Histomorphometric Studies of the Urinary Tubules of the African Grasscutter (*Thryonomys swinderianus*)

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Abstract

The study was carried out to elucidate the luminal diameters of some segments of the urinary tubules in the grasscutter (*Thryonomys swinderianus*). Seven adult, apparently, healthy grasscutters were used in this study. They were anaesthesized with chloroform, dissected and the kidneys removed. The kidneys were fixed and processed by routine histological procedures. The various segments of the nephron were identified by their cell types and their diameters were measured. The data obtained were subjected to Pearson's correlation analysis. The diameters of the Bowman's capsule and glomerulus were 11.24 ± 0.460 and 9.21 ± 0.347 µm, respectively. There was a very strong positive correlation between the diameters of the Bowman's capsule and those of the glomerulus ($r = 0.975$, $P < 0.01$). A significant positive correlation was also observed between the distal convoluted tubule and the thin segment of the loop of Henle ($r = 0.786$, $P < 0.05$), the collecting duct and the proximal convoluted tubule ($r = 0.781$, $P < 0.05$), and between the collecting duct and the thin segment of the loop of Henle ($r = 0.821$, $P < 0.05$). This study has attempted to present base-line data of the diameters of the various segments of the nephron.

Keywords

Histomorphometry, kidney, Urinary tubules, grasscutter

Introduction

The African grasscutter (*Thryonomys swinderianus*), also known as the greater cane rat and cutting grass, is the second largest rodent after the porcupine. It is found only in Africa (Adoun 1993) and it is a very good source of high-quality animal protein. Unfortunately, the animal has been aggressively hunted over the years, resulting in a depletion of the species. Commercial production of grasscutters as micro-livestock (National Research Council, 1991) and as laboratory animals (Asibey and Addo 2000) may be beneficial in conserving the species in countries where it is irrationally overhunted. The domestication of the rat has been with varying degrees of success (Jori *et al.,* 1995). Various investigations have been carried out on the grasscutter all in an attempt to exhaustively understand the biology of the animal. There is no doubt that it is only after extensive research on the special features of the biology of the rat that explanations to questions that may arise in the course of domestication and breeding of the grasscutter may be provided.

The primary function of the kidneys is to maintain proper balance of water and minerals (including electrolytes) in the body (Charmi *et al.*, 2009). Additional functions include filtration and excretion of waste products from the processing of food, drugs, and harmful substances (toxins); regulation of blood pressure; and secretion of certain hormones (Cutler, 2006; Onyeanusi *et al.*, 2007). All of these functions contribute immensely to the maintenance of homeostasis. Certain features in the renal anatomy of different mammals and variations with the aridity of their habitat have been reported (Sperber, 1944). Studies have shown that animals from dry arid areas have a relatively well-developed renal medulla, which reflects their high capacity for reducing excretory water loss (Schmidt and O'Dell, 1961) as the length of the kidney medulla is suggestive of the length of the loop of Henle. Furthermore, Warui (1989) reported that the quantitative structural characteristics of the kidneys of mammals and birds may reflect the influence of environmental as well as many other factors on renal function. Although, the urinary tubules are the fundamental units of the organ structure, little is known about the mechanisms governing the size of the tubules (Germino, 2005). However, a good knowledge of their sizes is important as the morphology and function of the kidney have been modified through evolution to fulfil different physiological requirements (Charmi *et al.*, 2009).

Baylis and Corman, (1998) reported that the rat provides a useful experimental model for the study of the mechanisms of renal ageing. Again, the first step to this, is studying the normal structures in their peculiarities and dimensions. There is a dearth of information on the microscopic quantitative characteristics of the renal structure of the African grasscutter.

The specific aim of this study was to obtain quantitative data on some metric proportions of the main structural component of the kidney of the African grasscutter (*Thryonimys swinderianus*).

Materials and Methods

Animal and tissue preservation

Seven adult grasscutters (*Thryonomys swinderianus*) were obtained from local hunters in Otukpo (6º 49´ North, 8º 40´ East) Benue State, Nigeria. They had a weight range of 1.5 to 3.5 kg. The rats were transported in a motor car to the Anatomy Research Laboratory of the Department of Veterinary Anatomy, Ahmadu Bello University, Zaria, using standard laboratory grasscutter cages. They were given access to fresh elephant grass (*Pennisetum purpureum*) and water *ad libitum*. The animals were anaesthesized with gaseous chloroform in a closed container, after which a simple exploratory laparatomy procedure was done to remove the kidneys. The whole kidneys were preserved in 10 % buffered neutral-formalin in the ratio of one part of tissue to ten parts of formalin to achieve adequate fixation. The kidneys were left in the fixative for 24 hours.

Tissue processing and staining

Each kidney was cut in the saggital plane. Tissue processing was done according to the method described by Gordon (1990), as outlined below:

The fixed tissues were dehydrated in an ascending series of graded concentrations of alcohol (70 %, 80 %, 95 % and 100 %) with a time interval of 1 hour for each stage of dehydration. The tissues were then cleared with xylene for about 2 hours, infiltrated with molten paraffin wax at 50 ºC for 2 hours and embedded in paraffin blocks. Sections of 5 µm thick were then made using a microtome (Model 42339, Berlin, Germany), mounted on glass slides in the presence of egg albumin, deparafinized and dried at room temperature (25 ºC). They were stained with hematoxylin and eosin (H & E), dehydrated, and coverslipped using Permount as the mounting medium and viewed under a light microscope (Olympus® CH20) at the magnification of x 400.

Measurements

The diameters of each kidney segments considered were measured using an ocular micrometer (Leitz Wetzlar®, Germany) following a calibration of the microscope with a stage micrometer (Graticules Ltd., London) as described by Reid (1968). The various histologic parts of the kidney were recognised based on functional histology (Young *et al.*, 2006). Bowman's capsules that were considered for measurement were those with conspicuous vascular poles. This was done to reduce the risk of measuring sections that were not in the middle of the Bowman's capsule, and consequently, improving the accuracy of the results. For each animal, the Bowman's capsules, Bowman's spaces, glomeruli, proximal convoluted tubules (PCT), thin segment of the loop of Henle (LOH), distal convoluted tubules (DCT) and collecting ducts (CD) were observed.

Results and Discussion:

The mean (± SEM) body weight and kidney weights for the animals are shown in Table 1. The weights of the rats ranged between 1.5 – 3.5 kg, and the weights of the kidneys ranged between 3.41 – 6.34 g.

The PCT was lined with simple low cuboidal epithelim that possessed conspicuous 'brush borders' (Figure 1). The thin segment of the LOH was lined by simple squamous epithelium (Figure 2) which was obvious under the microscope. The DCT was also lined by simple cuboidal epithelium (Figure 1), although, the 'brush borders' of the latter were absent and their lumina were more clearly defined.

The lumina of the DCT were larger than those of the PCT. The ranges for the diameters of the Bowman's capsule and glomerulus were 9.12 – 13.02 and 7.71 – 10.64 µm, respectively, while those of the PCT, LOH, DCT and CD were 2.71 – 3.47; 1.79 – 2.26; 3.09 -3.85 and 3.85 – 5.16 µm, respectively. The individual mean (± SEM) values are shown in Table 2. There was a very strong positive correlation between the diameters of the Bowman's capsule and those of glomerulus ($r = 0.975$, $P < 0.01$). Also, significant relationships were observed between the diameters of the CD and the PCT ($r = 0.781$, $P < 0.05$); the CD and the thin segment of the LOH ($r = 0.821$, $P < 0.05$).

From a physiological perspective, Du *et al.* (2004) reported that measurements of the luminal diameter are critical to assessing changes in hydrodynamic forces and torques. Therefore, a knowledge of the diameters of the tubules is useful in determining the rate of fluid flow through the urinary tubules. The larger sizes in the diameters of the

DCT and CD suggests that urine flows more slowly in the larger tubules (Endo and Kimura, 2005). However, the finding in this study is contrary to the observation of Bentley *et al.* (2007), who observed that the luminal diameter of the PCT was greater than those of the DCT and the CD in rats. The majority of clinical references concentrate on the functional evaluation of kidneys (Lauschova *et al.,* 2004). This may, however, be inadequate in thorough studies as functional aberrations are often accompanied by structural changes. Also, Damme and Koudstaal (1976) reported that in some kidney diseases it is important to know the diameter of the glomerulus. This is further buttressed by the work of Roman *et al.* (2004), who noted a significant increase in the diameters of the proximal tubules, distal tubules and collecting ducts of rats treated with cadmium. Normal kidneys are nearly always recognisable in survey radiography. However, an assessment of the histology of the renal pelvis and the ureter is not possible by radiography (SeyrekIntas and Kramer, 2008). Hence, the relevance of histological studies.
The method of using the ocular micrometer and a formalin fixed tissue section may hamper the accuracy of the measurements due to some level of shrinkage that occur during tissue processing with formalin (Baker, 1960). However, bearing in mind that the level of shrinkage in formalin fixed and paraffin embedded tissues is about 33.3 % (Baker, 1960), a working estimate may be deduced easily to reflect this tissue alteration.

The results have for the first time provided data on the tubular diameters of the various segments of the urinary tubule of the grasscutter.

Conclusion

In conclusion, this study has attempted to generate base-line data on the diameters of the various parts of the nephron. This may be useful as a relative control when studying the effect of certain drugs or toxins on the sizes of the various segments of the urinary tubules. However, further anatomic studies involving the use of more advanced equipment and methods like electron microscopy, radiology, ultrasonography, and, if necessary, computer tomography (CT) or magnetic resonance imaging are required for comprehensive elucidation of the kidneys in the grasscutter.

Table 1: Mean body weight and kidney weight (n = 7).

Variables (g)	Mean ± SEM
Body weight	2571.4 ± 0.283
Kidney weight	4.67 ± 0.400

Table 2: Mean diameters of the urinary tubule segments measured (n = 7).

Variables (μm)	Mean ± SEM	Minimum	Maximum
Bowman's capsule	11.244 ± 0.460	9.12	13.02
Glomerulus	9.213 ± 0.347	7.71	10.64
PCT	3.119 ± 0.106	2.71	3.47
LOH	1.921 ± 0.058	1.79	2.26
DCT	3.459 ±0.090	3.09	3.85
CD	4.356 ± 0.171	3.85	5.16

PCT- Proximal convoluted tubule, LOH- Loop of Henle, DCT- Distal convoluted tubule and CD- Collecting duct.

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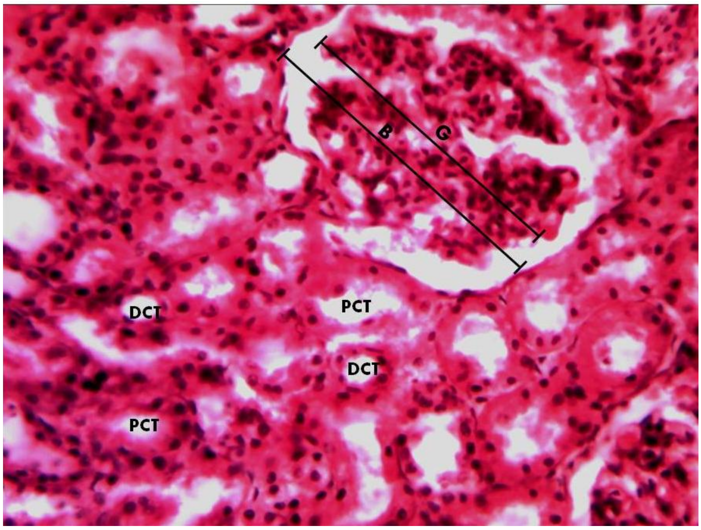

Fig (1): A section through the cortex of the kidney of the grasscutter (*Thryonomys swinderianus*) showing the glomerulus (G), the Bowman's capsule (B), the proximal convoluted tubule (PCT) and the distal convoluted tubule (DCT). (H & E, x 400)

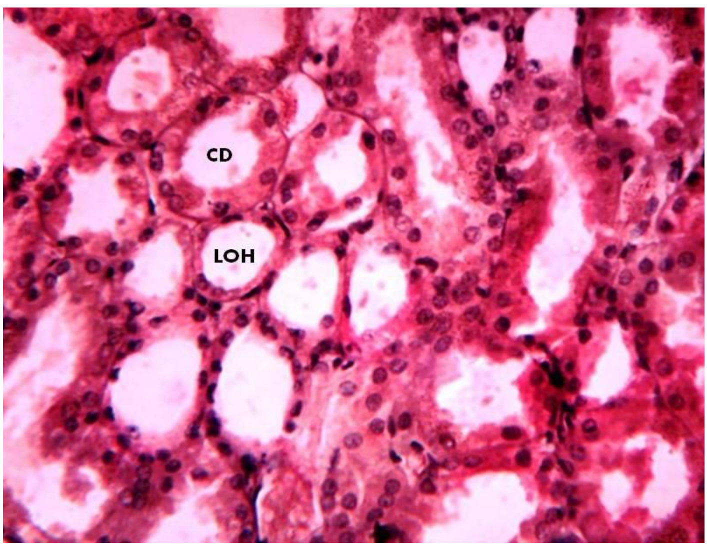

Fig (2): A section through the medulla of the kidney showing the collecting ducts (CD) and the thin segments of the loop of Henle (LOH). (H & E, x 400)