Histology of the Kidney and Urinary Bladder of *Siphonops annulatus* (Amphibia-Gymnophiona)

Elcinéa T. C. CARVALHO¹ and L. C. U. JUNQUEIRA²

Department of Biological Sciences¹, School of Pharmacy and Odontology of Alfenas, Minas Gerais ; and Cell Biology Laboratory², Department of Pathology, Medical School, University of São Paulo, Brazil

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Summary. The histology of the kidney and urinary bladder of *Siphonops annulatus* was studied by light microscopy in semithin sections of tissue embedded in hydrophilic resin. The kidney's nephron comprises the renal corpuscle, neck segment, proximal tubule, intermediate segment, distal tubule and collecting tubule. Nephrostomes are present. This structure, the neck segment, and intermediate tubules present long cilia, and probably play important roles in the propulsion of the peritoneal fluid and glomerular filtrate. The proximal tubule cells possess loosely packed microvilli and contain abundant polymorphic granules and vesicles that assume the aspect of lysosomes in different stages of intracellular digestion. The distal tubules are characterized by large, vertically disposed mitochondria assuming the aspect of ions transporting cells. The urinary bladder is lined with a transitional epithelium, whose aspect varies according to the quantity of urine.

Siphonops annulatus is a Gymnophiona adapted to terrestrial life. It is a burrowing animal like an earthworm and is the most widely distributed caecilian in South America, where it inhabits humid and semiarid areas. It has an annulated body, no tail and some features suited to this type of life such as a copulatory organ, eyes covered with skin, and the production of eggs with a shell that develop without contact with water.

The initial references to the Gymnophiona kidneys are from SPENGLER (1876) and WIEDERSHEIM (1879). A comprehensive review of this organ's morphology was published by WAKE (1970a). A detailed analysis of the structure of the kidney of *Typhlonectis compressicauda* was published by SAKAI et al. (1986, 1988a, b).

WELSCH and STORCH (1973) described the ultrastructure of the nephron of *Ichthyophis kohtaoensis*.

This paper presents the results obtained by studying the structure of the kidney and urinary bladder of *S. annulatus*.

MATERIAL AND METHODS

Ten adult specimens of both sexes of *Siphonops annulatus*, MIKAN (1820), classified according to TAYLOR (1968), were captured in the region of Itabuna and Ilhéus, State of Bahia, Brazil, at the Cacao Research Center (CEPEC-CEPLAC). (Some of the specimens used have been preserved in the collection of the Zoological Museum of the University of São Paulo under numbers 71.227, 71.278 and 71.279.) These animals weighed 23-48 g and measured 28-43 cm. They were adapted to life in a terrarium being fed periodically with new born mice and minced meat. After being submitted to a temperature of 4°C for 30 min and injected intraperitoneally with 4% paraformaldehyde dissolved in 0.1 M phosphate buffered saline, they were dissected after an interval of 10 min and further fixed in the same solution. Tissues were embedded in Technovit 7100 resin (Heraeus Kulzer, Philipp Reis Strasse 81, 6393-Werheim) and sectioned with a glass knife at 1 and 2 µm. Sections were stained with toluidin blue and fuchsin. To study the distribution of neutral or acid protein associated with polysaccharides, the periodic acid Schiff (PAS) and Alcian blue methods were used according to BANCROFT and STEVENS (1990).

As will be seen, thin sections of this material fixed in our conditions produced figures with higher resolution as compared with paraffin-embedded material. This permits a better understanding of the function of the cells and tissues studied (JUNQUEIRA, 1995).

RESULTS

The kidneys form a long, brown ribbon-like structure adherent to the dorsal wall of the animal by a meso. They extend from the heart to the urinary bladder. The central region contains a large artery and a vein presenting one of the kidneys at each side. The adrenals are located between the large vessels and the kidney parenchyma. The Wolffian and Müllerian duct are at its lateral region (Fig. 1). The kidney is covered with a thin layer of connective tissue. It presents a clear cut segmentation in its initial (cranial) portion. These segments are bound by thin strands of connective tissue and gradually fuse as they progress in a caudal direction (Fig. 2). Their general structure is similar to the one described by SAKAI, BILLO and KRIZ (1986), whose nomenclature has been adopted in this paper.

Renal corpuscles

These structures are of variable size (average diameter $95\text{ }\mu\text{m}$) and consist of a tuft of capillaries surrounded by the Bowman's capsule formed in its internal part by typical podocytes, while the external layer consists of a simple squamous epithelium. The diameter of the capillaries is wide and variable (average $16\text{ }\mu\text{m}$). The tissue present between the capillaries—the mesangium—is particularly developed, containing cells and an amorphous substance. Frequently large, irregular cells containing well stained granules of variable size are observed in the mesangium (Fig. 3).

The filtration barrier of the glomerulus due to the presence of abundant extracellular matrix is much thicker and assumes a diffuse and irregular aspect

(Fig. 12).

Occasionally groups of small lymphocyte-like cells with scarce cytoplasm are found in the urinary space (between the two layers of Bowman's capsule) (Fig. 4). None of the usual components of the juxtaglomerular apparatus (juxtaglomerular cells and macula densa) were observed in our material.

The nephrostomes

These frequent structures are clearly visible in *S. annulatus*. They appear as patent tubules that initiate as invaginations in its surface parenchyma. They are formed by cubic epithelial cells characterized by long cilia (average $28\text{ }\mu\text{m}$) directed to the interior of the organ, ending in the proximal renal tubules. (Figs. 1, 2, 5).

The renal tubules

The following segments are present: neck segment, proximal tubule, intermediate segment, distal tubule and collecting tubule. The neck segment begins abruptly at the urinary pole of the renal corpuscle and is formed by cubic epithelial cells that also contain long cilia disposed in the direction of the flow of the glomerular filtrate. (Figs. 5, 6). They end at the proximal tubule that presents a distinctly larger diameter than the previous segment and is formed by cubic or prismatic cells characterized by the presence of microvilli on their surface. These structures are not closely aggregated, so that individual microvilli can be clearly distinguished (Fig. 7).

The cytoplasm of these cells presents a diverse aspect: it can be homogeneous containing no inclusions, or exhibit a variable amount of dark granules of various sizes or assume the aspect of vacuoles that can contain dark granules or present no visible con-

Fig. 1. Transverse section of the kidneys. In the center are the artery (A) and collapsed vein (V), and the organs meso (arrow). The Wolffian (W) and Müllerian ducts (M) are laterally disposed. An arrowhead shows a nephrostome and an asterisk adrenal tissue. $\times 55$

Fig. 2. Longitudinal section on a region where fusion occurs between adjacent kidney segments. The arrowhead shows a renal corpuscle from which a neck segment originates. An asterisk shows a adrenal tissue. Loose connective tissue lies between the two renal segments. A diagonally cut nephrostome appears in the surface (arrow). $\times 150$

Fig. 3. Renal corpuscle with its capillaries (C), and visceral (arrowhead) and parietal (asterisk) layers. A large cell with polymorphic granules (arrow) in the mesangium. $\times 420$

Fig. 4. Segment of a renal corpuscle with an aggregate of lymphocyte-like cells in the urinary space (arrowhead). C glomerular capillaries. $\times 420$

Fig. 5. Nephrostome showing the disposition of its elongated cilia. Below, two ciliated segments. $\times 250$

Fig. 6. Section showing two transitions of the neck to proximal tubule. Note the intense vacuolization of the proximal tubule cells. $\times 420$

Fig. 7. Showing two proximal tubules (PT) with dark stained cytoplasm and distinct border of microvilli. $\times 420$

Figs. 1-7. Legends on the opposite page.

Figs. 8 and 9. Proximal tubules (*PT*) cells showing different aspects of their cytoplasm. *DT* a distal tubule, *B* blood vessels. Fig. 8: $\times 1,050$, Fig. 9 $\times 420$

Fig. 10. Distal tubule (*DT*) cells characterized by the striated aspect of their cytoplasm. $\times 1,050$

Fig. 11. Collecting tubule (*CT*) cells that bulge into the lumen and present a visible partition between the cells. The *asterisk* shows a thin endothelial cell separating a capillary lumen from strands of connective tissue. $\times 1,050$

tent. When these inclusions are large and abundant, the cells assume a swollen aspect (Figs. 6-9). The high diversity of the cells of the proximal tubule shown in Figures 7-9 was not observed in any specimens studied. This may be related to the feeding cycle of these animals. The proximal tubule ends abruptly at the intermediate segment that presents the same aspect described for the neck segment. The following one, the distal tubule, is formed by prismatic cells of uniform aspect characterized by the striated aspect of its cytoplasm due to the presence of elongated mitochondria disposed as a palisade (Fig. 10). The surface of these cells presents small and scarce microvilli. The distal tubule ends in the collecting tubule that presents the largest diameter of all segments and is also formed by prismatic cells which do not have a striated aspect. They present a less dense

cytoplasm and clearly visible cell boundaries (Fig. 11). This structure ends in the Wolffian duct. The inner surface of this duct is formed by a layer of cubic or slightly flattened epithelial cells surrounded by a layer of irregular connective tissue (Fig. 13).

Urinary bladder

This is a highly vascularized structure comprising a single lobe whose wall is formed by loose connective tissue containing an irregular layer or smooth muscle cells. Its outer surface is covered with a thin layer of squamous mesothelium.

When empty, this organ is lined with a typical transitional epithelium characteristic of the urinary system. When full, it assumes the aspect of a simple squamous epithelium (Figs. 14, 15). The arrector cloacal muscle that binds the bladder to the animals wall is

Fig. 12. Glomerulus. Its capillaries (C) are separated by an irregular, thick layer of the extracellular matrix. The light stained nuclei are of the mesangial cells. $\times 1,600$

Fig. 13. Section of the Wollfian duct. No evident secretory activity is observable in its cubic and flattened cells. At left, Müller's duct and at right, renal tubules. $\times 90$

Figs. 14 and 15. Sections of the empty (**Fig. 14**) and full (**Fig. 15**) urinary bladder. B blood vessels. Arrows show the different aspects of the bladder epithelium under these conditions. Note the smooth muscle cells and mesothelium (arrowheads). $\times 300$

essentially formed by smooth muscle cells.

DISCUSSION

The kidney segmentation observed in our material is in accordance with the observations by SPENGEL (1876) for *Siphonops*, BRAUER (1902) and TONUTTI (1931) for *Hypogeophis*, and GARG and PRASSD (1962) for *Uraeotyphlus*.

The general features we observed in the renal corpuscle agree with the observations by WAKE (1970a), WELSCH and STORCH (1973) and SAKAI, et al.

(1988a). However, the large cells present in the mesangium and containing conspicuously well stained polymorphic granules were not, to our knowledge, previously described. Their variable morphology suggests that they may be cells in different phases of intracellular digestion, since in mammals mesangial cells have been shown to have a phagocytic activity (FAWCETT, 1994). In the liver of *S. annulatus* we have observed cells with a very similar aspect in the site of its macrophages (Kupffer cells). While the absence of components of the juxtaglomerular apparatus in *Siphonops* coincides with the results of SAKAI and KAWAHARA (1983) in *Triturus*, we did

not observe in our material the peripolar granulated cells they described in *Triturus*. Our observations regarding the filtration barrier of the glomerulus agree with the description presented by SAKAI and KAWAHARA (1983) in *Triturus*, as the presence of abundant extracellular matrix promotes a less detailed image than what is observed in mammals.

In our material nephrostomes opening to the coelom as described by SPENGEL (1876) and BRAUER (1902) were frequently observed although WAKE (1970a) did not find them in a series of more than 50 species, including *S. annulatus*. This discrepancy might be due to the problems of fixation and preservation to which she called attention in her paper.

The structure we observed in the nephrostomes, neck, and intermediate segments of the renal tubules is similar to that observed by WELSCH and STORCH (1973) and SAKAI, et al. (1988a) and considered by them to be responsible for the propulsion of peritoneal fluid and glomerular filtrate within the tubules.

The structure observed in the proximal tubule coincides with the description by WELSCH and STORCH (1973) who report the presence of loosely packed microvilli and lysosomes of various sizes and aspects. These characteristics suggest that the main activity of these cells is probably related to the intracellular digestion of plasma proteins that have crossed the basement membrane, a phenomena known to occur also in the mammalian kidney. The presence of primary and secondary lysosomes in the cells of this segment was also described by SAKAI and KAWAHARA (1983) in *Triturus*. Microvilli are known to be related to processes of metabolite reabsorption, but their moderate amount does not suggest that this process occurs intensely in these cells. In contrast with SAKAI, et al. (1988a), only one cell type was found in the proximal tubule in our material.

The morphology of the distal tubule observed here coincides with the observations by SAKAI et al. (1988b), who described cells with large elongated mitochondria disposed in palisades and abundant basal cell membrane invaginations. These are characteristics of ion transporting cells and suggest that, in *S. annulatus*, the distal tubule has this main function. Surprisingly, the distal tubules of *Ichthyophis* do not present these characteristics (WELSCH and STORCH 1973).

No tubule with the characteristics of the connecting tubule described by SAKAI et al. (1988b) was found in our material. The collecting tubule and Wolffian duct in *S. annulatus* have only one type of epithelial cell that does not present characteristics suggesting other functions beside the transport of urine. While our description of the collecting duct coincides with that

of SAKAI and KAWAHARA (1983) in *Triturus*, no structure which presents an intense secretory epithelium surrounded by a layer of smooth muscle cells—a urether—was observed in *Siphonops* and was previously described in other Gymnophiona.

The position of the Wolffian duct in our material was lateral and not dorsal as described by WIEDERSHEIM (1897), coinciding therefore with the observations by WAKE (1970a).

The only published description on the structure of the Gymnophiona urinary bladder is found in the comprehensive paper by WAKE (1970b); our observations in *S. annulatus* agree with her. Our observation on the epithelial layer of this organ when empty or full shows that in *S. annulatus* it also presents the capacity to change its thickness, a characteristic of the transitional epithelium of vertebrates.

Summing up, the presence of nephrostomes that probably drain liquid from the peritoneal cavity has been observed in *S. annulatus*. This liquid plus the urinary filtrate derived from the renal corpuscle are propelled by the cilia of the nephrostomes, neck, and intermediate segments through the renal tubules. The proximal tubule presents cells with characteristics that suggest metabolite reabsorption and active intracellular digestion, while the morphology of distal tubule cells speaks in favor of intense processes of ion transport.

No patches of lymphoid tissue as described by WAKE (1970a) in the renal parenchyma were observed in *S. annulatus*.

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Prof. L. C. U. JUNQUEIRA
Santiago, 99
Poços de Caldas-MG
37701-373 Brazil
Tel: +55-035-7142984
Fax: +55-035-7142951