BIOCELL
2003, 27(1): 19-28

ISSN 0327 - 9545
PRINTED IN ARGENTINA

Structure of the kidney of *Bufo arenarum*: Intermediate segment, distal tubule and collecting tubule

ALEJANDRO FARÍAS, GLADYS NOEMÍ HERMIDA, AND LUISA ELEONORA FIORITO

Laboratorio de Histología Animal; Departamento de Biodiversidad y Biología Experimental. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Buenos Aires, Argentina.

Key words: Amphibia, kidney, ultrastructure, renal tubules.

ABSTRACT: The ultrastructure of the intermediate segment (IS), distal tubule and collecting tubule (CT) of the south american toad *Bufo arenarum*, was studied by light and transmission electron microscopy. The IS is composed of cubical ciliated cells which propel the urine along the renal tubule. The distal tubule is divided into two portions: the early distal tubule (EDT) and the late distal tubule (LDT). The EDT is characterized by only one type of cells with well developed basolateral interdigitations and numerous elongated mitochondria, which are oriented normal to the basal surface. The "macula densa - like" is a specialized zone of the EDT in contact with the vascular pole, where cells are more tightly packed than in the rest of the tubule. The LDT shows two types of cells called dark and light cells according to the appearance of their cytoplasm. Dark cells have microplicae and few but long microvilli at their luminal surface, and abundant mitochondria in their cytoplasm. Light cells show basal and lateral infoldings and few mitochondria. The CT, which is composed of dark and light cells, exhibits an enlarged lumen with an undulated surface and dilated spaces between neighbouring cells.

This work is a contribution to the knowledge of the kidney of *B. arenarum*; frequently used as an experimental model for physiological and biochemical studies.

Introduction

The amphibian kidney is regarded as the opisthonephros (Kardong, 1995). The microscopic structure of the amphibian kidney has been investigated in Urodela (Clothier *et al*., 1978; Hinton *et al*., 1982; Sakai and Kawahara, 1983), Gymnophiona (Sakai *et al*., 1986; Sakai *et al*., 1988 a,b; Carvalho and Junqueira, 1999) and Anura (Meseguer *et al*., 1978; Uchiyama *et al*., 1990; Møbjerg *et al*., 1998).

The renal corpuscle and the renal tubule constitute the nephron. The renal tubule is divided into the neck segment, the proximal tubule, the intermediate segment (IS) and the distal tubule, which in turn is differentiated into two portions, the early distal tubule (EDT) and the late distal tubule (LDT). The epithelium of the EDT consists of one type of cells with striated appearance; functionally, it reabsorbs NaCl, is almost impermeable to water, and participates in urine dilution (Stoner, 1977). The epithelium of the LDT is composed of two cell types with different structure called light and dark cells, this epithelium plays a role in acid secretion (Stanton, 1984). The LDT opens into the collecting tubule (CT) which displays a similar heterocellular pattern when compared with the LDT, although intercellular spaces are wider (Sakai *et al*., 1988 a; Møbjerg *et al*., 1998) and the

Address correspondence to: Alejandro Farías. Laboratorio de Histología Animal, Dpto. Biodiversidad y Biología Experimental, 4° Piso, Pabellón II. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Av. Güiraldes s/n. (C1428EHA) Buenos Aires, ARGENTINA.
E-mail: chango@bg.fcen.uba.ar / ghermida@mail.retina.ar
Received on April 29, 2002. Accepted on August 26, 2002

strands of the tight junctions are more numerous (Biemesderfer *et al*., 1989).

Internally, the renal parenchyma can be divided into the ventromedial and dorsolateral zones (Farías *et al*., 1998). The ventromedial zone is mainly composed of distal tubules, whereas the dorsolateral zone is made up of the above mentioned segments of the renal and collecting tubules.

Bufo arenarum is a south american toad that lives in both arid and humid environments up to 2,400 m a.s.l. (Cei, 1980).

In a previous study we described the organization of the kidney of *B. arenarum*, in particular the structure of the renal corpuscle, neck segment and proximal tubule (Farías *et al*., 1998). The aim of this work is to give a more detailed information on the structure of the IS, the EDT, the LDT and the CT of this species, which is frequently used as an experimental model for physiological and biochemical studies.

Materials and Methods

Female and male adults of *B. arenarum* with body weights ranging from 45 to 92 g, were collected in the vicinity of temporary ponds during spring (Tigre, Buenos Aires, Argentina). Toads were kept in glass containers with free access to water. Before dissection, animals were anaesthetised with tricaine methansulphonate (0.01 g/100 ml distilled water).

For transmission electron microscopy (TEM), the kidney was sectioned into small pieces which were fixed by immersion in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 at 4ºC for 3 h. Pieces were then rinsed in 0.1 M cacodylate buffer and postfixed in 1% osmium tetroxide in that same buffer for 1 h. Following postfixation, pieces were dehydrated through ethanol gradient series and embedded in araldite resin. Semithin sections for light microscopy (LM) were stained with 1% toluidine blue in 1% Na_2CO_3; ultrathin sections were double-stained with uranyl acetate - lead citrate (Reynolds, 1963) and observed under a Zeiss (Oberkochen, Germany) EM 109 T transmission electron microscope at 80 kV.

Results

a) *Intermediate Segment (IS):*

The proximal tubule of the nephron abruptly connects to a short IS, which courses dorsoventrally to join the EDT (Fig. 1). This segment is located between the dorsolateral and ventromedial zones of the organ, close to the renal corpuscles. The IS is lined with a simple cuboidal ciliated epithelium (Fig. 2a) in which cells have an irregular nucleus with peripheral heterochromatin. The cytoplasm contains ovoid and elongated mitochondria (0.26 – 0.72 μm and 0.78 – 1.69 μm in diameter, respectively) which are scattered throughout the lateral and supranuclear cytoplasm of the cell. Basal bodies of the cilia are connected to short and long striated rootlets which are next to mitochondria. Electron - dense bodies are mainly found in the apical part of the cell (Fig. 2b). Numerous vacuoles are distributed throughout the cytoplasm.

b) *Early Distal Tubule (EDT)*:

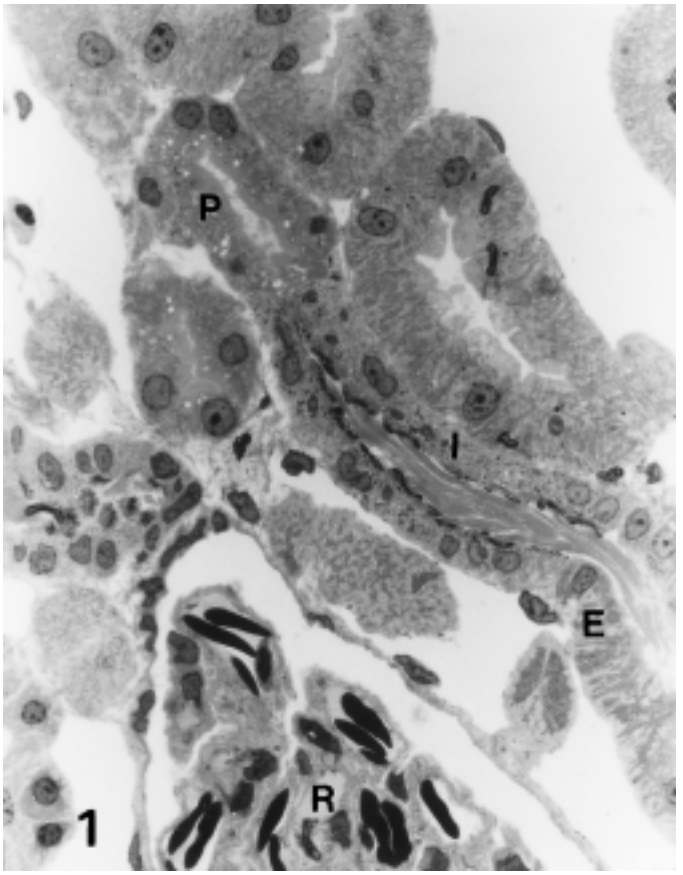
The LM observation reveals that the cytoplasm of the EDT epithelial cells shows striations perpendicular to the basal lamina (Fig. 1, 3a).

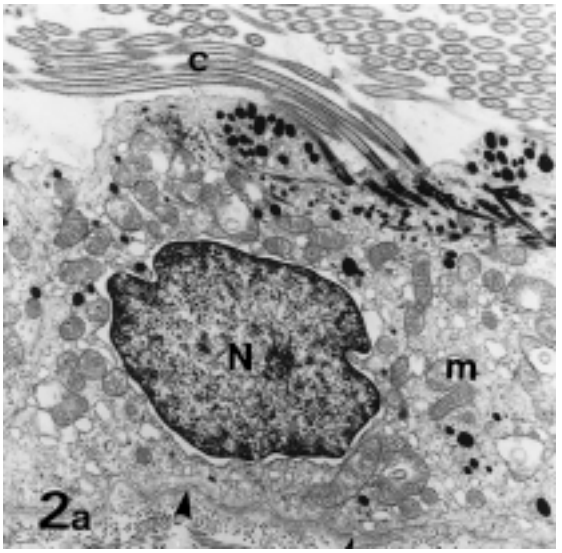
Adjacent cells are joined through a *zonula occludens* which decreases in number towards the transition between the EDT and the LDT. The apical surface of the cuboidal cells has a small number of short, thick and irregular microvilli (Fig. 3b). The number of microvilli decreases as the tubule continues in the ven-

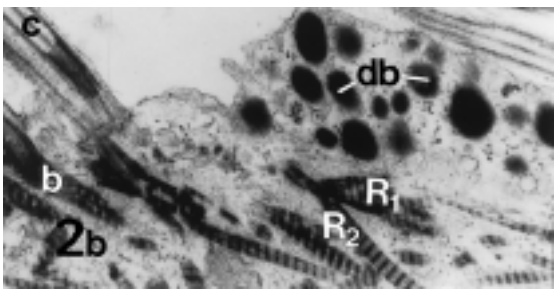
FIGURE 1. Semithin sections showing the early distal tubule (E); intermediate segment (I); proximal tubule (P) and renal corpuscle (R). X 640

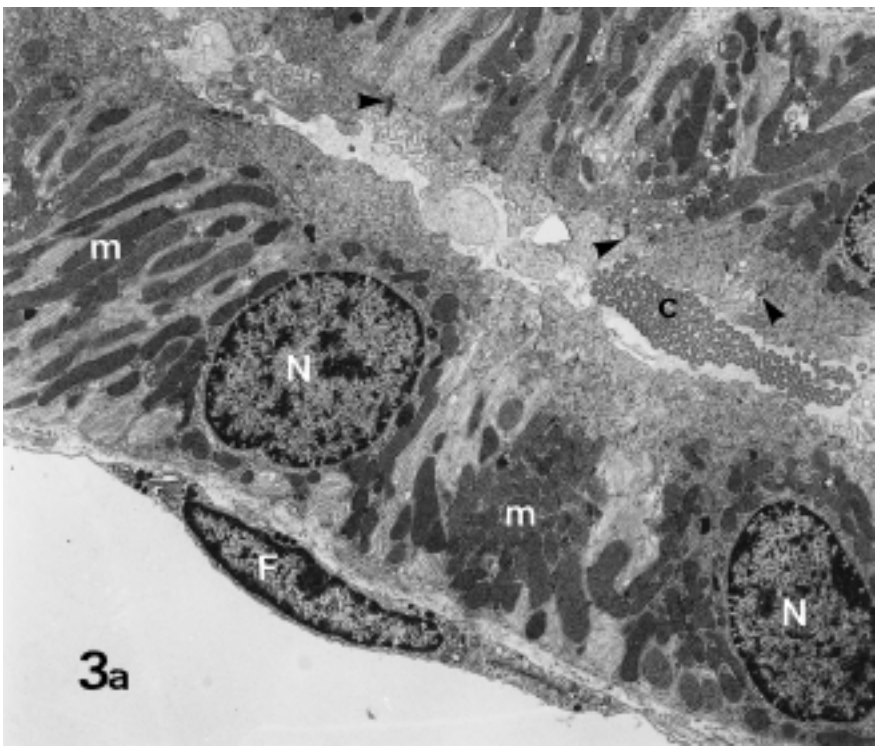
FIGURE 2. a) Epithelial cell of IS. c, cilia; m, mitochondrion; N, nucleus. Arrowhead: basal lamina. X 7,300
b) Detail of the apical region of an IS cell. b, basal corpuscle; db, dense body; R_1, short rootle; R_2, long rootle. X 21,400

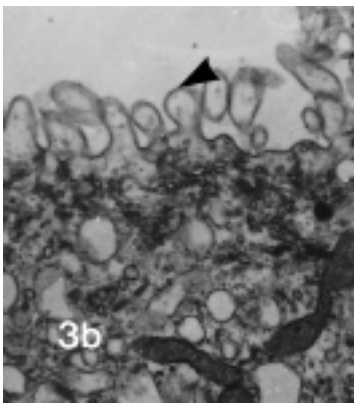
FIGURE 3. a) Cells of the first portion of the EDT. Cilia (c) present in the tubular lumen belong to IS. m, mitochondrion; N, nucleus. Arrowhead, zonula occludens. X 4,100
b) Detail of apical surface of the EDT cells. mv, microvilli. X 22,300
c) Detail of basal surface of the EDT cells. Arrowhead, basal lamina. X 10,100

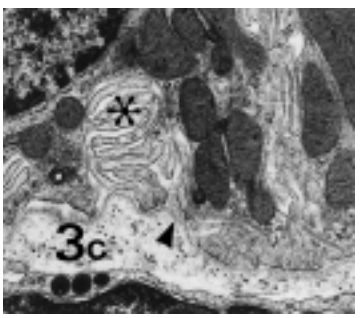

P
l
E
R
1


c
N
m
2a


c
db
b
R_1
R_2
2b


m
N
c
m
F
N
3a


3b


3c

tral direction of the kidney (Fig. 4). The cells have extensive basolateral interdigitations due to infoldings of the cell membrane (Fig. 3c). These infoldings either interdigitate with neighbouring cells or enclose mitochondria. The cytoplasm contains abundant elongated mitochondria (0.5 – 4.79 μm) oriented normal to the basal surface. Abundant smooth endoplasmic reticulum, free ribosomes and electron - dense bodies of lysosomal nature are observed. Lamellar and multivesicular bodies are occasionally seen.

The terminal portion of the EDT, where afferent and efferent arterioles are located, reaches the vascular pole of the renal corpuscle. In this region, the epithelium of the EDT includes cells that are referred to as *macula densa* - like cells (Fig. 5). These cells are easily recognizable because they are pyramidal in shape, are taller and narrower, and are more densely distributed than the rest of the EDT cells (Fig. 6). As is the case in all EDT cells, adjacent *macula densa* - like cells are joined together by *zonula occludens*. The apical cell surface has prominent and expanded microvilli. The basolateral cell membranes are characterized by conspicuous and deep infoldings. There are elongated or ovoid mitochondria (0.83 – 5.09 μm and 0.41 – 1.9 μm in diameter, respectively) in the cytoplasm. The cells of the middle layer of the afferent arteriole contain membrane - bounded granules of different sizes and shapes (Fig. 7). The content of the granules is heterogeneous, showing lamellar structures or electron - dense foci.

c) *Late distal tubule (LDT):*

The last portion of the distal tubule contains two different types of cells, represented by light cells with a clear appearance, and by dark cells with a obscure cytoplasm (Fig. 8). The light cells are cuboidal in shape and are characterized by basal and lateral infoldings of the plasma membrane. Their apical region comprise a junctional complex followed by interdigitations between neighbouring light cells. The luminal cell surface lacks microvilli, but has short and thick expansions of the apical cytoplasm containing few cytoplasmic organelles. Few mitochondria of different shapes (0.29 – 2.14 μm in diameter) are regularly arranged around the central nucleus (Fig. 9). Mitochondria lack between basal infoldings. Rough endoplasmic reticulum, vacuoles of different sizes and free ribosomes are present in the cytoplasm. On the other hand, dark cells are distinguished by their electron - dense cytoplasm and granules, the abundance of small and ovoid mitochondria (0.23 – 1.56 μm in diameter) and by their scarce lateral cell processes (Fig. 10a). The apical surface has cytoplasmic folds called microplicae, as well as few and long microvilli. The apical cytoplasm contains clear vesicles of various shapes and sizes (Fig. 10b). On the basal plasma membrane there are shallow infoldings, which are not associated with mitochondria (Fig. 10c). The extensions of cytoplasm alternate with basal foot processes that rest on the basal lamina.

d) *Collecting Tubule (CT):*

This tubule runs dorsoventrally along the dorsolateral zone of the kidney, next to the proximal tubules. Each CT connects to a collecting duct in the dorsal part of the kidney, which in turn drains into the archinephric duct along the lateral external border of the organ.

The CT exhibits a wide lumen with an undulated surface (Fig. 11). The epithelium is composed of light and dark cells of variable shape. Dilated spaces are found between neighbouring cells. The light cells lack microvilli and possess a basal nucleus, scattered mitochondria of variable sizes (0.20 – 1.78 μm in diameter) and free ribosomes (Fig. 12a). The lateral membrane shows infoldings that do not interdigitate with either light or dark neighbouring cells. Dark cells have an electron - dense cytoplasm which contains abundant mitochondria (0.28 – 1.29 μm in diameter). Vacuoles and

FIGURE 4. Cells of the final portion of EDT. mv: microvilli. Arrowhead, *zonula occludens*. X 7,500

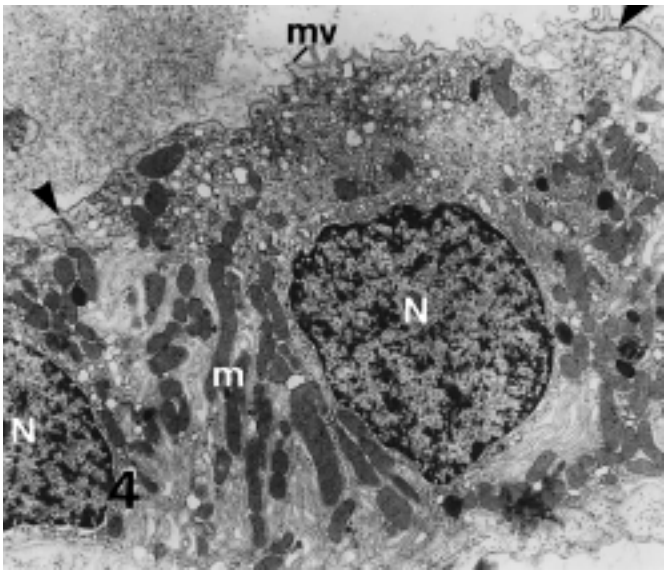
FIGURE 5. Semithin section. aa, afferent arteriole; md, *macula densa - like* cells; R, renal corpuscle. X 3,200

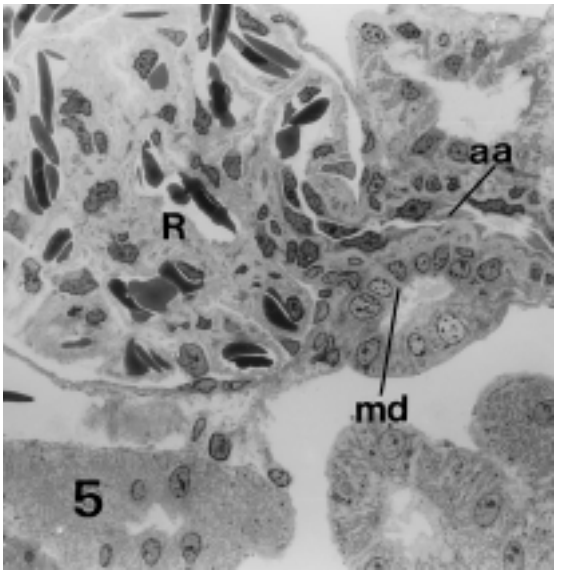
FIGURE 6. "*Macula densa - like*" cells. mv, microvilli. Arrowhead, *zonula occludens*. X 3,300

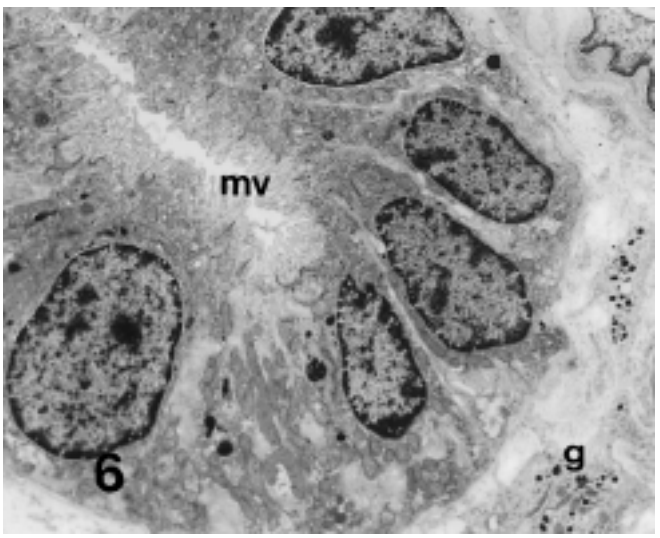
FIGURE 7. Detail of media cell of afferent arteriole. g, granules. X 18,000

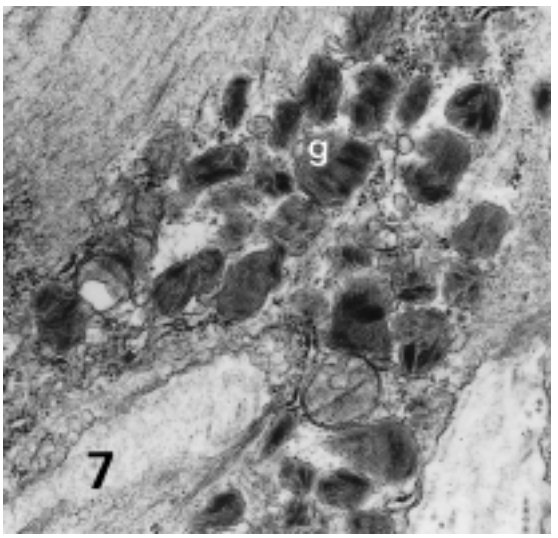
FIGURE 8. Semithin section of LDT in transversal view (asterisk). D, dark cell; E, early distal tubule; L, light cell. X 750

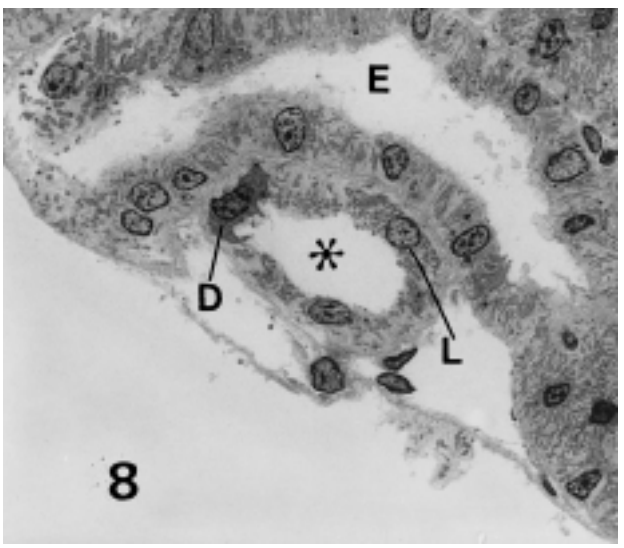
FIGURE 9. Light cell of LDT. m, mitochondrion; n, nucleolus. Arrowhead, basal lamina; asterisk, basal infoldings. X 7,500

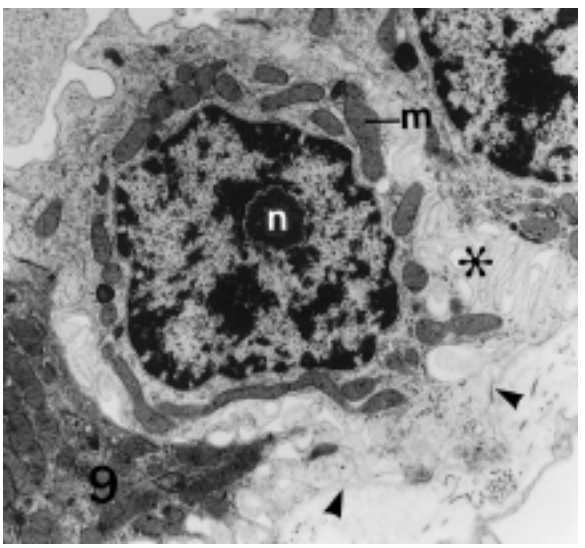

mv
N
m
N
4

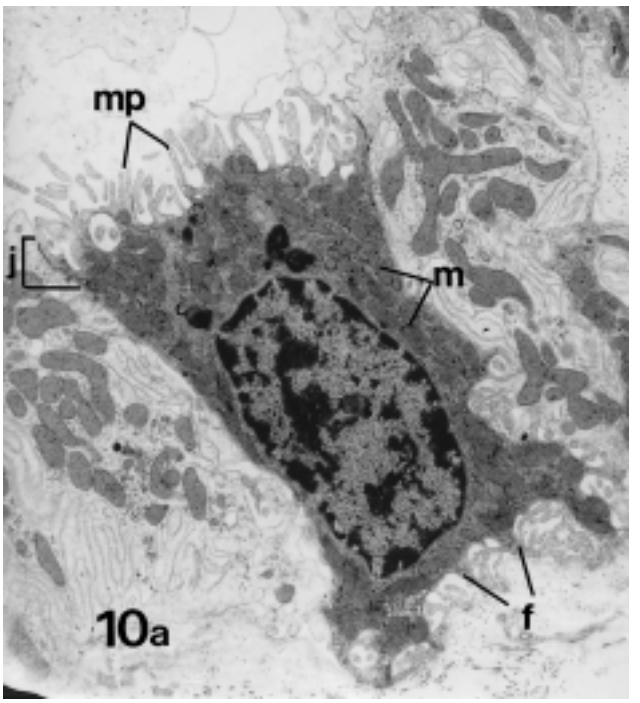

aa
R
md
5

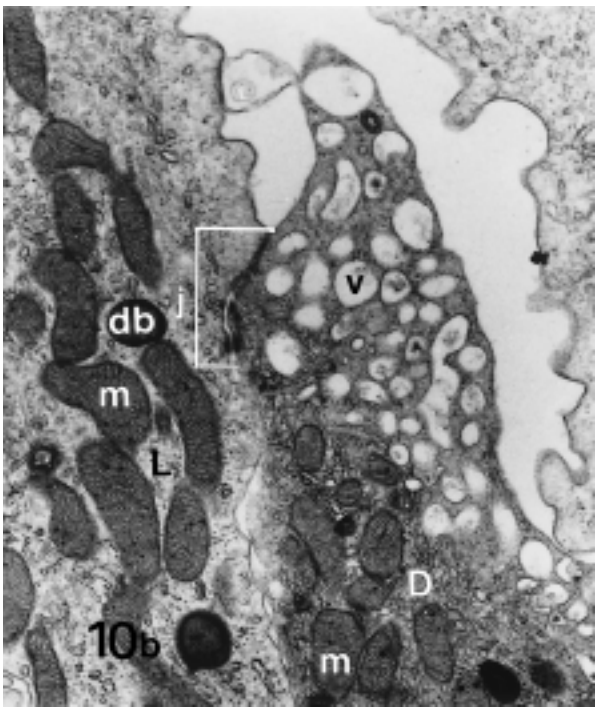

mv
6
g

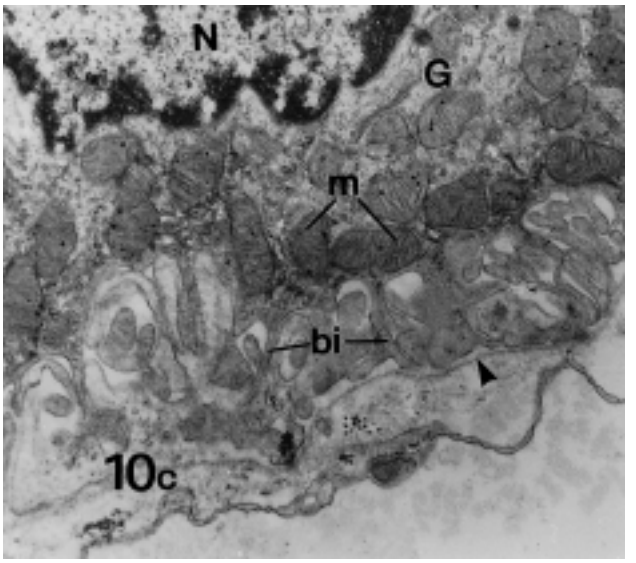

g
7


E
D
L
8


m
n
9







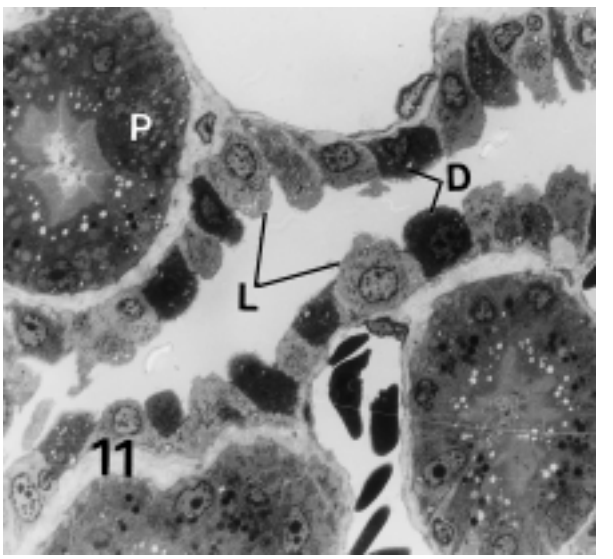
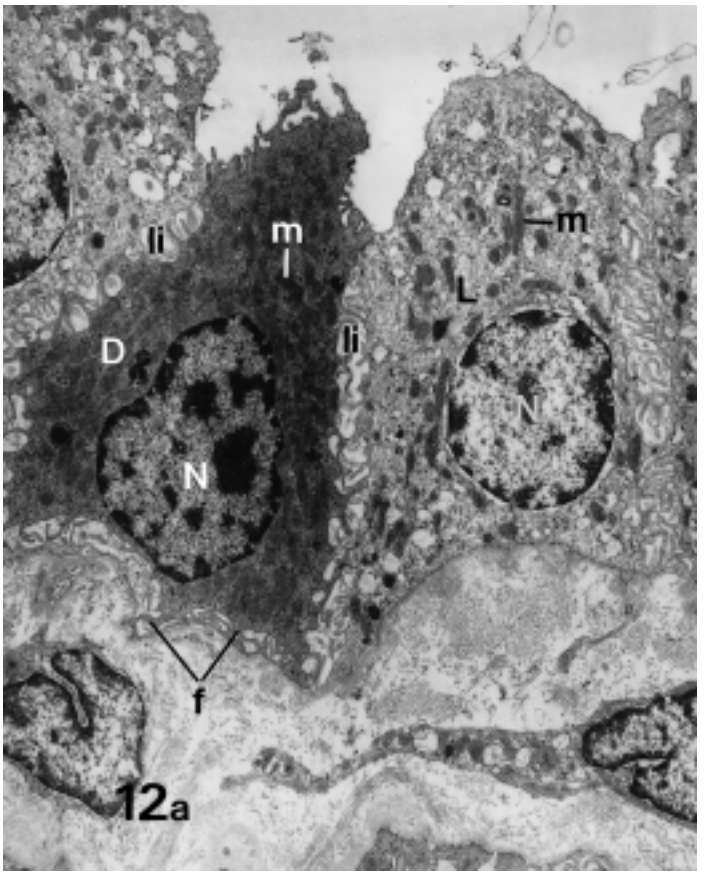
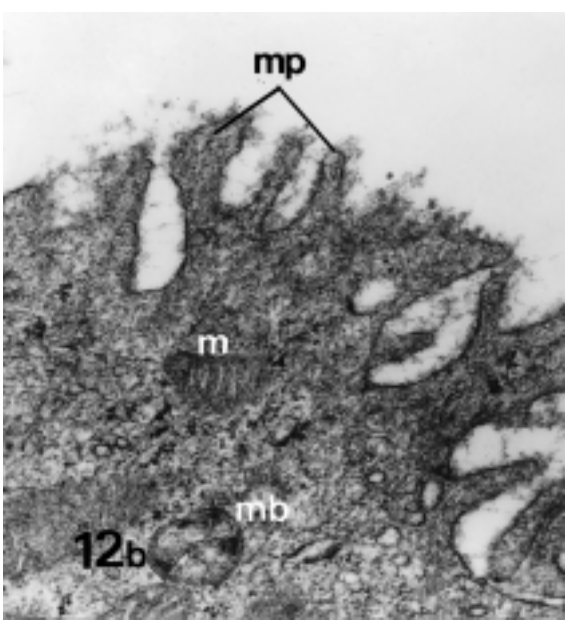


FIGURE 10. a) Dark cell of LDT. f, basal process; j, junctional complex; m, mitochondrion; mp, microplicae. X 5,800
b) Detail of apical limit between light (L) and dark (D) cells. J, junctional complex; m, mitochondrion; v, vesicles. X 18,000
c) Detail of the basal portion of the dark cell. bi, basal infoldings; G, Golgi complex; m, mitochondrion. Arrowhead, basal lamina. X 12,200

FIGURE 11. Semithin section of CT in longitudinal section. D, dark cell; L, light cell; P, proximal tubule. X 640





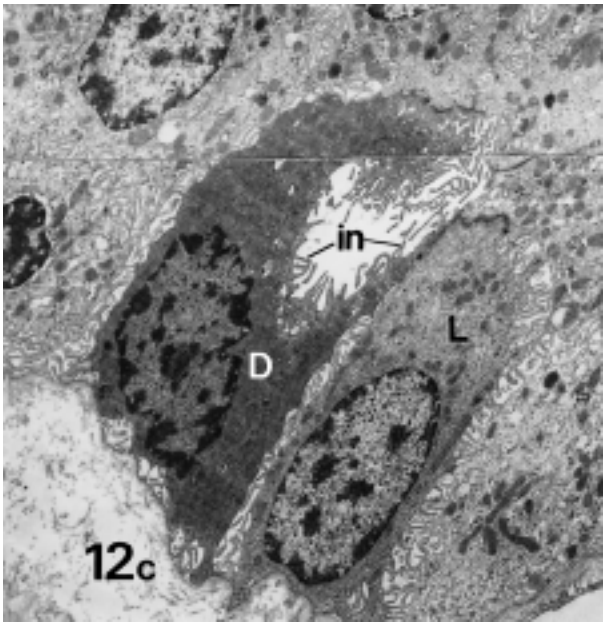


FIGURE 12. a) Light cell (L) and dark cell (D) of the CT. f, basal process; li, lateral intercellular space; m, mitochondrion; N, nucleous. X 6,000
b) Detail of apical surface of the dark cell. m, mitochondrion; mb, multivesicular body; mp, microplicae. X 37,600
c) Dark cell of CT with invaginations of the apical border (in). X 6,600

multivesicular bodies are found in the apical region. The apical surface shows microplicae (Fig. 12b), microvilli, and invaginations are occasionally found (Fig. 12c). Both light and dark cells possess basal infoldings.

Discussion

As far as we know, this is the first study of the ultrastructure of the IS, EDT, LDT and CT in *B. arenarum*. In a previous work we have described the cell types of the renal corpuscle, neck segment and proximal tubule in that toad species (Farías *et al*., 1998).

At the level of the renal corpuscle, a narrow IS separates the proximal tubule from the EDT. The IS resembles the neck segment, which is also located in the same zone of the kidney, both in cytological structure and in urine transport. In both segments, urine is propelled along the renal tubules by ciliar movement. The energy required for this activity is supplied by mitochondria located in the lateral and supranuclear cytoplasmic regions of the IS cells.

The distal tubule is divided into two parts. The first portion (EDT) is composed of one cell type and the second portion (LDT), of two cell types. The striated appearance of the EDT cells is due to basolateral interdigitations and infoldings of the cell membrane enclosing mitochondria with a palisade arrangement. In *Ambystoma tigrinum*, Stoner (1977) reported that this tubule exhibits a rapid NaCl reabsorption rate and that chloride is actively transported. Because the EDT epithelium is impermeable to water, salts are reabsorbed and excess water is eliminated, this segment has been termed the "diluting segment" (Stoner, 1977; Hinton *et al*., 1982).

Direct or indirect active transports driven by the Na^+/K^+ - ATPase system are located on the basolateral cell membranes (Jørgensen 1980; Katz, 1982). The ATP molecules for the functioning of such transports are provided by mitochondria (Himmelhoch and Karnovsky, 1961). Well developed basolateral interdigitations of the cell membrane amplify the surface area for the Na^+/K^+-ATPase activity and permit spatial proximity of tight junctions in order to facilitate the circular current of paracellular Na transport and transcellular Cl transport (Kyte, 1976).

The distal tubule returns to the vascular pole of the same renal corpuscle where the nephron starts. The EDT epithelial cells adjacent to the afferent arteriole differ from those in the rest of the tubule. This group of cells can be compared with the macula densa of mammals (Lamers *et al*., 1973). The middle layer of the afferent arteriole shows disperse juxtaglomerular cells (media cells), containing granules with a lamellar content. These granules are located at the opposite pole of the vascular lumen, in the neighbourhood of the "*macula densa* - like" cells and the adventitial nerve fibers (Lamers *et al*., 1974). In *Bufo* sp., media cells store renin or a renin - like compound in the granules (Lamers *et al*., 1985 a,b). In *B. arenarum*, Nolly and Fasciolo (1971) reported that granules contain a renin - like substance. This finding, together with the morphological similarities between the granules observed by us and those reported for *Bufo* sp., may confirm the presence of a renin - like content in these type of granules.

The second portion of the distal tube has been designated with different names, such as LDT (Stoner, 1977; Stanton *et al*., 1984; Biemesderfer *et al*., 1989; Møbjerg *et al*., 1998), distal region 2 (Clothier *et al*. 1978), distal part of distal tubulus (Taugner *et al*., 1982), junctional segment (Hinton *et al*., 1982), connecting tubule (Sakai *et al*., 1986, 1988a; Uchiyama *et al*., 1990), or terminal segment of distal tubulus (Fenoglio *et al*., 1996). In the LDT of *B. arenarum*, *zonula occludens* increase in depth and number of strands in direction of the CT. The presence of deep *zonula occludens* and numerous strands also occur in the LDT of *Rana esculenta*, in which the epithelium was characterized as a very tight one (Taugner *et al*., 1982).

Arginine vasotocin (AVT) V_2 - type receptors have been localized in the LDT of *Rana catesbeiana* (Uchiyama, 1994). The AVT stimulates the constriction of preglomerular vessels and the increase of tubular reabsorption (Pang *et al*., 1980). In dehydration, this hormone increases the permeability of tubules to water until equilibrium is reached between tubular and intercellular fluids and the urine becomes isosmotic (Dantzler, 1985).

Variations in the epithelial structure of the LDT reflect functional differences. Dark cells, which may be involved in urine acidification (Brown and Breton, 1996) are common constituents of both the EDT and CT. These have been called "intercalated cells" in caecilians (Sakai *et al*., 1988a), urodeles (Hinton *et al*., 1982; Stanton *et al*., 1984) and anurans (Møbjerg *et al*., 1998). Their apical cell membrane is characterized by the presence of microplicae, few microvilli and an electron-dense coat. On its inner side, the apical membrane shows characteristic specializations with the appearance of globular particles or "studs" (Taugner *et al*., 1982; Stanton *et al*., 1984; Sakai *et al*., 1988a). Some vesicles located in the apical region of the cytoplasm are coated with an electron - dense material, which is similar to that of the apical membrane. In the apical region, such vesicles

increase the number of proton pumps by exocytosis, and are removed by endocytosis (Brown and Breton, 1996). In the rat collecting tubule, the cytoplasm of dark cells also contain rod - shaped particles which are involved in ionic transport activities (Brown, 1978). Due to the presence of apical microvilli and microplicae, electron dense cytoplasm and abundant mitochondria, dark cells have been classified as "mitochondria - rich" (MR) cells (Brown and Breton, 1996).

In addition to dark or intercalated cells, "canaliculi" or flask cells have been described principally in the CT of various species. These cells were observed in *Xenopus laevis* (Brown, 1978), *Rana esculenta* (Bargmann and Welsch, 1972; Taugner *et al*., 1982) and *Rana cancrivora* (Bargmann and Welsch, 1972; Uchiyama *et al*., 1990). Canaliculi cells resemble the parietal cells of the gastric glands due to invaginations of their apical plasma membrane which forms either ramified intracellular canaliculi or a pear - shaped lumen. We have not observed these type of cells in *B. arenarum*. Although dark cells with apical invaginations are occasionally seen in the CT, their morphology differs from that of canaliculi cells.

The light cells of the CT of *B. arenarum* share some common characteristics with those of other amphibian species, such as: smooth apical surface, dilated intercellular spaces and lateral infoldings that do not interdigitate with neighbouring membrane cells (Sakai *et al*., 1988a; Møbjerg *et al*., 1998).

Further research is needed to explore relationships between morphology and function.

Acknowledgements

The authors would like to express their thanks to Mrs. Isabel Farías for their technical assistance and Silvia Pietrokovsky for revising the English translation.

References

Bargmann W, Welsch U (1972). *Über kanälchenzellen und dunkle zellen im nephron von anuren*. Z Zellforsch 134: 193-204.

Biemesderfer D, Stanton B, Wade JB, Kashgarian M, Giebisch G (1989). *Ultrastructure of Amphiuma distal nephron: evidence for cellular heterogeneity.* Am J Physiol 256 (4) (1): C849-C857.

Brown D (1978). *Freeze-fracture of Xenopus laevis kidney: rod-shaped particles in the canalicular membrane of the collecting tubule flask cell.* J Ultrastruct Res 63:35-40

Brown D, Breton S (1996). *Mitochondria-rich, proton-secreting epithelial cells*. J Exp Biol 199: 2345-2358.

Carvalho ETC, Junqueira LCU (1999). *Histology of the kidney and urinary bladder of Siphonops annulatus (Amphibia-Gymnophiona)*. Arch Histol Cytol 62(1): 39-45.

Cei JM (1980). *Amphibians of Argentina.* Monit Zool Ital 2: 609.

Clothier RH, Worley RTS, Balls M (1978). *The structure and ultrastructure of the renal tubule of the urodele amphibian Amphiuma means.* J Anat 127(3): 491-504.

Dantzler WH (1985). *Comparative aspects of renal function.* In: The Kidney: Physiology and Pathophysiology. Seldin and Giebisch Eds. Raven Press, New York. pp: 333-364.

Farías A, Fiorito LE, Hermida GN (1998). *Structure of the Bufo arenarum kidney: renal corpuscle, neck segment and proximal tubule.* Biocell 22(3): 187-196.

Fenoglio C, Vaccarone R, Chiari P, Gervaso MV (1996). *An ultrastructural and cytochemical study of the mesonephros of Rana esculenta during activity and hibernation*. Eur J Morph 34: 107-121.

Himmelhoch SR, Karnovsky MJ (1961). *Oxidative and hydrolytic enzymes in the nephron of Necturus maculosus. Histochemical, biochemical, and electron microscopical studies*. J Biophys Biochem Cytol 9: 893-908.

Hinton DE, Stoner LC, Burg M, Trump BF (1982). *Heterogeneity in the distal nephron of the salamander (Ambystoma tigrinum): A correlated structure function study of isolated tubule segments*. Anat Rec 204: 21-32.

Jørgensen PL (1980). *Sodium and potassium ion pump in kidney tubules.* Physiol Rev 60: 864-917.

Kardong KV (1995). *The urogenital system*. In: Vertebrates, comparative anatomy, function evolution. Wm C Brown publishers, Dubuque. Chapter 4, pp 533-583.

Katz AI (1982). *Renal Na – K – ATPase: its role in tubular sodium and potassium transport.* Am J Physiol 242: F207-F219.

Kyte J (1976). *Immunoferritin determination of the distribution of (Na^++K^+) ATPase over the plasma membranes of renal convoluted tubules. I. Distal segment.* J Cell Biol 68: 287-303.

Lamers APM, Stadhouders AM, Verhofstad AAJ, Michelakis AM (1985 a). *Immunoelectron microscopic localization of renin in the juxtaglomerular cells of the amphibian Bufo bufo.* Gen Comp Endoc 60: 380-389.

Lamers APM, Van Dongen WJ, Van Kemenade JAM (1973). *The morphology of the juxtaglomerular apparatus in the toad Bufo bufo.* A light microscopy study. Z Zellforsch 138: 545-555.

Lamers APM, Van Dongen WJ, Van Kemenade JAM (1974). *An ultrastructural study of the juxtaglomerular apparatus in the toad, Bufo bufo* (1974). Cell Tissue Res 153: 449-464.

Lamers APM, Verhofstad AAJ, Stadhouders AM, Michelakis AM (1985 b). *Immunohistochemical demonstration or renin in the juxta-glomerular apparatus of three Bufo species.* Cell Tissue Res 239: 677-682.

Meseguer J, Agulleiro B, Llombart Bosch A (1978). *Structure and ultrastructure of the frog's nephron (R. ridibunda). II. Proximal convoluted tubule, distal convoluted tubule and segment of connection*. Morfología Normal y Patológica Sec A 2: 41-60.

Møbjerg N, Larsen EH, Jespersen Å (1998). *Morphology of the nephron in the mesonephros of Bufo bufo (Amphibia, Anura, Bufonidae)*. Acta Zoologica (Stockholm) 79(1): 31-50.

Nolly H, Fasciolo JC (1971). *The renin - angiotensin system in Bufo arenarum and Bufo paracnemis*. Comp Biochem Physiol 39A: 823-831.

Pang PK, Galli-Gallardo SM, Collie N, Sawyer WH (1980). *Renal and peripheral vascular responsiveness to arginine vasotocin in bullfrog, Rana catesbeiana.* Am J Physiol 239(1): R156-R160.

Reynolds ES (1963). *The use of lead citrate at high pH as an electron-opaque stain in electron microscopy.* J Cell Biol 17:208-212.

Sakai T, Billo R, Kriz W (1986). *The structural organisation of the kidney of Typhlonectes compressicaudus (Amphibia, Gymnophiona)*. Anat Embriol 174: 243-252.

Sakai T, Billo R, Kriz W (1988 a). *Ultrastructure of the kidney of a South American caecilian, Typhlonectes compressicaudus (Amphibia, Gymnophiona). II. Distal tubule, connecting tubule, collecting duct and Wolffian duct.* Cell Tissue Res 252: 601-610.

Sakai T, Billo R, Nobiling R, Gorgas K, Kriz W (1988b). *Ultrastructure of the kidney of a South American caecilian, Typhlonectes compressicaudus (Amphibia, Gymnophiona). I. Renal corpuscle, neck segment, proximal tubule and intermediate segment.* Cell Tissue Res 252: 589-600.

Sakai T, Kawahara K (1983). *The structure of the kidney of Japanese newts, Triturus (Cynops) pyrrhogaster.* Anat Embriol 166: 31-52.

Stanton B (1984). *Electroneutral NaCl transport by distal tubule: evidence for Na^+/H^+-Cl^-/HCO_3^- exchange.* Am J Physiol 254 (Renal Fluid Electrolyte Physiol. 23): F80-F86.

Stanton B, Biemesderfer D, Stetson D, Kashgarian M, Giebisch G (1984). *Cellular ultrastructure of Amphiuma distal nephron: effect of exposure to potassium.* Am J Physiol 247: C204-C216.

Stoner L (1977). *Isolated, perfused amphibian renal tubules: the diluting segment.* Am J Physiol 253: F438-F444.

Taugner R, Schiller A, Ntokalou - Knittel S (1982). *Cells and intercellular contacts in glomeruli and tubules of the frog kidney*. Cell Tissue Res 226: 589-608.

Uchiyama M (1994). *Sites of action of arginine vasotocin in the nephron of the bullfrog kidney.* Gen Comp Endocrinol 94: 366-373.

Uchiyama M, Murakami T, Yoshizawa H, Wakasugi C (1990). *Structure of the kidney in the crab-eating frog, Rana cancrivora.* J Morph 204: 147-156.