Bull. Inst. Zool., Academia Sinica 26(4): 271-277 (1987)

RENAL MORPHOLOGY OF THE EURYHALINE TELEOST, *OREOCHROMIS MOSSAMBICUS*¹

PUNG-PUNG HWANG² and SU-MEI WU

Institute of Zoology, Academia Sinica, Taipei, Taiwan 11529, Republic of China

(Received May 8, 1987) (Revision received July 24, 1987) (Accepted July 29, 1987)

Pung-Pung Hwang and Su-Mei Wu (1987) Renal morphology of the euryhaline teleost, *Oreochromis mossambicus. Bull. Inst. Zool., Academia Sinica* 26(4): 271-277. Morphology of nephron was studied in tilapia (*Oreochromis mossambicus*) with light microscopy. Tilapia nephron is composed of a renal corpuscle, a short neck segment, bisegmental proximal tubule segments with PAS-positive brush border, a distal tubule segment and a tall columnar epithelial collecting tubule segment which connects with the system of collecting duct surrounded with thick connective tissue. A presumable undifferentiated tubule was found in some nephrons. These results were compared with the previous observations on other freshwater teleosts.

Kidney and gill are the most important organs responsible for osmoregulation in teleosts (Evans, 1979; Eddy, 1982). In seawater, teleosts passively lose water and gain salt. For compensation they drink seawater and actively excrete the most of monovalent ions the gills, and small amounts of divalent ions through the kidneys. Freshwater teleosts, in exactly reverse situation, drink little or no water since large amounts of water diffuse inward. To get rid of the excess water their kidneys produce large amounts of hypotonic urine and their gills actively absorb salts from the environment.

Tilapia (Oreochromis mossambicus), a euryhaline teleost, has provided a valuable model system for studies of the mechanism of osmoregulation because of the excellent adaptability of this species to various salinities (Forskett *et al.*, 1981; Assem and Hanke, 1984; Hwang, 1987). Morphology and physiology of the chloride cells in tilapia branchial regions have been extensively studied (Forsett *et al.*, 1981; Hwang, 1987). However, study concerned with the kidneys of tilapia is not yet available.

In the present work, structures of segments in tilapia nephrons were studied histologically.

MATERIALS AND METHODS

Tilapia (*Oreochromis mossambicus*) used in this study were 20-25 g in body weight 10-12 cm in total length. The fish were offered from Tainan Fishery Station, and reared in freshwater for over one month before sampling.

The fish were killed by destruction of the brain. The kidneys were prefixed *in situ* in Bouin's fixative for 5-10 min. After excision, they were cut and trimmed in proper size and postfixed in the same fixative for 24 h. Paraffin sectons of 5-7 μ m thickness were prepared and stained in (1) Periodic-Acid-Schiff counterstained with Mayer's hematoxylin and orange G, (2) Mayer's hema-

1. Paper No. 295 of the Journal Series of the Institute of Zoology, Academia Sinica.

^{2.} To whom reprint request should be sent.

toxylin and eosin. Observations were made with Nikon Optiphot optic microscop.

RESULTS

According to Ogawa (1962), the kidney of tilapia belongs to type I. It lays in a retroperitoneal poistion on the dorsal and caudal surface of the body cavity, and forms a single midline structure except in the head kidney where it splits in two small projections.

The tilapia kidneys do not show any zonal structures as cortex and medulla in mammals. Rather, it is composed of numerous nephrons arranged in unspecific pattern (Figs. 1, 5 and 8). The nephron consists of a renal corpuscle, a neck, two (first and second) proximal tubule segments with brush borders, a distal tubule segment and a collecting tubule segment which connects with the system of collecting duct (Figs. 1 and 5).

Renal Corpuscle

The main part of the renal corpuscle is a vascular capillary glomerulus that is enclosed by Bowman's capsule (Fig. 1). The glomeruli normally occupy most of the lumen enclosed by Bowman's capsule (Figs. 1 and 5.) In some cases, the glomeruli are much smaller than the capsules, leaving a marked space between the glomerulus and the capsular epithelium.

Neck

The neck segment is very short and contains tightly packed basophilic cuboidal epithelium and has no distinct brush border (Fig. 1). The squamous cells of the Bowman's capsule blend mmediately with the cuboidal cells lining the neck segment (Fig. 1).

First Proximal Tubule

Distally, the neck segment shows a sharp transition to the next segment, the first proximal tubule segment (Fig. 1). The latter begins with cuboidal, and ends in low columnar epithelium that has basally located nuclei. The supranuclear cytoplasm of the tubular cell stains less acidophilic than the basal cytoplasm, and shows numerous endocytic vacuoles (Figs. 3, 4, 6 and 7). The apical surface of the tubule cell has well-developed brush border with strong PAS positive reaction (Figs. 3, 4, 6 and 7). A diffuse PASpositivity also occurs in the supranuclear zone (Figs. 6 and 7).

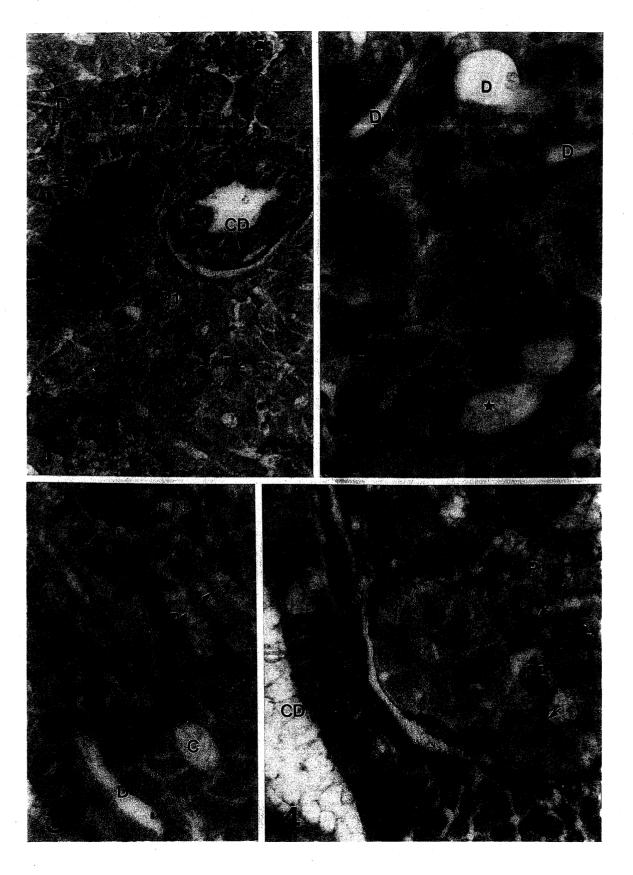
Second Proximal Tubule

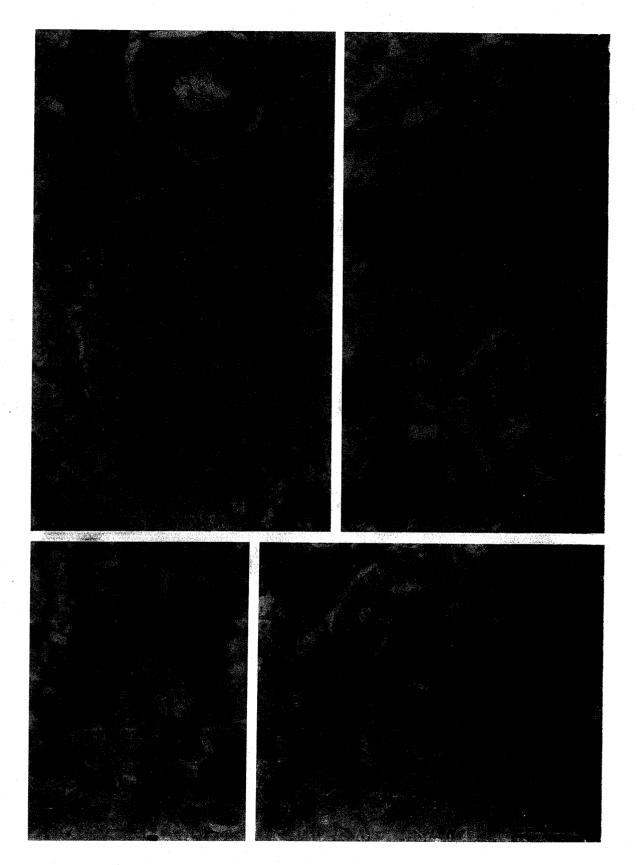
The columnar epithelium of the second proximal tubule segment reveals a homogenous, intensively acidophilic-staining cytoplasm (Figs. 1 and 2). These cells with midpositioned nuclei, also have prominent brush border which is less developed and less PASpositive than that in the first proximal tubule cells (Figs. 2, 4, 6 and 7). The second proximal tubule segment occupys the most part of the nephron (Figs. 1, 5 and 8). In the end part of the second proximal tubule, cell hight, brush border and acidophilia of the tubule cell are more or less reduced, showing a transition to the next segment, the distal tubule segment (Fig. 2).

Distal Tubule

The distal tubule is a short segment lined by low columnar or cuboidal epithelium. The cells have homogenerous, slightly acidophilic cytoplasm and base-located nuclei, but lack

Figs. 1-4. H and E stained kidney sections of Oreochromis mossambicus. B, Bowman's capsule; C, collecting tubule; CD, collecting duct; CN, connective tissue; D, distal tubule; G, glomerulus; N, neck segment; Pl, first proximal tubule; P2, second proximal tubule; U, undifferentiated tubule. The full features of the nephron can be viewed in Fig. 1. Brush border (arrow) was evident in the apical surface of P1 and P2 but not found in the other segments (Figs. 2, 3 and 4). The end part of P2 (star) showed transitional features to the next segment, D (Fig. 2). Magnification: 4000× in Fig. 1, 800× in Figs. 2-4.





a brush border nor distinct PAS-positivity in the apical surface (Figs. 2, 3 and 6).

Collecting Tubule

The collecting tubule segment is distinguished from the distal tubule segment by an increase cell of height (tall columnar), tubular diameter and cellular basophilia (Figs. 1, 3, 5 and 8). As in the distal tubule, the cells in the collecting tubule have base-located nuclei and do not show brush border nor PAS-positivity in the apical surface (Figs. 3 and 7).

Collecting Duct

The collecting tubules empty into two collecting ducts which finally fuse to form the urinary bladder. Pseudostratified columnar epithelium lines the collecting duct wall. These epithelial cells resemble those of the collecting tubule, however the former show distinct cilia and taller in cell height, and are surrounded with a thicker fibrous connective tissue (Figs. 1, 4 and 5). Moreover, lumen and tubular diameter of the collecting duct are much larger than the other segments of the nephron (Figs. 1 and 5).

Undifferentiated Tubule

A presumable undifferentiated tubule was observed in some nephrons (Figs. 1 and 8). The undifferented tubule has the smallest tubular and lumen diameter in the nephron, and consists of cuboidal epithelium whose cells showing extensive basophila in the cytoplasm, has base-located nuclei occupying the most part of the cell (Figs. 1 and 8).

DISCUSSION

Numerous observations on teleost nephrons have revealed that the extensive diver-

sity in morphology of the nephrons reflects the different needs of salt and water balance environments teleosts widely varied in (Edwards and Schnitter, 1933: inhabit Grafflin, 1937; Forster, 1953; Ogawa, 1962; Newstead and Ford, 1960; Hickman and Trump, 1969; Kendall and Hinton, 1974; Groman, 1982; Endo and Kimura, 1984). These observations stimulated comparative studies on physiology and morphology which enhanced the understanding of the renal In the freshwater, teleosts are function. hyperosmotic regulators, the regulation of the ionic composition of the blood is carried out mainly by the kidney which primarily conserves filtered electrolytes. The urine is dilute, often nearly free of sodium and chloride, and its volume must balance the quantity of water entering the body from the dilute environment. To perform this function, Hickman and Trump (1969) emphasized two tubular characteristics are essential: (1) a powerful monovalent ion reabsorptive mechanism which operates in conjunction with (2) a low tubular permeability to filtered plasma water. It has been asserted that, the renal corpuscle involves as a device to get rid the fish of excess water, the neck segment improves the efficiency of filtrate flow, the first proximal tubule segment involves in pinocytic uptake of filtered proteins and other macromolecules, the second proximal tubule segment participates in isosomtic sodium reabsorption and hydrogen ion secretion, the distal tubule segment participates in active sodium resorption and is important in improving the efficiency of monovalent ion retention and urinary dilution, the collecting tubule and duct are essential for the formation of a dilute urinary reasorbing monovalent ions from the filtrate (Smith, 1932;

Figs. 5-8. PAS counterstained with hematoxylin and orange G kidney sections of Oreochromis mossambicus. Abbreviations are the same as in Fig. 1. Figs. 5 and 8 showed the full features of the nephron. Strong PAS-positivity occured in the apical surface of P1 and P2 but not in the other segments (Figs. 6 and 7). Magnification: 400× in Figs. 5 and 8, 800× in Figs. 6 and 7.

Marshall, 1534; Hickman and Trump, 1969; Evans, 1979; Endo and Kimura, 1984).

The present study described new morphological features of teleostean nephron, although most of the findings agreed with the previous observations on other teleosts (Ogawa, 1962; Newstead and Ford, 1960; Fukusho, 1969; Hickman and Trump, 1969; Groman, 1982; Endo and Kimura, 1984). It is quite easy to distinct the various segments of tilapia nephron from size, shape and staining of epithelial cells, location of the nuclei, and presence of brush border and PAS-positivity in the apical surface of the epithelial cell.

In tilapia nephron, under H and E stains the end part of the second proximal tubule shows some transitional features to the next segment, the distal tubule. At the same location of the channel catfish (Ictalurus punctatus) nephron, a narrow ciliated intermediate segment was found by Kendall and Hinton (1974) with a special histological staining techniques (Pollak's polychrome and phosphotungstic acid hematoxylin) Kendall and Hinton (1974) emphasized the failure of H and E stains to reveal the presence of the intermediate segment. Moreover, Ogawa (1962) has stated that in the goldfish the presence of the intermediate segment is variable in different nephrons, and that in fact the presence of this segment is rare. With regard to the distal tubule segment, the cell is low columnar and slightly acidophilic in tilapia nephron, while in that of carp (Cyprinus carpio) large and relative clear cell was observed (Hickman and Trump, 1969). The undifferentiated tubule is a general appearance in tilapia nephron. It was also found in the rainbow trout nephron (Oguri, 1982) and in the developing nephron of tilapia larvae (our unpublished observations) but seldom reported in the literature. Reviewing numerous previous reports, Hickman and Trump (1969) has suggested the chief points of difference that were reported in the literature involve the neck, the intermediate segment and the distal segment. To ascertain the precise morphology of these segments, it is necessary to study the ultrastructure of tilapia nephron with electron microscopy in the further work.

Acknowledgements: This study was supported by the National Science Council of the Republic of China (NSC75-0201-B001-41). We would like to thank Dir. Y. Y. Ting and Mr. M. N. Lin, Tainan Fish Culture Station of Taiwan Fisheries Research Institute for their generous help in suppling tilapias. Thanks are also due to Mr. C. M. Sun for preparation of the micrographs.

REFERENCES

- ASSEM, H. and W. HANKE (1984) A comparison between the effects of cortisol and prolactin on the euryhaline tilapia (Sarotherodon mossanbicus). Zool. J. Physiol. 88: 423-431.
- EDDy, F. B. (1982) Osmotic and ionic regulation in captive fish with particular reference to salmonids. *Comp. Biochem. Physiol.* 73B: 125-142.
- EDWARDS, J.G. and C. SCHNITTER (1933) The renal unit the kidney of vertebrates. Am. J. Anat. 53: 55-87.
- ENDO, M. and M. KIMURA (1984) Structures and functions of segments in some teleostean nephrons. Japan. J. Ichthyol. 31: 71-78.
- Evans, D. H. (1979) Fish. In Comparative physiology of osmoregulation in animals (G. M. P. Maloiy, ed.). Academic Press, New York, 305-390.
- FORSKETT, J. K., C. D. LOGSDON, T. TURNER, T. E. MACHEN and H. A. BERN (1981) Differentiation of the chloride extrusion mechanism during seawater adaptation of a teleost fish, the cichlid Sarotherodon mossambicus. J. Exp. Zool. 93: 209-224.
- FORSTER, R. P. (1953) A comparative study of renal function in marine teleosts. J. Cellular Comp. Physiol. 42: 487-510.
- FUKUSHO, K. (1969) The specific difference of salinity tolerance among cichlid fishs genus *Tilapia* and histological comparison of their kidneys. Bull. Japan. Soc. Sci. Fish. 35 148-155.

- GRAFFLIN, A. L. (1937) Observations upon the structure of the nephron in the common eel. Am. J. Anat. 61: 21-62.
- GROMAN, D. B. (1982) Renal excretory system. In Histology of the striped bass. Am. Fish. Soc., Maryland, 48-52.
- HICKMAN, C. P. and B. F. TRUMP (1969) The kidney. In Fish physiology (W. S. Hoar and D. J. Randall, eds.). Academic Press, New York, 1: 91-240.
- HWANG, P. P. (1987) Tolerance and ultrastructural response of branchial chloride cells to salinity changes in the euryhaline teleost Oreochromis mossambicus. Marine Biol. 94: 643-649.
- KENDALL, M. W. and D. E. Hinton (1974) Renal tubular morphology in the chanal catfish (Ictakurus punctatus) kidney. J. Fish. Res. Board Can. 31: 346-347.

- MARSHALL, E. K. (1934) The comparative physiology of the kidney in relation to theories of renal secretion. *Physiol. Rev.* 14: 133-159.
- NEWSTEAD, J. D. and P. FORD (1960) Studies on the development of the kidney of the Pacific pink salmon Oncorhynchus gorbuscha (Walbaum).
 III. The development of the mesonephros with particular reference to the mesonephric tubule. Can. J. Zool. 38: 1-7.
- OGAWA, M. (1962) Comparative study on the internal structure of the teleostean kidney. Sci. Rept. Saitama Univ. **B4**: 107-129.
- OGURI, M. (1982) Kidney. In Atlas of fish histology (T. Hibiya, ed.). Kou Dan Shia, Tokyo, 94-103.
- SMITH, H.W. (1932) Water regulation and its evolution in the fishs. Quart. Rev. Biol. 7: 1-26.

鹽廣性眞骨魚類吳郭魚 (Oreochromis mossambicus) 腎臟形態學之研究

黄鵰鵰 异淑美

本報告以光學顯微鏡術研究吳郭魚(Oreochromis mosambicus) 腎元之形態。吳郭魚腎元是由:腎 小體,一段極短之頸節,二段具 PAS 陽性刷子緣之近位細尿管,一段遠位細尿管,一段高柱狀上皮之 集合細尿管及與其相通之集合管系統等所組成。另外,在一些腎元中發現一種可能是未分化的細尿管。 這些觀察結果與過去有關其他魚類之報告進行比較。