endothelium of the afferent arteriole (Fig. 7). Mesangial cells were not observed.

The renal tubule was subdivided into short neck segment, first and second portion of proximal segment, an intermediate segment, the distal segment and collecting tubule that was connected to the mesonephric duct (Fig. 2).

The short neck segment had a narrow lumen and was lined with a single layer of columnar cells showing spherical basally located nuclei and slightly basophilic cytoplasm (Fig. 2). The apical border of these cells carried prominent brush border that showed PAS-positive reaction (Fig. 8). At the EM level, the cells lined the neck segment were low columnar with basal euchromatic electron-lucent nuclei showing prominent nucleoli. The apical border of the cell presented prominent microvilli. Basally the cell membrane exhibited slight infoldings that house mitochondria with dense matrices. Laterally, the opposed cell membrane of the adjacent cells acquired numerous desmosomes (Fig. 9). Free ribosomes, cisternae of rER, multivesicular bodies and electron dense granules were scattered in the cytoplasm (Fig. 9).

The initial portion of the proximal segment had a slightly wider diameter than that of the neck segment. It was lined by columnar cells with apically located spherical nuclei and prominent apical brush border (Fig. 2). The cytoplasm was vacuolar and acidophilic. The supranuclear cytoplasm was less acidophilic than the basal cytoplasm. The luminal border of the cells and the brush border showed an intense PAS-positive reaction (Fig. 8). By EM, the apical cytoplasm con-

tained numerous electron dense vacuoles of different sizes, lysosomes, smooth and rough endoplasmic reticula and free ribosomes. The apical surface of the cells was modified into long and densely packed microvilli (Fig. 10). The basal cytoplasm contained numerous filamentous mitochondria that were lodged in the deep basal infoldings (Fig. 11).

The second portion of the proximal segment had a relatively narrower luminal diameter than that of the initial portion. It was lined with columnar cells showing apical brush border that exhibited an intense PAS-positive reaction. The cells had an acidophilic cytoplasm with spherical or oval nuclei located towards the cell lumen (Fig. 12). With TEM, the cytoplasm contained electron dense granules, rER, free ribosomes and elongated electron-dense filamentous mitochondria that were mainly encountered parallel to the cell axis in the basal cytoplasm in association with the numerous basal infoldings (Fig. 13).

The intermediate segment was short with a narrow lumen. It was lined by a single layer of columnar cells without obvious apical brush border. The cytoplasm was vacuolar, and lightly acidophilic (Fig. 2). The nuclei were spherical in shape and basally located. Fine structure of these cells revealed the presence of euchromatic nuclei. The cytoplasm showed free ribosome, few rER and small vesicles. Basal infoldings holding less electron dense curved mitochondria were obvious (Fig. 14).

The distal segment was recognized as a short tubule with a wide lumen. It was lined by low columnar cells with weakly visible brush border (Fig. 2 & 15). The cytoplasm was slightly acidophilic. The nuclei were large spherical in shape and basally situated. TEM examination showed that the microvilli were shorter and more random caudally. The mitochondria were fewer, scattered throughout the cytoplasm and tended to be perpendicular to the basement membrane and parallel to plasmalemmal infoldings (Fig. 14). The number of small vacuoles and free ribosomes were increased.

The collecting tubules were lined with columnar cells with basally located ovoid nuclei and slightly basophilic cytoplasm (Fig. 12). The apical border of the cells was distinct. The tubules were surrounded by a thin layer of smooth muscle cells and connective tissue elements (Fig. 15). The basement membrane and the smooth muscle layer showed PAS-positive reaction. With EM, the cytoplasm exhibited electron dense mitochondria, sER, few rER, free ribosomes, and electron dense membrane bound granules. Also cytoplasmic vacuoles of different sizes were observed (Fig. 16). Well-developed tight junctions were encountered among the lateral cell membranes of the opposing cells (Fig. 16).

The mesonephric duct was lined with pseudostratified columnar epithelium. Its lumen was wide and irregular in appearance. A thin layer of smooth muscle fibers and connective tissue ele-

ments surrounded the duct (Fig. 18). The basement membrane and smooth muscle layer showed PAS-positive reaction (Fig. 19).

DISCUSSION

The present study showed that the posterior kidney of *Oreochromis niloticus* was not differentiated into cortex and medulla as in mammals. Rather it consisted of numerous nephrons and ducts arranged in no specific pattern. The nephron was composed of a renal corpuscle and renal tubules. The renal corpuscle was formed from glomerulus and Bowman's capsule. The same result was recorded in fresh water fishes (Edwards, 1935; Grafflin, 1937; Ogawa, 1962; Bulger and Trump, 1969; Kendall and Hinton, 1974; Anderson and Loewen, 1975; Roberts, 1978; Endo and Kimura, 1982; Groman, 1982; Hendricks, 1983; Di and Yu, 1986 and Takashima and Hibiya, 1995).

The renal tubules of *Oreochromis niloticus* were divided into a short neck segment, first (initial) and second portion of the proximal tubule, an intermediate segment, distal tubule and collecting tubule. There was no thin segment. The renal corpuscles in fresh water fishes were comparatively larger and more numerous than those in marine fishes (Nash, 1931; Friedman et al., 1942; Kempton, 1943; Harder, 1975 and Browne, 1985). Harder (1975) suggested that the fresh water fishes need a large filtration surface, where the concentration of salts in their body fluids is higher than that in the surrounding medium. However, the present risk of passive increase in the volume of the body fluid which would damage the tissue, so that water must constantly be drawn out of the body. Browne (1985) added that, in seawater low filtration rates were associated with a small number of filtering glomeruli compared with much larger population of filtering glomeruli in fish adapted to fresh water. Thus it was presumed that the kidney of fresh water fishes produce a dilute or hypotonic urine to maintain salts in their body fluid constant.

The visceral epithelial cells (podocytes) of Bowman's capsule in *Oreochromis niloticus* consisted of a spherical cell body with several large processes that give rise to smaller ones (pedicels) which interdigitate with other pedicels forming a perivascular layer, similar to that in mammals. Similar findings were previously demonstrated by Bulger and Trump (1969) in English sole and by Anderson and Loewen (1975) in fresh water trout. Podocytes are likely to be found wherever ultrafiltration occurs (Heath-eyes and Mcmillan, 1974) in hagfish. Moreover the podocyte cytoplasmic vesicles that have been revealed in the present study might indicate a transport of glomerular filtrate through the cell from the subpodocytic space (Ellas et al., 1965) and could be formed either by pinocytosis or glomerular filtration pressure which form lacunae in the pedi-

cles. Podocytes also may reabsorb protein from Bowman's space and presumably return it to the blood (**Heath-eves and Micmillam, 1974**) in hagfish.

The present study revealed that the glomeruli had a single afferent arteriole. Glomeruli with two afferent arterioles were recorded in rainbow trout (**Browne, 1985**).

As in the other fresh water species (**Capreol and Sutherland, 1968; Oguri and Sakabe, 1968; Harder, 1975, Oguri, 1980 "a" and "b"**), the juxtaglomerular cells in *Oreochromis niloticus* were identified in the wall of afferent arterioles. These cells contain secretory granules which stained by PAS technique. **Takashima and Hibiya (1995)** added that the hormone rennin is secreted from these cells. On the other hand, **Oguri, (1993)** in the aglomerular kidney of the red batfish, found that juxtaglomerular cells were localized as clusters in several regions within the kidney. In the present study, the cytoplasm of the juxtaglomerular cells contained electron dense secretory granules which stained positively with PAS, consequently these granules could be equivalent to renin which partially control the blood flow to the afferent arteriole in fish (**Harder, 1975**).

In the posterior kidney of *Oreochromis niloticus*, the neck segment was lined with low columnar cells carried prominent brush border, but there were no cilia in this region. This was in contrast to **Edwards (1935)** in bony fish, **Romer (1962), Bulger and Trump (1968), Youson and McMillan (1970), Anderson and Lowen (1975), Lacy and Reale (1991) and Takashima and Hibiya (1995)** who recorded that the neck segment was lined with columnar ciliated cells. The later authors added that the presence of microvilli at these cells could indicate absorptive function, while the presence of electron dense granules, rough endoplasmic reticulum and free ribosomes suggested secretory function too.

The proximal tubule was the largest portion of the nephron, composed of segment I and segments II. This result confirmed those recorded in fresh water fish (**Anderson and Lowen, 1975; Groman, 1982; Hendricks, 1983 and Takashima and Hibiya, 1995**). The first proximal segment was lined with columnar cells with spherical to oval nuclei. The supranuclear cytoplasm stained less acidophilic than the basal cytoplasm. The same finding was mentioned in fresh water fish (**Anderson and Mitchum, 1974; Anderson and Lowen, 1975; Ottosen, 1978; Groman, 1982; Hendricks, 1983 and Feeraz et al., 1993**). In fact the proximal segment in fresh water fish is composed of two parts; segment I and II for reabsorption of salt and eliminate excess water and further urine dilution in distal tubules. This in accordance with the statement of **Hendricks (1983)** who recorded that the major functions of fresh water teleost nephron are conservation of salt and elimination of excess water. According to TEM study in the present investigation, the presence of apical microvilli and electron dense vacuoles of different sizes in these

cells could reflect the absorptive function of the proximal tubules. Moreover, the presence of filamentous mitochondria in the basal cytoplasm with basal infoldings may confirm transport function of the proximal tubules (Anderson and Lowen, 1975).

The second proximal segment was lined by taller columnar cells that showed an increase in the outer tubular diameter as well as a decrease in the luminal one. The same architecture was described in fresh water trout Anderson and Mitchum (1974) and Anderson and Lowen (1975), striped bass (Groman, 1982), teleosts Endo and Kimura (1982), Hendricks (1983) and Hickman and Trump (1969), elasmobranch Grizzle and Rogers (1979), Elger and Hetschel (1986) and Lacy and Reale (1991). The latter suggested that the second proximal segment might be responsible for divalent-ions secretion. In the present study, the second proximal segment seemed to have a well-developed absorptive function as indicated from the possession of a distinct brush border on the majority of its cells.

Cells of the intermediate segment in *Oreochromis niloticus* were non-ciliated columnar with spherical nuclei and acidophilic cytoplasm. Borghese (1966); Bulger and Trump (1969) and Kendall and Hinton (1974) stated that no intermediate segment was observed in some elasmobranch fish, in English sole fish and in channel catfish respectively, but it is found in the striped bass (Groman, 1982) and in carp kidneys (Takashima and Hibiya, 1995). In the present study, the ultrastructural investigation of this segment revealed the presence of few rER, free ribosomes and vesicles beside a clear basal infoldings that were associated with long curved mitochondria. According to Anderson and Mitchum (1974) the association of such organelles in this segment might indicate that this segment plays crucial roles in the process of salts conservation in fresh water fish.

Unlike Ogawa (1962) and Bulger and Trump (1968) who stated that the distal segment was absent in marine fish and Beitch (1963) who found no distal segment in the striped bass from seawater, we recorded the presence of this segment in our investigation. The present study showed that the distal segment was lined with columnar cells whose cytoplasm contained vacuoles, few mitochondria and basal infoldings. In the distal segment, the number and size of mitochondria were markedly decreased. These phenomena and their association with the plamalemma system could involve this segment in the dilution of urine by reabsorption of sodium and chlorine in fresh water teleosts (Tampi, 1959; Morris, 1960 and Hickman, 1965).

The present investigation revealed that the collecting tubules were lined by columnar cells with basophilic cytoplasm and oval basally located nuclei. The basement membrane and the smooth muscle layer surrounding the tubule showed PAS-positive reaction. The same results were observed in English sole (Bulger and Trump, 1968). Fine structure of the collecting seg-

ment of **Oreochromis niloticus** revealed that these cells had fewer organelles than those of distal tubules. Moreover the intercellular spaces between its lining epithelium seemed to be reduced with the presence of a well developed tight junction between the opposing membranes. Such ultrastructural peculiarities might suggest an active role in the process of protein transport as was previously demonstrated by **Bulger and Trump (1969)** in teleosts.



Fig. 1 : A photomicrograph of a section in posterior kidney of **Oreochromis niloticus** showing: thin connective tissue capsule (C) containing smooth muscle fibers (arrow) and renal tubules (R). Crossman's stain X 200.

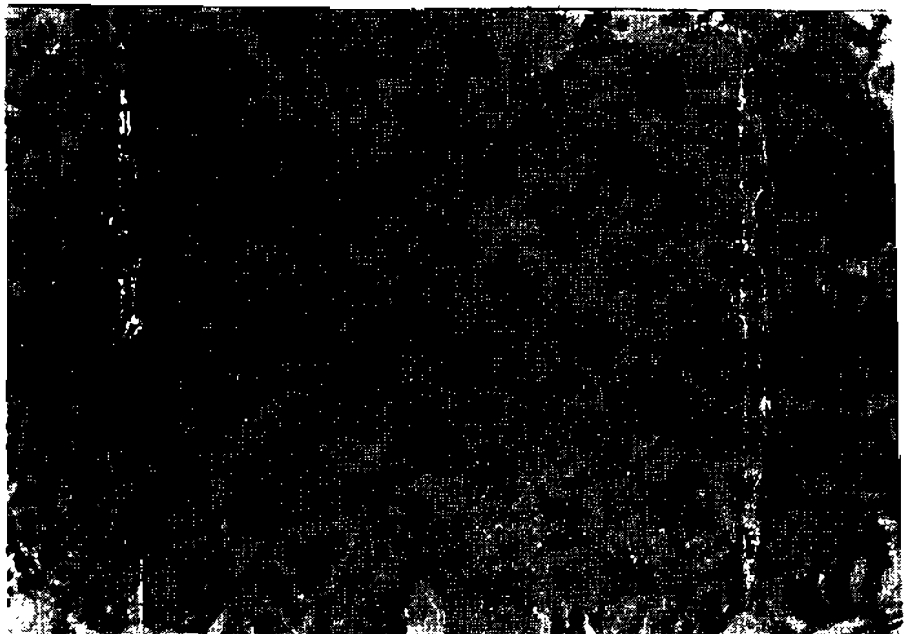


Fig. 2 : A photomicrograph of a section in posterior kidney of **Oreochromis niloticus** showing: glomerulus (G), neck region (n), first proximal segment (p1), second proximal segment (p2), intermediate segment (IS) and distal tubule (d) H & E stain X 400.

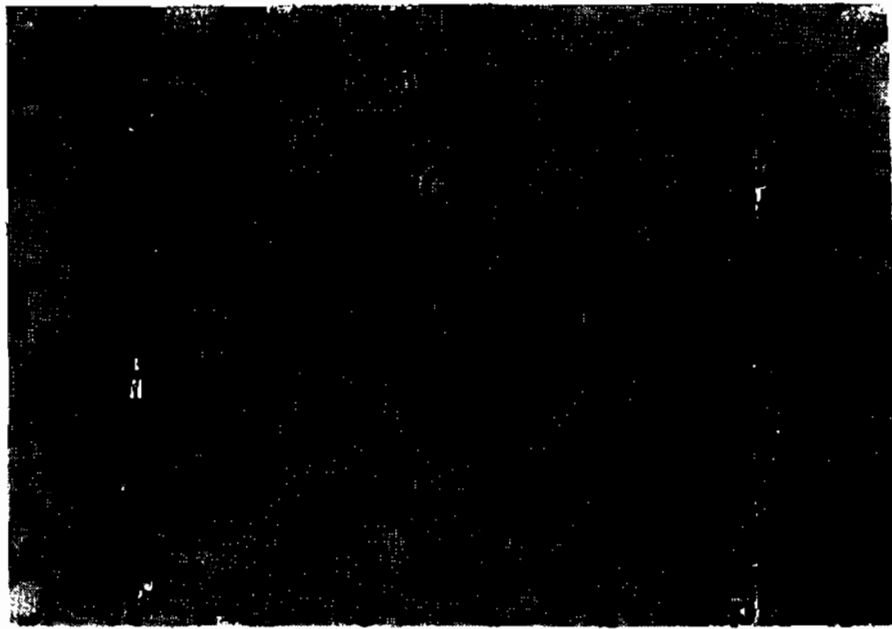


Fig. 3 : A photomicrograph of a section in posterior kidney of *Oreochromis niloticus* showing: Bowman's capsule (B), PAS-positive reaction in glomerulus (G), microvilli brush border of neck region (n) and proximal tubule (p). PAS-technique X 400

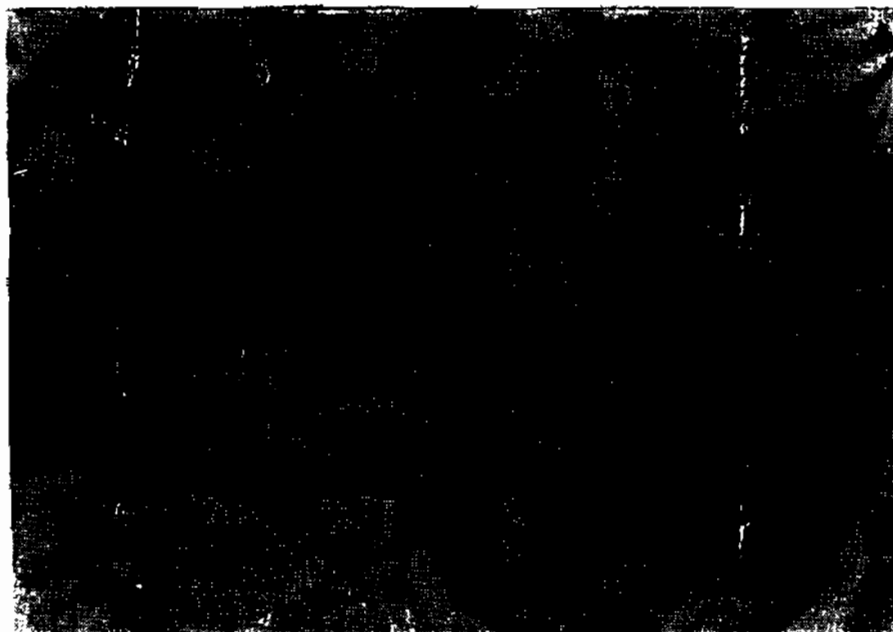


Fig. 4 : High magnification of Fig. 3 showing: PAS-positive reaction in the cytoplasm of podocytes (o) and smooth muscle layer of afferent arteriole (f). Note also squamous cells (s) of the outer parietal layer. PAS-technique X 1000

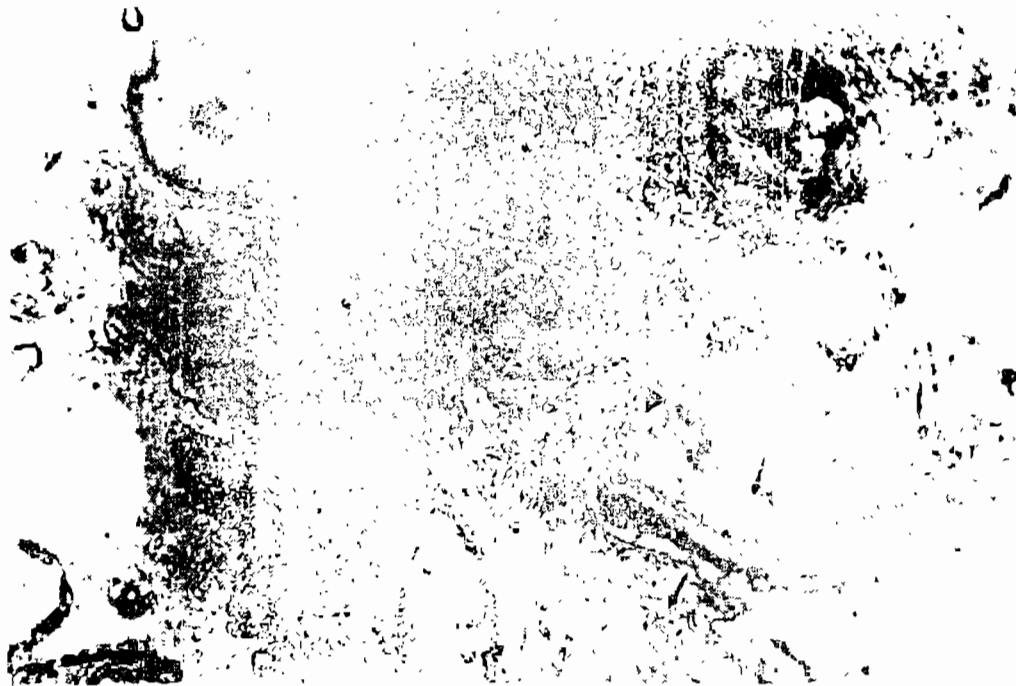


Fig. 5 : Electron micrograph of podocytes from the *Oreochromis niloticus* posterior kidney showing podocytes (arrow) and its 2ry processes (arrow.) X 9500



Fig. 6: Electron micrograph of glomerular tuft from the Kidney of *Oreochromis niloticus* showing: 2ry processes (arrow), podocyte, basement membrane (b), endothelium (c). Note also Golgi sacs (g), and mitochond (m) and (b)R X 32300



Fig. 7 : Electron micrograph of a part of afferent arteriole from the posterior kidney of **Oreochromis niloticus** showing electron dense granules (g) in modified smooth muscle cell (J.G. cell), lumen of arteriole containing erythrocyte (l) and endothelial cell (E). X 6120



Fig. 8: A photomicrograph of a section of posterior kidney of **Oreochromis niloticus** showing intense PAS-positive reaction at the brush border of the cells lining neck region (n) and first (P1) & second (P2) proximal segments and the basement membrane of the renal tubules (arrow). PAS- reaction, x 1000

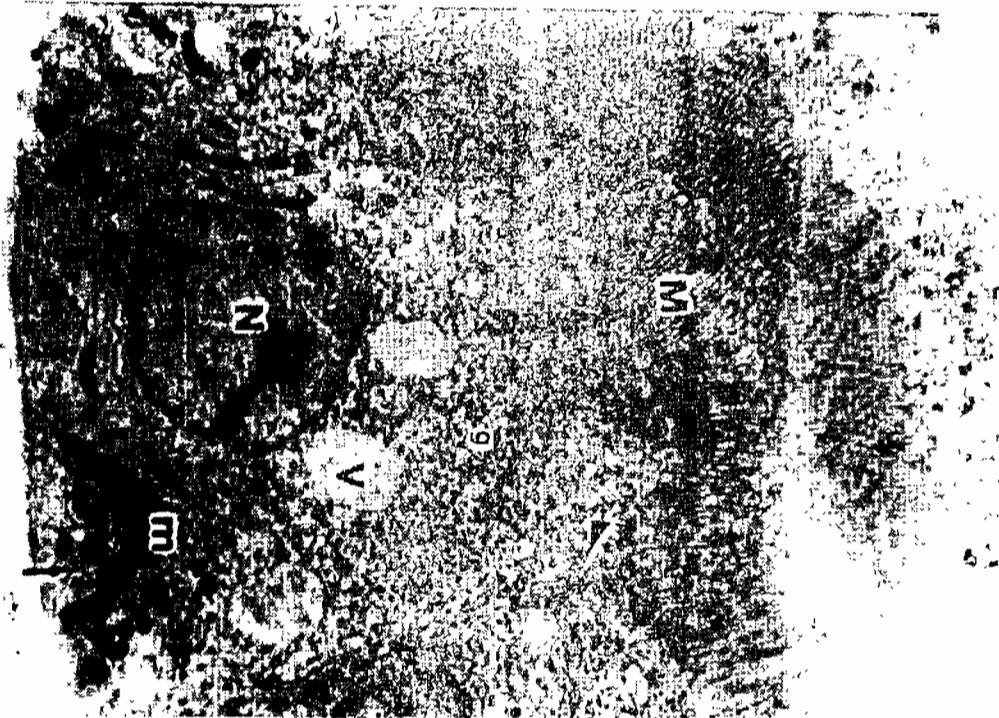


Fig. 9 : Electron micrograph of a part of neck segment from the posterior kidney of **Oreochromis niloticus** showing numerous long and densely backed microvilli (M) on the apical region of the cells, nucleus (N), mitochondria (m), vesicles (V) and electron dense granules (g). X 8600

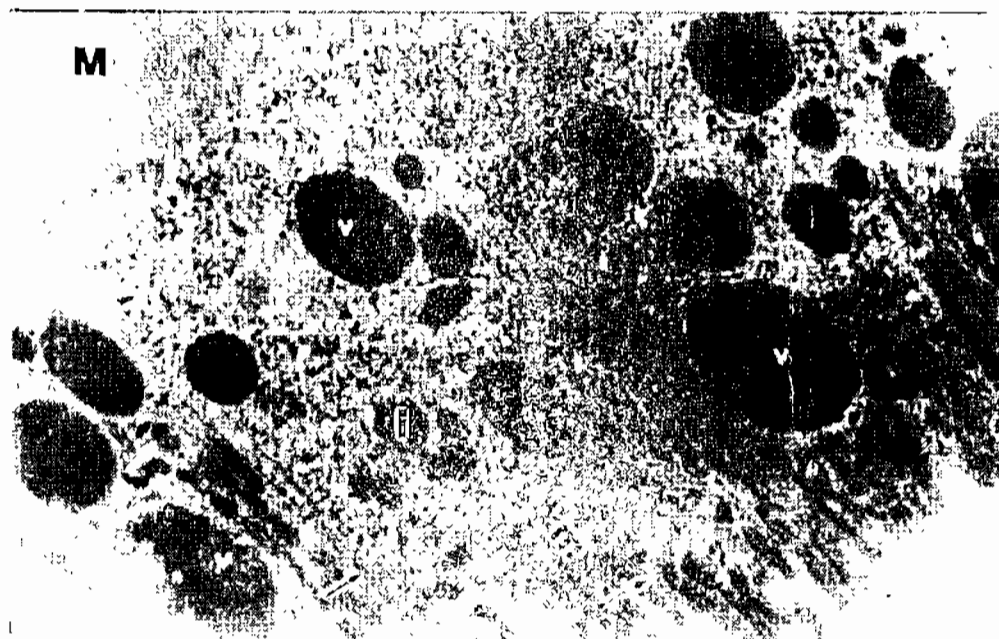


Fig. 10 : Electron micrograph of a part of first proximal segment from the posterior kidney of **Oreochromis niloticus** showing basal mitochondria (m), densely packed apical microvilli (M), electron dense large vacuoles (v), and other organelles. X 9860



Fig. 11 : Electron micrograph of a basal part of first proximal segment from the posterior kidney of **Oreochromis niloticus** showing filamentous mitochondria (m), basal infoldings (arrow), nucleus (N) and basal lamina (c). X 17000

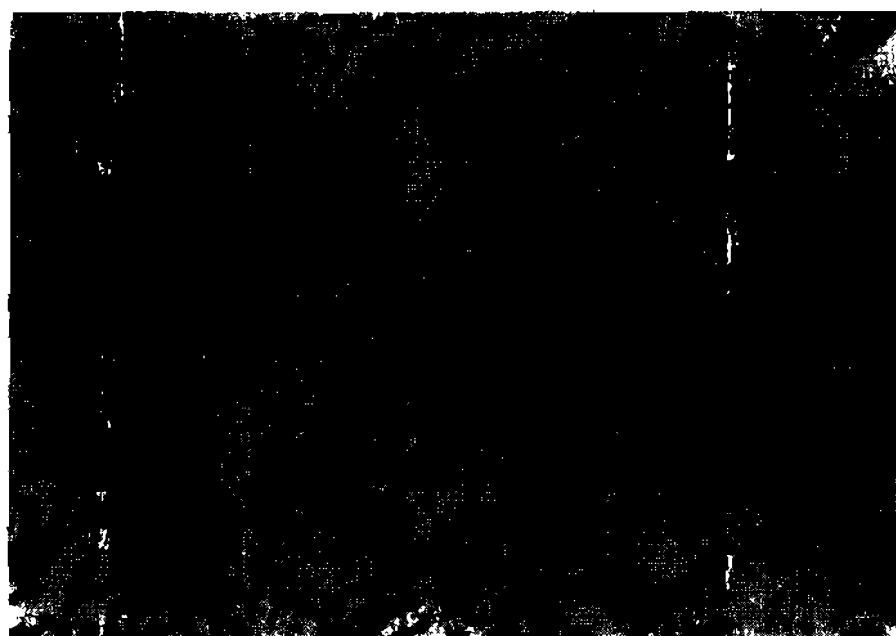


Fig. 12 : A photomicrograph of a section in posterior kidney of **Oreochromis niloticus** showing first (p1) and second (p2) segments of proximal tubule, distal (d) and collecting tubule (c). H&E stain. X 400.

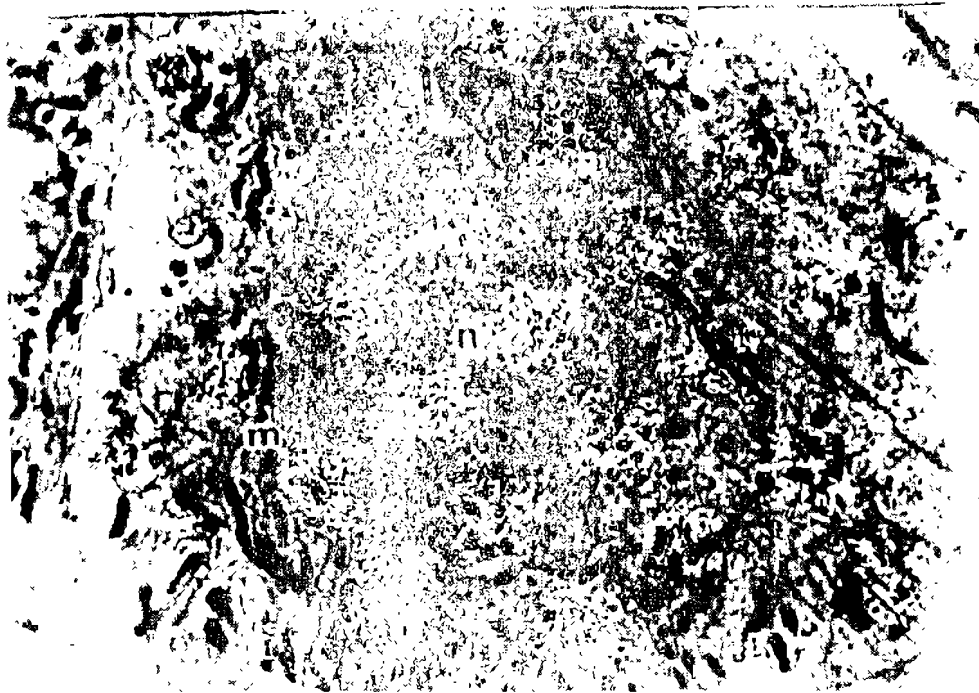


Fig. 13 : Electron micrograph of a part of second proximal segment from the posterior kidney of **Oreochromis niloticus** showing filamentous mitochondria (m), basal infoldings (arrow), nucleus (n) and electron dense granules (g). X 9860

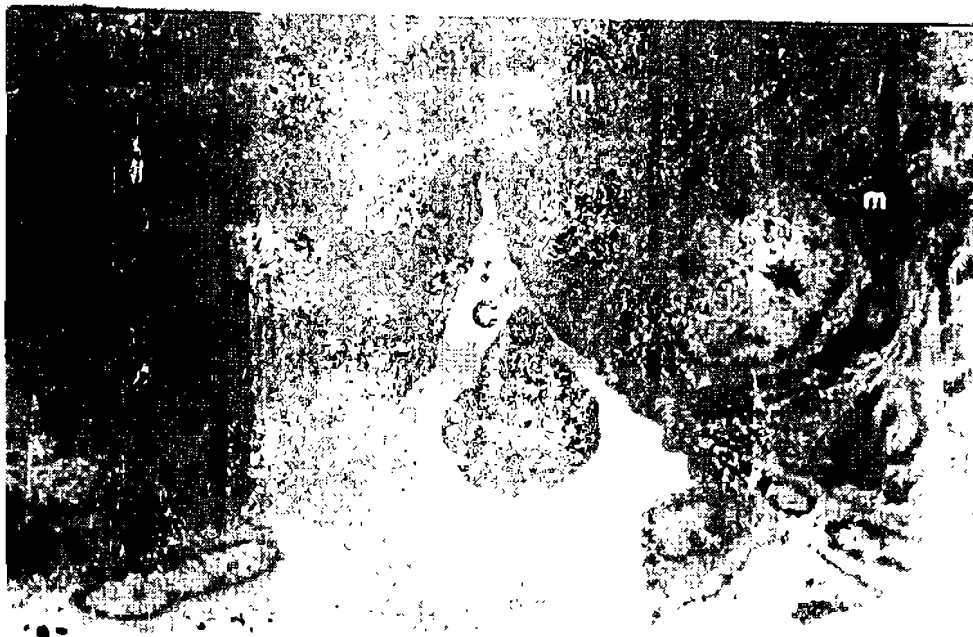


Fig. 14 : Electron micrograph of a part of distal tubule (right), intermediate segment (left) and blood capillary (c) from the posterior kidney of **Oreochromis niloticus**. Note mitochondria (m) X 6120



Fig. 15 : A photomicrograph of a section in posterior kidney of **Oreochromis niloticus** showing first (P1) and second (P2) proximal segments, distal tubule (d), collecting duct (c) lined by high columnar cells with microvilli (arrows) and smooth muscle layer (m).

II&E stain X 400

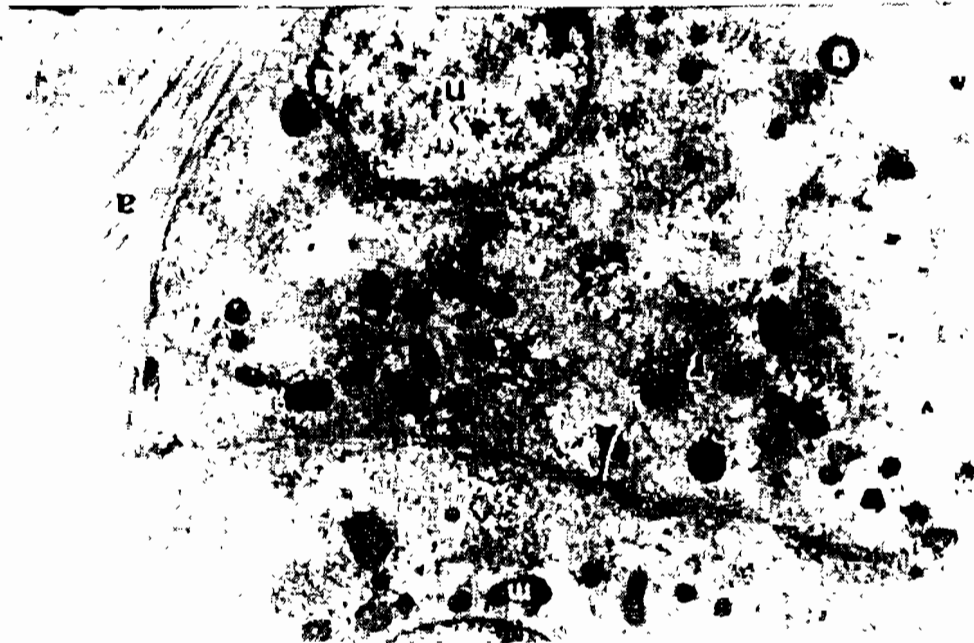


Fig. 16 : Electron micrograph of a part of collecting tubule from the posterior kidney of **Oreochromis niloticus** showing mitochondria (m), electron-dense granules (g), vesicles (v), rER (r), tight junction (arrowhead), smooth muscle cell (a) and nucleus (n). X 9860



Fig. 17 : A photomicrograph of a section in papillae kidney of **Oreochromis niloticus** showing collecting tubule (c) joined to a nephron (n) and a duct (M) which lined by pseudostratified columnar epithelium and surrounded by basement and smooth muscle layer (m).

Crossmor's stain X 200.

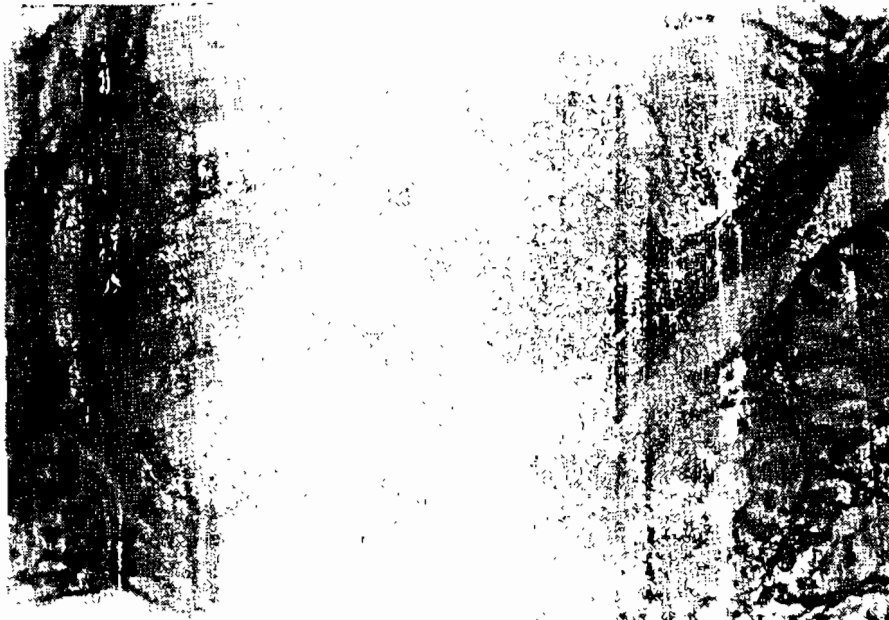


Fig. 18 : A photomicrograph of a section in papillae kidney of **Oreochromis niloticus** showing PAS-positive reaction in the smooth muscle layer (m) surrounding the mesonephric duct and the basement membrane of renal tubules (arrows). PAS-technique X 400.

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الملخص العربي

دراسات بواسطة الميكروسكوب الضوئي والميكروسكوب الإلكتروني النافذ
على الكلية الخلفية لأسماك البلطي النيلي

تم دراسة تركيب الكلية الخلفية لأسماك البلطي النيلي وذلك باستخدام كل من الميكروسكوب الضوئي والميكروسكوب الإلكتروني النافذ. أوضحت تلك الدراسة عدم تميز الكلية الخلفية في أسماك البلطي النيلي إلى قشرة ولب كما هو الحال في كلى الحيوانات الثديية، حيث تميزت تلك الكلى باحتوائها على العديد من الوحدات التركيبية المعروفة بالنيفرون بالإضافة إلى الأوعية الناقلة، تكون كل نيفرون من منطقتين أساسيتين وهما الكرة والأنبيبات الكلوية. إحتوت الكريات الكلوية على شبكة شعيرية محاطة بمحفظة بومن. أما الأنبيبات الكلوية فلقد تم تمييزها إلى عدة مناطق أساسية وهي جزء الرقبة القصير والجزء الأول والثاني من الأنبيبات الدنيا distal tubules ، الأنبيبات القصى Proximal tubules ، الجزء الوسيط intermediale tubules ، حيث إلتحمت نهايات وحدات الأوعية الناقلة المجمعة collecting ducts مع أنواع الناقل للكلية الوسيطة mesonephric duct .