Microanatomical Investigations of Renal Cortex in African Sideneck Turtle (*Pelusios castaneus*)

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***Abstract*—The tropics is endowed with a diverse species of freshwater turtles among which is the African sideneck turtle (Pelusios castaneus) commonly captured as food. The anatomy of the reptilian kidney exhibits some similarities with higher vertebrates, though slight variations exist. The study therefore sought to describe the basic histological features as well as the ultrastructural components of the renal cortex of the African sideneck turtle. To achieve this, a total of 10 adult male turtles with an average body weight of 620 g picked up from river drainages in Ibadan, Nigeria, were used for the study. Subsequently, the turtles were anaesthetized, kidney samples were harvested and processed for routine staining, periodic acid Schiff (PAS), Masson's Trichrome (MT), Verhoeff's techniques. Ultra-thin sections were also prepared for electron microscopy. The results showed the typical histomorphological pattern of the renal cortex with renal corpuscles and tubules. The Bowman's capsule was observed to enclose the glomerulus, described as a tuft of capillaries. The renal capsules, corpuscles, blood vessels, and connective tissue of the African sideneck turtle were strongly Verhoeff's stain-positive indicative of the high presence of elastic fibers while corpuscles, blood vessels, and connective tissue were strongly MT stain-positive showing their wealth in collagen. With the PAS stain, the Bowman's capsule with its content as well as the connective tissue and boundary membranes of the corpuscles, tubules, and ducts enclosed within the parenchyma of the kidney were all strongly positive implying the high presence of carbohydrates in these structures. The histological and histochemical features of the kidney of the African sideneck turtle are similar to those of mammals with the exception of the loop of Henle. The findings of this work could be applied in the comparative histology and cytology of the reptilian kidney and serve as a baseline for microanatomical investigations of the kidney of turtles.**

Keywords—African sideneck turtle; Bowman's capsule; Renal corpuscle; Renal cortex

I. INTRODUCTION

The reptilian kidney is involved in the elimination of waste products as well as the osmoregulation of the body fluids [1, 2]. In all vertebrates, including reptiles, the basic structural and functional unit of the kidney is the nephron [3]. However, it has been documented that the reptilian nephron lacks the loop of Henle, the portion of the mammalian kidneys that produces concentrated urine, and therefore cannot produce urine hyperosmotic to plasma [4, 5]. The nephron of the reptiles is composed of the glomerulus with the Bowman's capsule connected by a short ciliated neck segment to the proximal tubule, a short ciliated thin intermediate segment and a distal tubule which opens into the collecting duct [6]. Some studies on the reptilian histology of the kidney have described the terminal part of the distal segment as the connecting segment because of its distinct structure and function compared to those of mammals [7, 8].

The African sideneck turtle (*Pelusios castaneus*), a small to medium in size freshwater turtle widely distributed in West African [9]. The animal has received considerable research attention in the last decade with previous reports on its reproductive and nervous systems [10, 11, 12]. Apart from its recent application as a research animal especially in anatomical investigations, among local hunters and farmers, the animal has gained wide popularity due to its frequent use in traditional medicine, and not often captured for meat [13].

Detailed anatomical investigations abound on the mammalian renal structure and function with only a handful of reports in reptiles [3]. Among the available reptilian studies of kidney function and structure, those of turtles are very rare in literature. The histology and ultrastructure of the kidney have been described in freshwater turtle (*Pseudemys scripta*) and desert tortoise, *Gopherus agassizii* [14], the green turtle, *Chelonia mydas* [1] and the soft-shelled turtle, *Pelodiscus sinensis* [3]. There is no available published study on the microanatomy of the kidney of turtles in the tropical environment. Therefore, this study was designed to describe the histology, histochemistry, and transmission electron microscopic features of the renal cortex in the African sideneck turtle.

II. MATERIALS AND METHODS

A. Ethical Approval

Procedures adopted in the current study were approved by the University of Ibadan (Ibadan, Nigeria) Animal Care and Use Research Ethics Committee (UI ACUREC: 12/13/05).

B. Study Design

Authors identified this turtle using shell morphology and body features earlier described by [9]. Following a series of anatomical dissections and comparative studies of the shells of the males and female turtle, features of sex determination and adulthood were established and reported [11]. Ten adult male African sideneck turtles (*Pelusios castaneus*) with an average body weight of 620 g, picked up from river drainages in Ibadan, Nigeria, were used for the study. Shell characteristics of the turtle were used in the determination of sex and adulthood, and were thereafter anesthetized using an intramuscular injection of ketamine-HCl (Sigma, St. Louis, MO, USA) at a dose of 25 mg/kg body-weight followed by sacrifice by cervical decapitation [11]. Kidney samples were retrieved at necropsy.

C. Light Microscopy

Renal samples from five turtles were fixed in buffered neutral formalin and embedded in paraffin blocks. Sections 3-5 µm thick were stained with Haematoxylin and Eosin, Periodic Acid Schiff (PAS), Masson's Trichrome (MT) and Verhoff's stain as previously described by [13]. Five slides were obtained for each staining technique used. Verhoeff's stain was chosen because of its ability to demonstrate elastic tissue in organs; PAS for the demonstration of carbohydrates such as mucin and glycogen in tissues and MT was chosen due to its ability to demonstrate collagen fibres in tissues. The slides were then examined under a light microscope at x40 and x400 magnifications (Olympus BX63, Germany, with a DP72 camera).

D. Transmission Electron Microscopy (TEM)

Renal tissues were fixed in glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 4 hours at 40C as previously described by [11]. Briefly, renal samples were then thoroughly washed in the same buffer, post-fixed in 1% osmium tetroxide. Samples were dehydrated in a graded series of ethanol solutions followed by clearance with propylene oxide, infiltrated with a 1:1 solution of propylene oxide:epoxy resin, 1:2 solution of propylene oxide:epoxy resin. This was followed by subsequent placement in 100% epoxy resin for 36 hours under vacuum. Embedding was done in fresh epoxy resin and cured at 600C for 48 hours. Toluidine blue was used to stain semi-thin sections of tissues and observed under the light microscope (Olympus BX63 with a DP72 camera). Ultra-thin sections (70-80 nm) were cut with a diamond knife on an ultramicrotome (Ultracut-Reichert, Austria). Sections were then double-stained with uranyl acetate and lead acetate. The copper grids were examined under a transmission electron microscope (Philips CM 10 TEM) operating at 80 kv. Representative micrographs of different sections of the kidney were taken using a Gatan 785 Erlangshen digital camera (Gatan Inc., Warrendale, PA, USA). Analysis and assembling of composite micrographs were carried out using Adobe Photoshop CS5 (Adobe Systems, San Jose, CA).

III. RESULTS

A. Histological findings

The cross section of the renal tissue of the African sideneck turtle revealed a surrounding capsule, an outer cortex and an inner medulla (Fig. 1). A prominent feature observed in the section of the cortex was the renal corpuscles. All through the regions of the cortex, there were Bowman's capsules which surrounded a meshwork of capillaries otherwise known as the glomerulus (Fig. 2). Each Bowman's capsule had a space located between it and the glomerulus. Further, the Bowman's capsule consisted of two spaces opposite each other; the vascular and urinary spaces. Other structures found were the proximal and distal convoluted tubules, blood vessels and connective tissues. However, no Loop of Henle was found within the renal cortex of all the turtles investigated.

B. Histochemistry of the renal tissue

The renal capsules, corpuscles, blood vessels, and connective tissue of the African sideneck turtle were strongly Verhoff's stain-positive (Fig. 3) while corpuscles, blood vessels, and connective tissue were strongly Masson's Trichrome stain-positive (Fig. 4). With the PAS, the Bowman's capsule with its content as well as the connective tissue and boundary membranes of the corpuscles, tubules, and ducts enclosed within the parenchyma of the kidney were all strongly positive (Fig. 5).

C. Transmission electron microscopic features of the renal cortex

Transmission Electron Microscope section of the Bowman's capsule revealed that it was bounded by a well outlined membrane and enclosed a parietal epithelium which was squamous with Bowman's space between the parietal epithelium and podocytes (Fig. 6). In addition to podocytes, the Bowman's capsule comprised a capillary of glomerulus and simple columnar epithelial cells known as the mesangial cells. In the endothelium of capillaries of the glomerulus, mesangial cells were found as well as the juxtaglomerular apparatus outside the glomerulus. The distal convoluted tubule (DCT) of the turtle was bounded by a distinct membrane enclosing cuboidal epithelial cells having nuclei. Some of the nuclei presented with centrally located prominent nucleoli (Fig. 7). The apical cytoplasm of the epithelial cells of the DCT presented with numerous mitochondria. The lumen of the DCT was clear and devoid of apical brush borders. The proximal convoluted tubule (PCT) was bounded by a distinct membrane, comprised simple cuboidal to columnar epithelial cells (Fig. 8). The nuclei of the epithelial cells had lesser nucleoli compared to those of the DCT. However, numerous mitochondria were seen in the subnuclear and supranuclear portions of the cytoplasm of the PCT. The lumen of the PCT, unlike the DCT had prominent apical brush borders (Fig. 9).

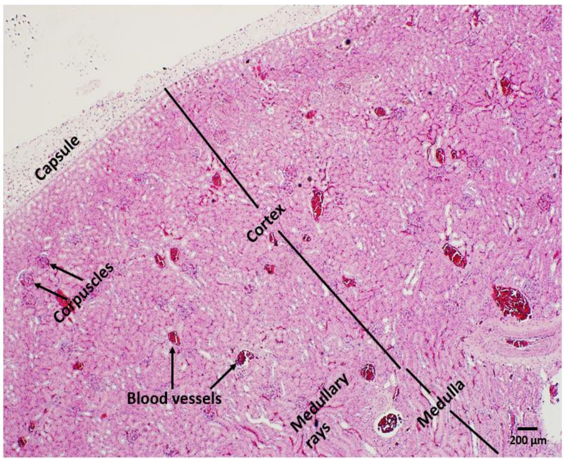

Fig. 1. *A section of the kidney of the African sideneck turtle showing the capsule, cortex and medulla (H&E; X40).*

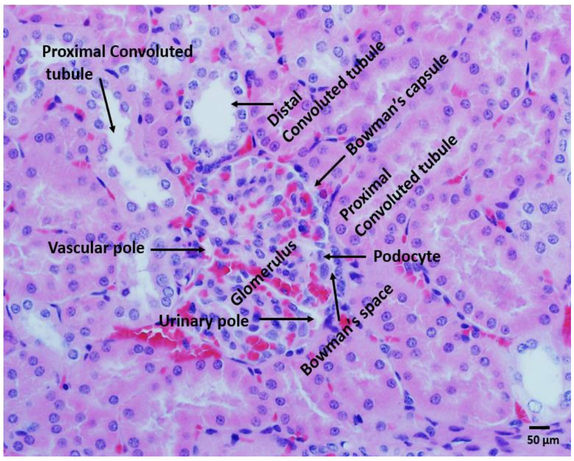

Fig. 2. *A cross section of the renal cortex of the African sideneck turtle showing contents of the Bowman's capsule and associated structures (H&E; X400).*

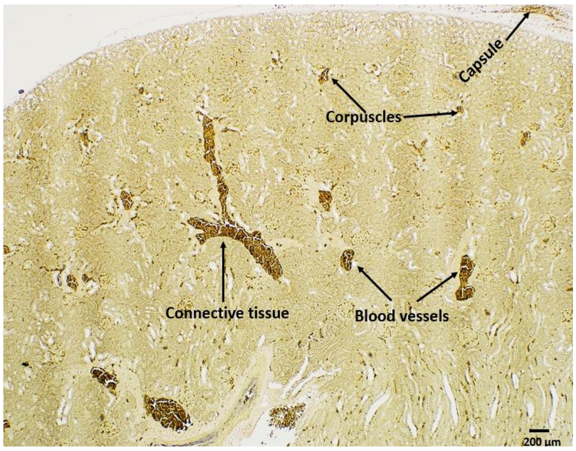

Fig. 3. *A section of the kidney of the African sideneck turtle showing the capsule, corpuscles, connective tissue and blood vessels as being Verhoff's stain-positive (Verhoff's stain; X40).*

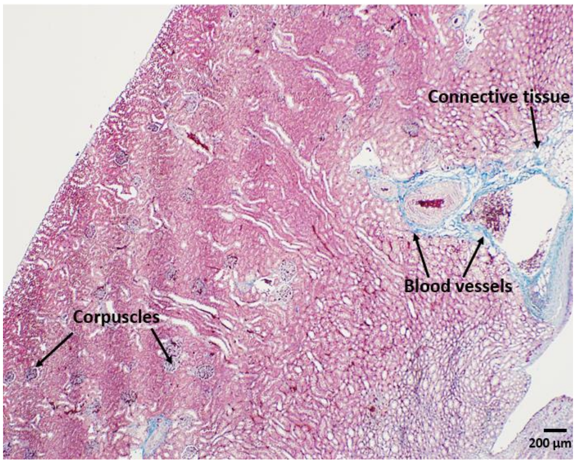

Fig. 4. *A section of the kidney of the African sideneck turtle showing the corpuscles, connective tissue and blood vessels as being Masson's Trichrome stain-positive (Masson's Trichrome stain; X40).*

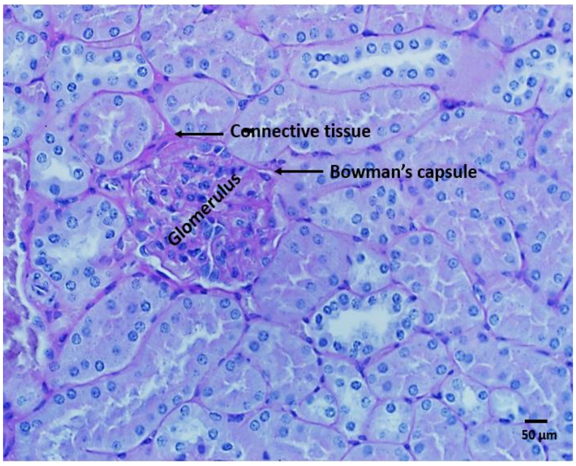

Fig. 5. *A section of the kidney of the African sideneck turtle showing the Bowman's capsule and its content and connective tissue as Periodic Acid Schiff's stain-positive (Periodic Acid Schiff stain; X400).*

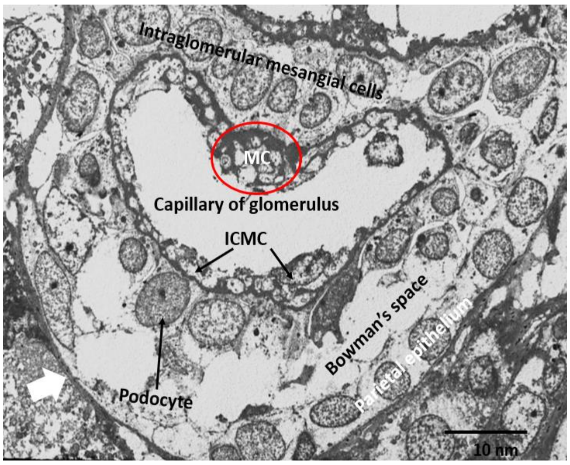

Fig. 6. *TEM section of the Bowman's capsule of the African sideneck turtle. Observe the basement membrane (White arrow head) and the contents of the capsule. Note the Juxtaglomerular apparatus (Red sphere area) enclosing some mesangial cells (MC) as well as the intracapillary mesangial cells (ICMC) and the intraglomerular mesangial cells.*

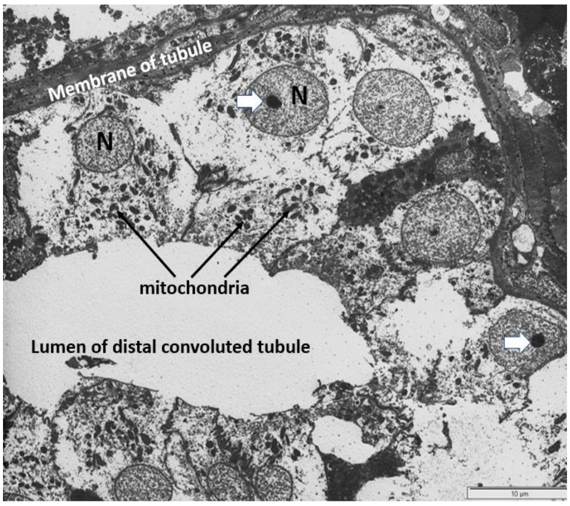

Fig. 7. *TEM section of the distal convoluted tubule of the kidney of the African sideneck turtle showing a lumen void of apical brush borders, nucleus (N) of tubular eithelial cells with its nucleolus (White arrows). The apical cytoplasm of the tubular epithelial cells bears numerous mitochondria (black arrows).*

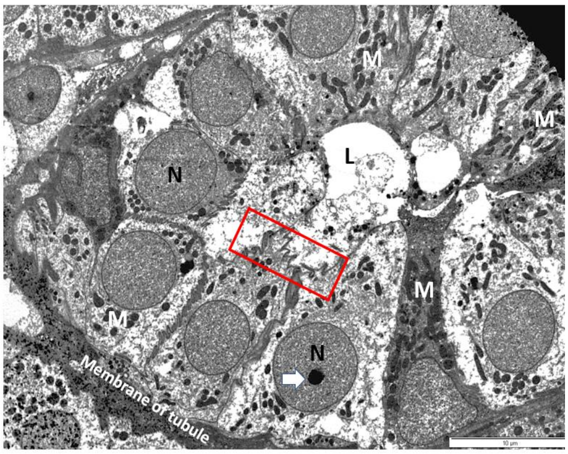

Fig. 8. *TEM section of the proximal convoluted tubule of the kidney of the African sideneck. There is a typical apical brush borders (area captured in rectangle), nucleus (N) of tubular epithelial cells with its nucleolus (White arrow). The cytoplasm of the tubular epithelial cells bears numerous mitochondria (M). L: Lumen of proximal tubule.*

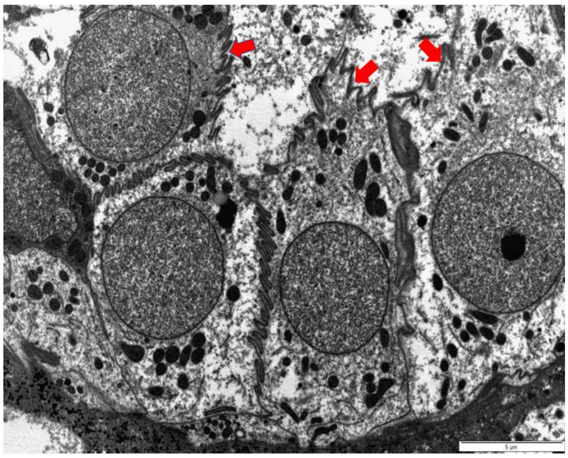

Fig. 9. *TEM section of the proximal convoluted tubule of the kidney of the African sideneck turtle showing a lumen having typical apical brush borders (Red arrows).*

IV. DISCUSSION

The histological features of the kidney of the African sideneck turtle observed in the study presented with close similarities with those already described in other turtles and reptiles generally [5,8]. Unlike the kidney of sea turtles that had been reported to lack a distinct cortex and medulla [4], an evaluation of the kidney of African sideneck turtle in the present study clearly showed that the cortex can be differentiated from the medulla. The renal corpuscles were the most prominent structures of the histology of the renal cortex of the animal. The contents of the Bowman's capsule of the turtle is similar to those earlier described in other turtles and reptiles [3,4,8]. These also bear close similarity with the structures of the mammalian kidney [15].

The renal capsule, connective tissue, blood vessels and membrane of renal corpuscles being strong Verhoeff's stain positive implies that these structures are rich in elastic tissue. The presence of elastin in the blood vessels is meant to facilitate pressure wave propagation of blood flow [16]. The fact that the renal corpuscles, blood vessels, and connective tissues show a strong affinity for MT stain implies that these structures are rich in collagen fibres. Collagen, an important component of the extracellular matrix has been demonstrated to be involved in several biological processes inclusive of the regulation of intracellular calcium. In response to the accumulation of collagen in the kidney, pathologies such as renal fibrosis and vascular calcification have been observed [17].

The entire renal connective tissue and blood vessels as well as the membrane of the BC of the African sideneck turtle were strongly PAS-positive indicative of the presence of carbohydrates, especially glycogen. The percentage of renal glycogen has been proposed to be a function of how renal cells generate the energy needed for its functions including ultrafiltration and water retention among others [18]. Hence, the strong PAS-positivity observed in the renal structures of the turtle is suggestive of a high level of energy generation for the execution of the functions of the kidney especially in the absence of the Loop of Henle in the turtles.

The intra- and extraglomerular location of mesangial cells observed in the current study is similar to their locations in mammals and reptiles [3,15]. Mesangial cells of the Bowman's capsule are contractile cells that made up the central stalk of the glomerulus. When found in the proximity of the capillary lumen, mesangial cells do make direct contact with the glomerular endothelium without an intervening basement membrane [3]. These cells have been demonstrated to function as specialized pericytes, being responsible for the stabilization of the glomerular endothelial structure and function [16].

From the capsule enclosing the cortex and then the innermost medulla, the histological structures of the African sideneck turtle are similar to those of mammals with the exception of the loop of Henle found in mammalian kidneys [15]. The lack of loop of Henle causes the distal convoluted tubules of the animal to drain dilute filtrate into the collecting duct via connecting segment and this may impart on the ability of the turtles to produce concentrated urine. Previous studies have shown that turtles and other reptiles cannot produce urine hyperosmotic to plasma [3,4].

The lack of loop of Henle in reptiles can be regarded as a case of structural adaptation to function because the intermediate tubule segment of the kidney has been described to be short in reptiles and amphibians. The absence of loop of Henle has been documented for the kidneys of reptiles investigated to date [3,4,5]. However, water conservation and the reabsorption of sodium chloride from the renal filtrate in mammals leading to the production of concentrated urine are the functions of the loop of Henle [19].

In conclusion, the kidney of the African sideneck turtle has similar histological pattern and histochemical features with those previously reported in other reptiles only differing in some segments from those of mammals. The findings of this study could be applied in the comparative histology and cytology of the reptilian kidney and serve as a baseline for microanatomical investigations of the kidney in turtles.

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