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A COMPARISON OF THE RENAL STRUCTURES OF THE ANACONDA AND THE BALL PYTHON

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Abstract

The renal vascular system of the Ball python (*Python regius*) and the anaconda (*Eunectes noteus*; Serpentes - Squamata) has been investigated using light microscopy, transmission electron microscopy (TEM), and scanning electron microscopy (SEM) of vascular corrosion casts and critical-point dried non-corroded specimens. The average glomerular diameters of these two species differ significantly (anaconda: 59.1 μm, python: 124.3 μm). Also, the relative proportions of the renal tubules are different. These findings can be related to the different habitats of the two species (aquatic versus terrestrial environment).

Key Words: Kidney, snakes, microcorrosion casts, glomerulus, electron microscopy, blood vessels, reptiles, renal anatomy.

Introduction

The capacity of water conservation, among other factors, is an important prerequisite for the permanent terrestrial life of reptiles in a variety of biotopes. However, compared to mammals, their differing renal structure and function (e.g., lack of Henle's loops, uric acid as a major excretory component), have resulted in alternate adaptational pathways.

Renal anatomy reflects this physiologic adaptation at the tubular and vascular level. The development of a renal portal system in reptiles (and birds) has long been regarded as a primitive feature (e.g., see Fox, 1977). However, physiological studies (Perschmann, 1956; Dantzler and Schmidt-Nielsen, 1966; Schmidt-Nielsen and Davis, 1968; Dantzler, 1982a,b; Schmidt-Nielsen, 1988) indicate that the renal portal system plays an important role in tubular secretion, especially of uric acid.

The reptilian renal lobes form morphologically distinct units, supplied by a central artery giving rise to afferent glomerular arterioles, a central draining vein and several peripheral afferent portal veins that supply most of the peritubular capillary system. The renal tubules form S-like loops starting at the glomeruli near the center of the lobule and enter the ureteral branches at its periphery. The arrangement of the renal lobes differs in the various reptilian species.

The size of the renal vascular tuft was regarded by some scientists as a parameter closely related to the water supply of the habitat (Marshall and Smith, 1930; Marshall, 1934). However, earlier studies on turtles, lizards and birds (Ditrich and Splechtna, 1986, 1987a,b, 1994; Guo *et al.*, 1996), indicated that the relation between glomerular size and water supply to a species is probably more complicated.

Several studies have been performed on the structure and function of snake kidneys (e.g., Bishop, 1959; Peek and McMillan, 1979a,b; Dantzler, 1982a,b; Yokota and Dantzler, 1990). A mathematical model of a snake glomerulus has been published recently (Papenfuss *et al.*, 1992). The authors of the latter study affirm that in addition to several physiological parameters, detailed

morphological data on the glomerulus are required to generate a valid model of the glomerular microcirculation. Generating a mathematical model of a glomerulus can help in explaining many experimental data. Histological results, e.g., those yielding reconstructions of serial sections, are inherently less accurate due to shrinkage and compression by sectioning than analyses based on microcorrosion casts.

Microcorrosion casting using acrylic resins and the scanning electron microscope (SEM) was initially developed to investigate the renal vascular system of the rat (Murakami, 1971). Subsequently, a notable number of studies applied this technique in morphological studies on the kidney in other vertebrates and in investigations on renal pathology and pharmacological processes (for reviews see Ditrich and Splechtna, 1990; Lametschwandtner *et al.*, 1990; Konerding, 1991).

The vascular system can be regarded as the key determinant of renal structure and function in snakes and other reptiles. Anacondas live under semi-aquatic conditions, and thus have mainly the problem of removal of excess water from the body, while the Ball python lives in an arid, although not desert biotope, that requires water conservation. This study was carried out to compare the renal anatomy of these two snake species from the boidae family, adapted to different environmental conditions. It may help in understanding the specific constructional principles underlying the structure-function relationship when coping with differing habitats.

Material and Methods

A total of 16 juvenile specimens of the anaconda (*Eunectes noteus*, approximately 250 g - 500 g body weight) and the Ball python (*Python regius*; Squamata - Serpentes; approximately 1000 g - 1560 g body weight) were used in this study in conformity with the relevant legal regulations. The animals were obtained from a commercial pet shop and kept in the laboratory for one to two weeks before the preparation. The conditions for keeping the animals were adapted to the biological requirements of the species (i.e., aquatic for anacondas and terrestrial for pythons, temperature, diet, etc.).

The animals were injected with 0.3-0.5 ml heparin (5000 I.E., Novo Ind., Vienna, Austria) and anaesthetized with a terminal dose of sodium-pentobarbital (10 mg i.p./100 g body weight; Nebutal, Serva, Heidelberg, Germany).

The animals were then perfused with buffered saline (pH 7.3; 300 mOsm; 22°C; flow approximately 25-35 ml/min) using a peristaltic laboratory pump (PA-SF2, IKA, Staufen, Germany) with a polyethylene-catheter tied into the left aortic arch. The right aortic arch was clamped. The sinus venosus was opened to allow drainage of the blood and saline. The exposed vessels, especially near the liver, were monitored with a light microscope during perfusion and injection to assure a natural filling-state of the vessels (i.e., no excessive dilation or collapse). Nine animals (four pythons and five anacondas) were injected supravitally with Mercox® (Japanese Vilene Co., Tokyo, Japan) diluted with 25% methylmethacrylate using a mechanical press and further processed as described previously (Ditrich and Splechtna, 1986, 1987b). The amount of hardener-catalyst was reduced to approximately 1 vol% of the resin-mixture to prolong injection time up to approximately 15 minutes.

Seven other animals (four pythons and three anacondas) were perfusion-fixed for ten minutes with 2% formaldehyde and 0.5% glutardialdehyde in the same saline used for flushing. These specimens were quickly dissected; the kidneys were cut into 5 mm blocks, postfixed in the same fixative (4°C, 12 hours), dehydrated with graded ethanol and either transferred into acetone and critical-point (CP) dried for scanning electron microscopy or postfixed in 1% OsO_4 and embedded in Epon (Bal-Tec, Balzers, Liechtenstein) for standard light (LM) and transmission electron microscopy (TEM). One perfusion-fixed python was injected with the diluted Mercox unilaterally from the ureter in order to inject the renal tubular system.

Corrosion cast preparations were validated with light microscopy for completeness of filling, frozen in distilled water, cut and air dried. Measurements were carried out either with the line-width system on the microscope directly (SEM) or on photographic prints (LM, TEM). A stereological 168-point multipurpose test grid (Weibel, 1973) was used to estimate the relative structural densities in sections. Numerical data were evaluated with basic statistical methods (standard deviation, Student's t-test).

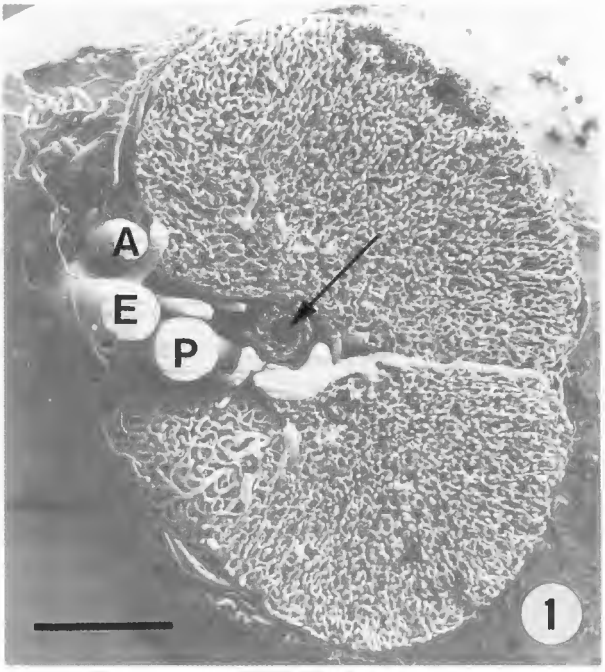
Results

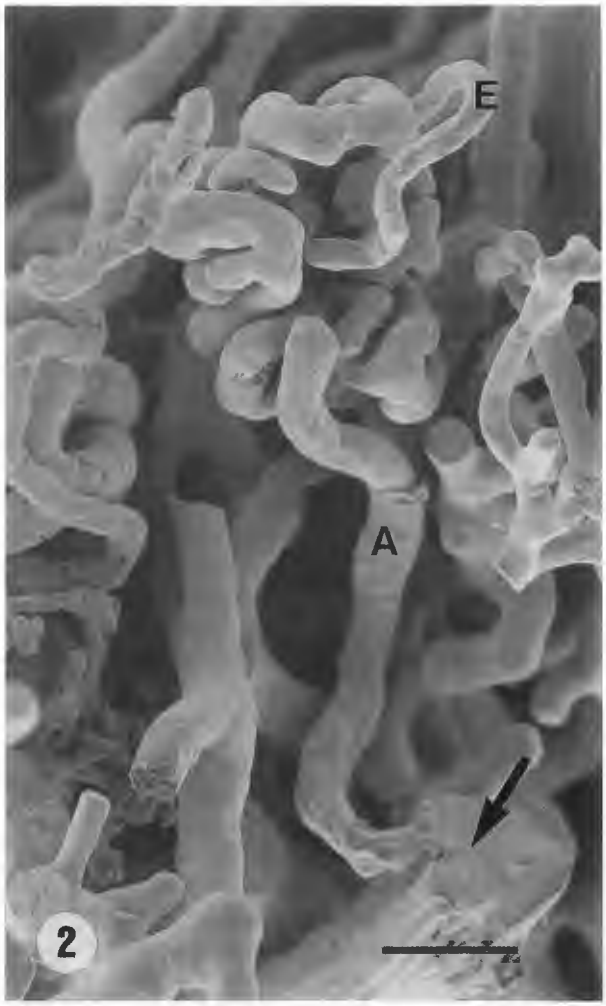
General anatomy

The kidney of snakes shows adaptations to the elongated body shape. One of the two kidneys, normally the right, is shifted cranially in relation to the other. The dorsal surface of the kidney shows pronounced sculpturing. These ripples represent the fan-like renal lobes that are roughly serially arranged.

Arterial supply is derived from a renal artery that branches from the aorta in a short distance anterior to the respective kidney. This artery branches further to supply the intralobular arteries.

A single, large renal efferent vein drains the blood from each kidney. It fuses shortly after the cranial end of the anterior kidney with the renal efferent vein from





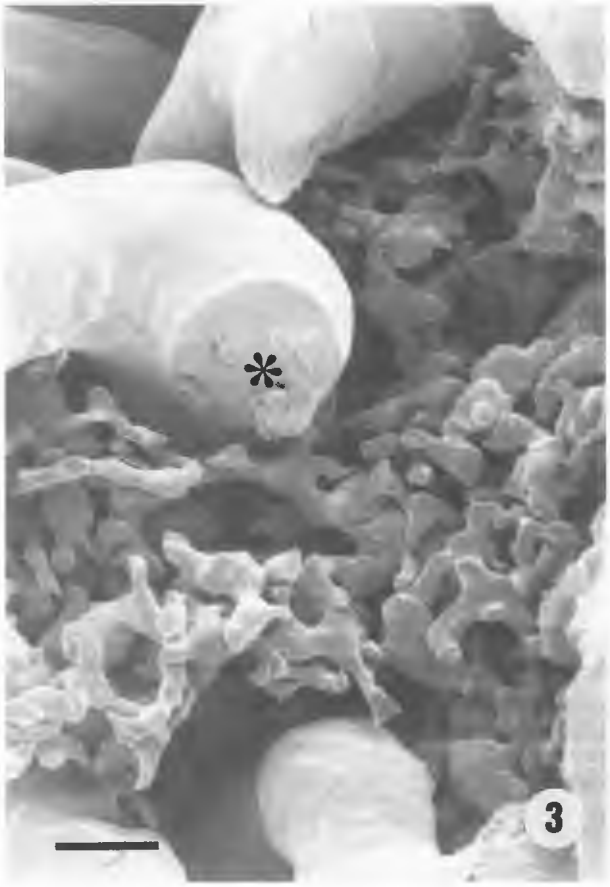


Figure 1. Transverse section of a cast left kidney of the anaconda (dorsal; right). Note the position of the ureter (arrow), the renal artery (A) and the portal (P) and efferent (E) veins. Bar = 1 mm.

Figure 2. Intralobular artery (arrow) of a Ball python giving rise to an afferent arteriole (A) of a glomerulus. E = efferent arteriole. Bar = 50 μm.

Figure 3. Fractured glomerulus (Ball python) showing intraglomerular lacuneous cast channels. The asterisk indicates fractured glomerular capillaries. Bar = 10 μm.

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the contralateral side, thus forming the V. cava posterior. The efferent renal vein runs on the ventral kidney surface parallel to the renal artery, medial to the renal portal vein (Fig. 1). The latter forms from a large branch of the caudal vein, contacting the kidney from the posterior end. The renal portal veins run cranially, thinning until they are nearly indiscernible at the cranial end of the kidney. Although, snakes lack legs and most of the associated structures, a larger tributary from lateral to the renal portal vein is present. This vessel may be homologized with the femoral or iliac vein and originates in the lateral musculature.

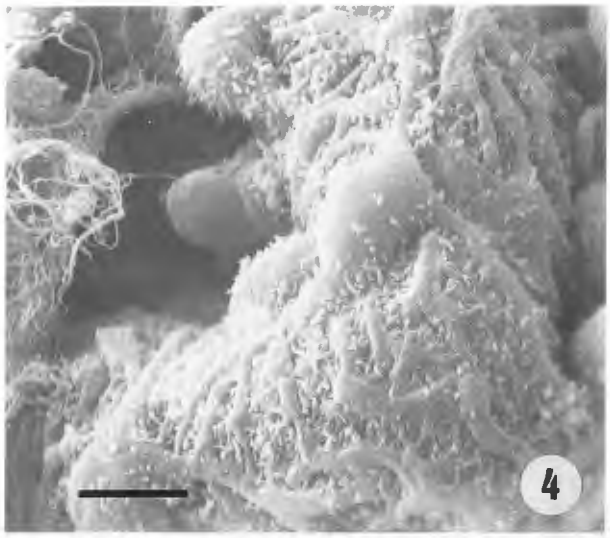
The ureter is also situated near the ventral surface of the kidney (Fig. 1). It is supplied by collecting ducts that distribute at the periphery of the lobule in a similar manner to the branches of the afferent veins.

Microscopical results

The serially arranged lobules show a very similar internal organization. The intralobular artery and the intralobular (efferent) vein run largely in parallel close to each other. The intrarenal artery gives off short afferent arterioles that supply the glomeruli (Fig. 2). Branching of the afferent arterioles has infrequently been observed. The glomerulus is formed by few, dichotomously branched capillary loops (Fig. 2). A dense lacuneous channel system can normally be found in the interior of the vascular tuft (Fig. 3). This network is probably the cast equivalent of the mesangial matrix channels. The latter allow access of the mesangial cells to macromolecular substances (e.g., antigen-antibody complexes) in the blood plasma. This lacuneous system was conspicuous in fractured glomerular casts. The glomerular core is about evenly filled with these channels; capillaries penetrating the interior of the glomeruli are hardly ever found. A single glomerular efferent arteriole connects with the peritubular capillary system after a short distance. Double efferent arterioles have not been found.

The glomerular visceral epithelium, as seen in CP dried preparations, shows numerous pericarya that frequently have microvilli-like projections at their surface (Fig. 4). The podocyte processes show branches of first and second order, however, branches of higher order are rare. The glomerular basement membrane is quite uniform in thickness (approximately, 0.2-0.4 μm) and shows an electron dense zone (Lamina densa) and a looser structured area (Lamina rara interna) below the capillary endothelium. However, a Lamina rara externa, i.e., a looser structured zone at the side of the podocytes seems minute or missing (Fig. 5). The capillary endothelium is of the flat, fenestrated type similar to the glomeruli of most vertebrates.

The parietal epithelium of Bowman's capsule is formed by flat, epitheloid cells. At the entrance of the renal tubule, these cells change to a higher prismatic type that is characterized by numerous cilia projecting into the tubular lumen (Fig. 6). This neck segment is continuous with the proximal tubule, that, in addition to numerous microvilli at the luminal surface shows frequently large intracytoplasmic droplets, probably of lipoid content (Fig. 7). The mostly cuboidal distal tubular cells show foldings of the lateral cellular membrane and variable intercellular spaces to the adjacent distal tubular cells. Frequently, numerous osmiophilic and empty vesicles are present at the luminal side (Fig. 8).



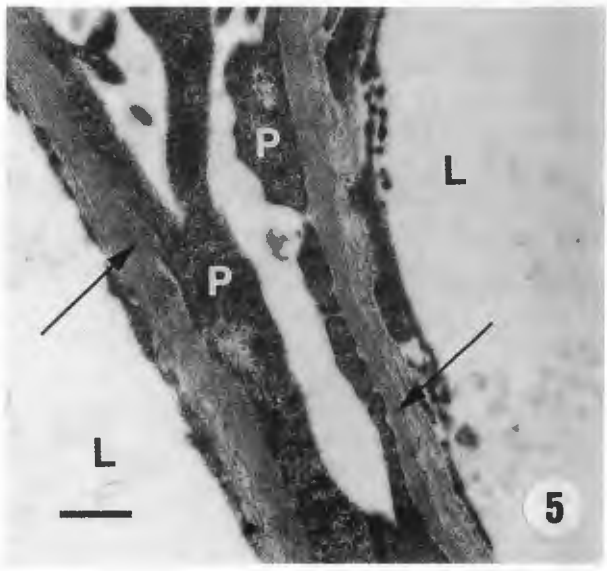
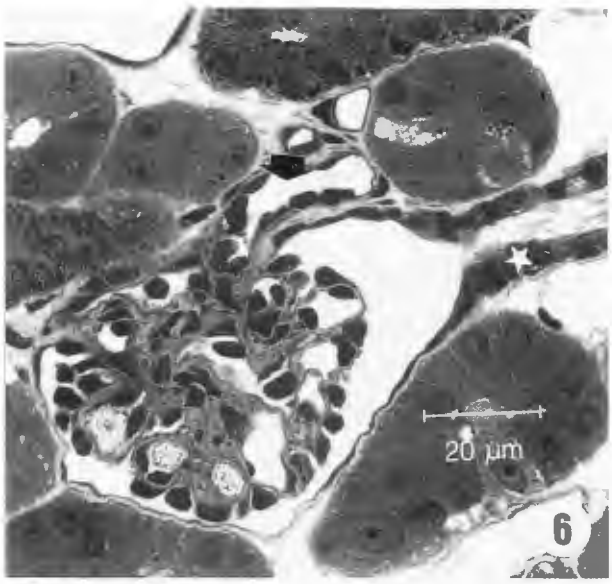


Figure 4. Critical point dried glomerulus of the Ball python. Note the irregularly distributed primary processes of the podocytes. Bar = 5 μm.

Figure 5. Transmission electron micrograph of two glomerular capillary walls from the anaconda. The podocyte processes rest on an apparently homogenous basal membrane (Lamina densa; arrows), while the fenestrated endothelium covers a looser structured zone (Lamina rara interna). L = capillary lumen; P = podocyte processes. Bar = 0.5 μm.

The architecture of the renal lobe of snakes facilitates the tracing of individual nephrons after tubular corrosion casting in the SEM, as the tubules are roughly arranged in parallel (Fig. 9). Generally, the nephron



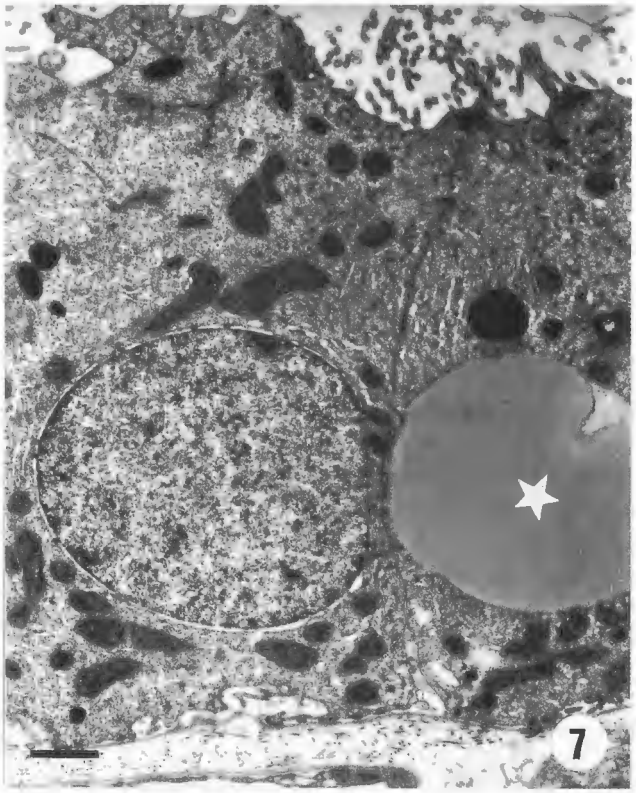


Figure 6. Semithin section (LM - 1 μm) of a Malphigian corpuscle of an anaconda. The epithelium of the Bowman's capsule is continuous with the ciliated neck segment (asterisk) of the nephron. Arrow indicates the efferent arteriole. Bar = 20 μm.

Figure 7. Transmission electron micrograph of a proximal tubular cell of the python. Note the microvilli covered luminal side, a large osmiophilic vacuole (asterisk) and numerous, irregularly shaped mitochondria. Bar = 1 μm.

Table 1. Comparison of glomerular diameters (μm), directly measured from corrosion casts in the SEM.

	Python	Anaconda
largest diameter		
average	135.6	68.2
standard deviation	± 13.5	± 9.6
n	21	20
smallest diameter		
average	113.0	50.5
standard deviation	± 16.2	± 7.7
n	21	25

begins near the center of the lobe and continues with some bends radially towards the periphery. Then, the nephron curves back with some turning loops towards the glomerulus of its origin. There, the tubule contacts the vascular pole of the glomerulus forming a juxtaglomerular apparatus and then moves again to the periphery of the lobe and connects to the other collecting ducts. Consequently, some parts of the nephrons, i.e., the distal part of the proximal tubule, intermediate segment, parts of the distal tubule and the collecting ducts are predominantly or exclusively supplied with venous (portal) blood.

Interspecific comparison

Although the microanatomy of the two investigated species is similar, there is considerable interspecific variation. In the Ball python, the glomeruli are comparatively large and well developed while they are significantly smaller ($p < 0.001$) in the anaconda (Table 1). As the glomeruli are ellipsoid in shape, the largest (a) and smallest (b) glomerular diameter of each glomerulus were measured and processed separately.

Estimating the filtration surface from these average values (Surface = $4ab\pi$; Volume = $4/3ab^2\pi$; glomeruli regarded as smooth ellipsoids) yields about a 4.5 times larger single glomerular filtering surface for the python compared to the anaconda. However, using stereological methods on semithin sections, the relative proportion of glomeruli per kidney volume turned out to be quite similar in the two species (compare Table 2). Accordingly, there must be about twelve times more glomeruli, and consequently also nephrons per unit volume in the anaconda kidney. This results in a more than double filtration area per kidney volume in the anaconda compared to the python. Also, more renal blood vessels per unit volume of kidney tissue (excluding glomerular vessels) are present in the anaconda while the proportion

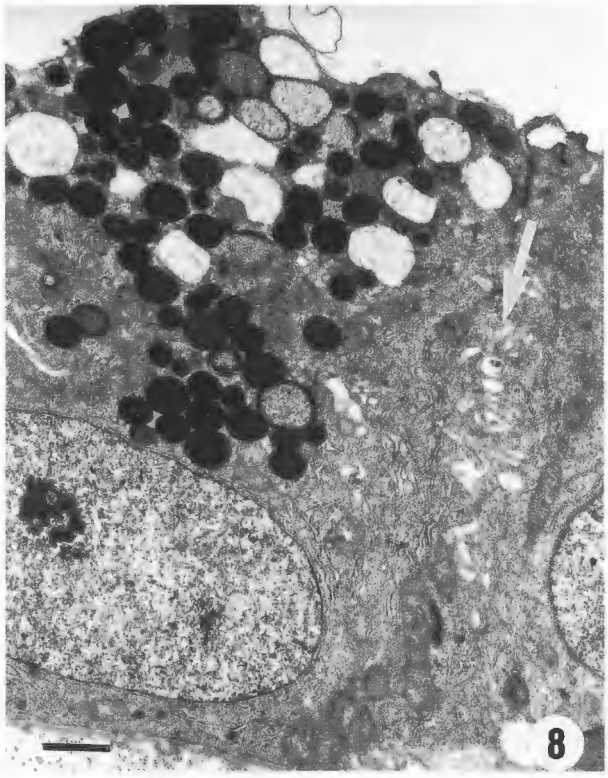


Figure 8. Transmission electron micrograph of a distal tubular cell of the python showing lateral membrane convolutions (arrow) and numerous vesicles at the luminal side. Bar = 1 μm.

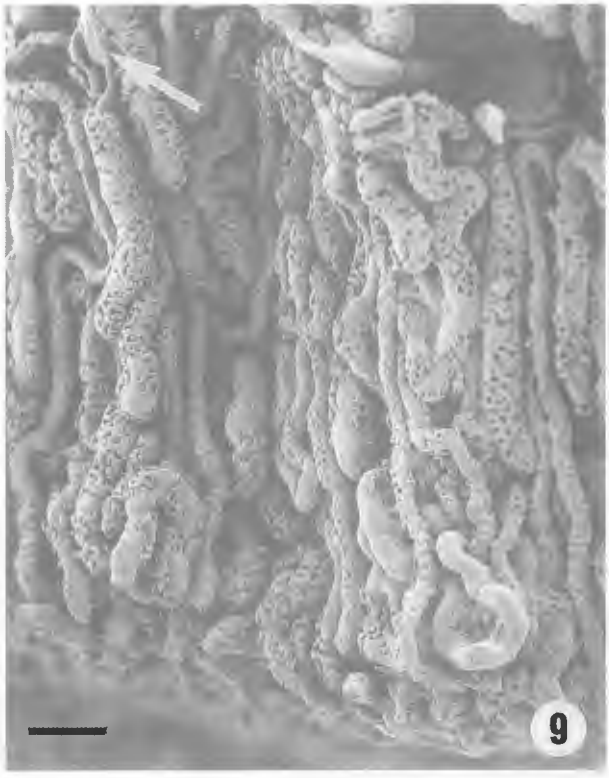


Figure 9. Tubular cast (Ball python) obtained from retrograde ureteric injection. The tubules are about parallel in arrangement starting near the center of the renal lobe (top) extending after some loops to the lobe's periphery (bottom). Arrow indicates the cast Bowman's capsule. Bar = 100 μm.

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of tubular elements is higher in the Ball python (Table 2).

Discussion

Due to the elongate form of the snake kidney, special problems of producing corrosion casts under "life-like" conditions (compare Ditrich and Splechtna, 1987b, 1990) had to be met. Choosing the heart as injection site, thus leaving the lower abdomen intact and maintaining a relatively low injection pressure, required the use of a low viscosity injection medium with delayed polymerization (see also Weiger *et al.*, 1986). Although strongly diluted injection resin may lead to casts of extraluminal structures (Castenholz *et al.*, 1982; Castenholz, 1983; Aharinejad and Böck, 1993, 1994), such surface structures (plastic sheets, plastic strips, endothelial rests, etc.) were normally absent in the casts used for this study.

Renal tubular casting was carried out by injecting from the ureter near to the cloaca. These casts allow the observation of the arrangement of the renal tubules within the single lobes of the kidney and their connection to the ureter (see also Boykin and Braun, 1993). However, no measurements were taken from these casts as the pertinent effects (completeness of filling, dilation or shrinkage) are not yet clear. Therefore, interpretations based on tubular casts remain critical (compare O'Shea *et al.*, 1993).

The anatomy, histology and several ultrastructural studies on snake kidneys are reviewed by Fox (1977). The anatomy and vascular supply of the two species investigated in this study generally corresponds to the pattern found normally in snakes. However, the strong lateral tributary to the portal venous system that might be homologous to the femoral or iliac vein seems more conspicuous in boid snakes than in other groups. Peek and McMillan (1979a,b) described in detail the ultrastructure of the kidney of the garter snake *Thamnophis sirtalis*. Several ultrastructural features like the structure of the glomerular podocytes and the mesangium seem quite similar comparing the garter snake and the two species investigated here. However, the glomeruli of the anaconda and the Ball python are more complex, as

Table 2. Comparison of renal components (vol%), measured from semithin sections with a stereologic test grid.

	Python	Anaconda
Renal Tubules*	61.55	51.65
Blood Vessels*	13.84	24.34
Interstitium	20.80	19.97
Capsular Lumen	1.46	0.99
Glomeruli	2.35	3.04

*Significantly different ($p < 0.001$).

glomeruli consisting of only one or two capillaries have not been found. Also, the structure of tubular components is quite similar to *T. sirtalis*. The proximal tubular cells of the latter also contain "dense bodies" (Peek and McMillan, 1979a), i.e., osmiophilic vesicles that may be secondary lysosomes. In the distal tubule, no apparent sexual dimorphism was found (compare Bishop, 1959). However, the animals used in this study were either juvenile or probably not in a reproductive phase. The lateral convolution (interdigitating lateral cellular membrane folds) described by Peek and McMillan (1979a) in the garter snake and by Schmidt-Nielsen and Davis (1968) in other reptiles were present, but not strongly developed in the two investigated species. Mucus secretion is commonly found in the renal tubule of uricotelic reptiles (e.g., Davis *et al.*, 1976) and may be required for the transport of uric acid precipitates. Thus, numerous apparently secretory cells can be found in the distal tubule and connecting ducts of the two snakes studied here.

A mathematical model of a glomerulus helps explain physiological data and allows the deduction of numerous rheological parameters. Due to its relatively simple morphology (Peek and McMillan, 1979b) such a model has been developed for the glomerulus of the garter snake (Papenfuss *et al.*, 1992). To obtain such a model for, e.g., mammalian glomeruli would be difficult because of the complicate angioarchitecture. In reptiles and birds, however, the pertinent mathematical models can be applied, avoiding many of the generalizations inherent in a more complicated system. Reconstructions of serial sections are less accurate due to shrinkage and compression by sectioning than analyses based on microcorrosion casts. Therefore, as detailed morphological data on the glomerulus are required to generate a valid model of the microcirculation, such measurements can probably be best obtained from microcorrosion casts.

The physiologic capacity of shifting the relative proportions of nitrogenous waste products (ammonia, urea, uric acid) in accord with the environmental parameters allows precise accommodation of a species (in some cases, also of an individual) to the water supply (Dantzler and Schmidt-Nielsen, 1966; King and Goldstein, 1985; Schmidt-Nielsen, 1988). This physiologic capacity is supplemented with corresponding development of the renal structures. The portal venous system of the sauropsidian kidney is of crucial importance in the adaptation to arid biotopes as it is the main pathway for uric acid excretion (Perschmann, 1956; see also Sykes, 1971). This nitrogenous waste product requires only little water for its removal and is mainly transported via proximal tubular secretion (Dantzler and Schmidt-Nielsen, 1966; Dantzler, 1982a; King and Goldstein, 1985). Glomerular filtration, on the other hand, is the main pathway for the removal of water from the body. However, single glomerular size was regarded as a key parameter for renal function in relation to the habitat's water supply (Marshall and Smith, 1930; Marshall, 1934). Instead, numerous studies indicate that regulation of the glomerular filtration rate plays a key role in renal water management (Dantzler and Schmidt-Nielsen, 1966; Dantzler, 1982b; Yokota *et al.*, 1985; Schmidt-Nielsen, 1988).

Caution is required when generalizing results derived from animals bred in captivity. However, disregarding detailed structural and physiological differences in the excretory system of the two species, it can be assumed that structures required for filtration and secretion of water (glomeruli, peritubular capillaries) are more developed in anacondas, while the renal tubules that are, among other functions, required for excretion of uric acid and resorptive processes, are relatively more developed in the Ball python.

From our previous studies (Ditrich and Splechtna, 1986, 1987a,b, 1990, 1992, 1994; Guo *et al.*, 1996) and from literature data (Bishop, 1959; Anderson, 1960; Davis *et al.*, 1976; Peek and McMillan, 1979b; O'Shea *et al.*, 1993; see also Fox, 1977) it may be concluded that non-mammalians predominantly coping with dry environments have comparatively larger, well vascularized glomeruli in a lower number per kidney volume. The tubular areas responsible for reabsorption as well as secretion of uric acid are well developed. Those animals that mainly have to remove water from their body fluids have comparatively smaller, but more glomeruli. Although obviously more studies are required to substantiate this hypothesis, comparative data on birds, turtles, lizards and also an urodelan amphibian (Ditrich and Splechtna, 1986, 1987a,b, 1992, 1994; Guo *et al.*, 1996) are in favor of this interpretation.

Several mechanisms (such as amnion, egg-shells, or strong epidermal keratinization) contribute to the capa-

bility of reptiles to inhabit a large variety of biotopes ranging from fresh water to extreme deserts and the sea. Comparisons of relatively closely related species may help in a better understanding of the pertinent adaptations involved.

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Discussion with Reviewers

A. Castenholz: In the **Discussion**, you point out that normally the corrosion casts used in your study do not possess any extraluminal structures possibly produced by diluted resin. In Figure 3, however, a lacuneous channel system is shown, which fills the glomerular core and is not supplied by blood capillaries. How can you explain this phenomenon, neglecting the fact that these structures are caused by an extravasation of resin?

M. Konerding: How can you be sure that these "lacuneous channels" are not artifacts?

Author: These structures appear constantly in the glomerular core in both of the species investigated here. It was also found in other reptiles (e.g., Ditrich and Splechtna, 1985, 1994). In other areas of the casts, similar structures were never found. The glomerular basement membrane is continuous with the mesangial matrix at the mesangial side of a glomerular capillary. Matrix gaps in this area allow blood-plasma and macromolecular substances to penetrate into the glomerular core (e.g., Jones *et al.*, 1962). Thus, it may be assumed that these structures are existing matrix channels filled with casting resin and not artifacts. However, it is true that these are extraluminal structures, as they are not lined with endothelial cells.

A. Castenholz: In the **Discussion**, a short remark was made with respect to the sexual dimorphism related to the distal renal tubules. In **Materials and Methods**, statements of the sexes of the animals of the species examined were not made. Did you evaluate the tissue specimens under the aspect of sexual differentiation? What kind of structural differentiation one can expect in adult snakes of both sexes?

Author: Male and female snakes were studied, however, as the species were juvenile, no sexual dimorphism was expected. The tubular differentiation in males was not seen in the LM or TEM preparations. Additionally, the reproductive cycle of tropical snakes probably could not have been simulated under the laboratory conditions used for keeping the animals. The cyclic secretory activity of parts of the distal tubules of males (sex segment, see Fox, 1977) is probably also more pronounced in species with a distinct seasonally influenced ethology like the garter snake.

A. Lametschwandtner: You mention that branching of the afferent (glomerular) arterioles was rare. Is it correct to conclude that the short afferent arterioles each supply one glomerulus only and that this holds for both species investigated?

Author: This is true in most of the observed cases. If branching of the glomerular afferent vessel is present, this leads to two independent glomeruli, thus double afferent glomerular vessels have not been found.

Reviewer I: The author mentions that only a Lamina rara interna was found in the basal lamina of the glomerular filtration membrane (no Lamina rara externa was present). Recent studies (for references, see Casotti and Braun, 1996) have shown that the three layers of the Lamina densa are the result of artifact due to rapid dehydration during processing of the tissue for electron microscopy. Hence, Figure 5 and the associated discus-

sion of this finding should be removed from the paper.
Author: There are indications that the trilaminar structure of the glomerular basement membrane (Lamina rara externa, Lamina densa and Lamina rara interna) may indeed be an artifact. Especially methods involving quick freezing of the tissue tend to support this conclusion (e.g., Reale and Luciano, 1990). However, these layers form reproducibly in all vertebrates (compare, e.g., Decker and Reale, 1991; Melman *et al.*, 1991) using conventional TEM processing. As this problem seems still controversial in the literature and was not specifically targeted in the present study, only findings based on the applied methods are presented.

M. Konerding: Obviously there are many extravasates in Figure 9. How do you explain this high amount?
Reviewer I: The author should mention the structures surrounding the tubular lumen in his legend of the micrograph (Fig. 9). It seems almost as if the peritubular capillaries have been filled as well.
Author: The interpretation of tubular casts seems critical in this respect. However, when compared to reconstructions of serial sections and isolated tubular preparations, the general form of the casts seems equivalent. Measurements of length, thickness, etc., of tubular casts have not been attempted in this study due to a lack of experience.

Reviewer I: The use of juvenile specimens is a concern. What is the meaning of juveniles in this instance? Does the word juvenile refer to the fact that the animals were not sexually mature? If so, then were the animals old enough so that the kidneys were past the stage of further development?
Author: "Juvenile" is used in the sense of sexually immature. As snakes (and other reptiles) grow permanently, developing glomeruli can be expected for their entire life, although in a diminishing quantity. Thus, it can only be assumed that the majority of the glomeruli measured in this study were in a fully developed state, and a small proportion was still growing.

Reviewer I: The author states that "Corrosion cast preparations were validated with light microscopy for completeness of filling..." By this, I presume that the author compared measurements between the corrosion cast preparations with that from the light histology to ensure complete filling of the corrosion casts.
Author: The casts were inspected in a (stereo-) light microscope in order to identify crude artifacts like extravasates or incompleteness of filling in order to exclude such preparations before SEM. Details like round ended vessels or microdroplet-like extravasates, however, can be only identified in SEM.

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