UNIVERSITI PUTRA MALAYSIA

THE NEPHRON OF SWIFTLET - AN ULTRASTRUCTURAL STUDY

NUR LIYANA BINTI LOKHMAN HAKIM

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CERTIFICATION

I hereby certify that I have read this project paper entitled "The Nephron of Swiftlet- An Ultrastructural Study", by Nur Liyana Binti Lokhman Hakim and in my opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999-Final Year Project.

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DEDICATION

I wish to dedicate this Final Year Project paper to

My father, Lokhman Hakim Bin Harun

My mother, Noorihan Binti Abdul Rahman

For their passion and compassion in nurturing

My supervisor

My family

My friends

Thank you for your continuous support.

May this will be your inspiration and motivation for your future endeavours

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I would like to express my deepest gratitude to my supervisor YBhg Dato' Dr. Tengku Azmi Bin Tengku Ibrahim for his guidance and suggestion in improving the report, invaluable interpretation of the electron micrographs, time and support throughout the research. His guidance had contributed to the completion of this research project.

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LIST OF ABBREVIATION

EBN	Edible bird nest
H&E	Hematoxylin and eosin
Cd	Collecting duct
Rc	Renal corpuscle

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999- Projek Tahun Akhir.

NEFRON BURUNG WALIT- KAJIAN ULTRASTRUKTUR

Oleh:

NUR LIYANA BINTI LOKHMAN HAKIM

Supervisor: Dato' Dr.TengkuAzmi Bin Tengku Ibrahim

Telah dilaporkan bahawa struktur organisasi nefron burung walit menyerupai nefron reptilia yang tidak "bergelung" – dengan ketiadaan anggota menurun dan menaik lengkungan Henle. Tanpa bergelung menjadikan korteks lebih luas dan di penuhi oleh tubul proksima. Hipotesis kajian ini adalah burung walit mempunyai nefron yang mudah daripada segi struktur tetapi efisient dari segi fungsi. Dengan yang demikian kajian ini meneliti morfologi nefron burung walit di tahap ultrastruktur dan kearah tujuan ini sampel buah pinggang burung walit yang diperolehi telah di proses untuk mikroskopi electron transmisi. Terdapat banyak mikrovilus dalam sitoplasma sel epitelium tubul proksima, insidens mitokondria yang tinggi disamping indentasi dan lipatan ke dalam membrane bes sel tubul proksima yang luas yang menunjukkan kecekapan tinggi serapan semula filtrat glomerulus dan penyalurannya ke lumina kapilari peritubul. Kecekapan nefron dipertingkatkan seterusnya oleh tubul segmen perantaraan yang juga mempunyai struktur yang sama seperti sel epitelium tubul

proksima – mikrovili, mitokondria dan indentasi bes dan lipatan kedalam membrane sel – yang menunjukkan masih lagi berlaku penyerapan filtrat glomerulus di bahagian tubul nefron ini. Tubul lingkaran distal dan tubul pengumpul juga menunjukkan bukti dari segi struktur penyerapan semula dan penyaluran filtrat glomerulus ke dalam kapilari peritubul. Cerapan menarik dalam kajian ini adalah terdapatnya dengan begitu banyak titisan lipid dalam lumina nefron. Mikrovilus mempunyai permukaan hujung yang luas berkemungkin terbentuk khas bagi penyerapan lipid dalam segmen perantaraan nefron. Dengan ini kesimpulan yang boleh diambil daripada kajian ini ialah nefron burung walit adalah mudah dari segi struktur kerana tidak mempunyai anggota menurun dan menaik lengkungan Henle tetapi mempunyai kecekapan yang tinggi mengambil kira penyerapan semula filtrat glomerulus berlaku di sepanjang nefron.

Kata kunci: *burung walit, ketiadaan lengkungan, nefron reptilia, segmen perantaraan, titisan lipid*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999- Final year Project.

THE NEPHRON OF SWIFTLETS - AN ULTRASTRUCTURAL STUDY

By:

NUR LIYANA BINTI LOKHMAN HAKIM

Supervisor: Dato' Dr. Tengku Azmi Bin Tengku Ibrahim

It has been reported that the structural organization of the swiftlet nephron resembled that of the reptilian which was "loopless" – absence of the descending and ascending limbs of the loop of Henle. Being loopless and with a large cortex occupied mainly by the proximal tubules it is hypothesized that the swiftlet's nephron is structurally simple but functionally efficient. Accordingly the present study examined the morphology of the swiftlet's nephron at the ultrastructural level and towards this objective samples of the swiftlet's kidney collected were processed for transmission electron microscopy. Numerous microvilli, a high incidence of mitochondria and an extensive indentation and infolding of the basal epithelium of the proximal tubule indicated enhanced reabsorption of glomerular filtrate and its release into peritubular capillary. Efficiency of the nephron is further augmented by the tubules of the intermediate segment which also showed similar structural evidences – microvilli, mitochondria and basal indentations and infoldings - indicating there was still considerable reabsorption of glomerular filtrate in this part of the nephric tubules. The distal convoluted tubules and collecting ducts also showed evidences of

reabsorption and release of glomerular filtrate into peritubular capillary. An interesting observation in this study was the presence of many lipid droplets in the lumen of the nephron. Microvilli with dilated apical ends could be specially designed for the absorption of lipids in the intermediate segment. Thus the nephron of the swiftlet is simple as it is loopless but highly efficient as reabsorption take place throughout the entire length of the nephron.

Keywords*: swiftlet, loopless. Reptilian nephron, intermediate segment, lipid droplet*

1.0 INTRODUCTION

In Malaysia, the nests of *Aerodramus fuciphagus* (white-nest swiftlet) and *Aerodramus maximus* (black-nest swiftlet) are harvested for commercial purposes (Looi Qi Hao *et al*., 2015). With a market price of about RM3000/kg the edible bird nest (EBN) is one of the most expensive animal products consumed by human (Rashid, 2015). Unlike other avian species, the swiftlet build their nests almost entirely from its salivary secretion (Ibrahim et al., 2009).

EBN is claimed to have many therapeutic properties (Syed and Norazlin, 2014) as it consists of the highly valued glycoprotein rich in amino acids, carbohydrate, calcium, sodium and potassium (Norhayati *et al.,* 2010). It also helps to promote one's immune system and enhances body metabolism. More recently, bird nests have been used as a component in cosmetic products (Zainab *et al*., 2013). More importantly, EBN is also known to contain the immuno-competent and antiviral properties (Norhayati et. al, 2010).

The swiftlet, from the time it leaves its nest at dawn to forage on insects in the upper strata of the atmosphere, is always in constant flight until dusk when it returns to its nest. During flight, for duration of 12 hours, there is very likelihood that it has limited access to water. Hence, it must have a urinary system which is highly efficient to conserve its body fluids.

1.1 OBJECTIVE

In a previous study (Raihan and Tengku Azmi, 2015), it was reported that the nephron of the swiftlet is loopless – absence of ascending and descending limbs of the loop of Henle. An ultrastructural study of the nephron would provide comprehensive information on the efficiency of the swiftlet kidney.

1.2 JUSTIFICATION

The present study is aimed at providing an understanding on the structural and functional relationship of the tubular parts of the swiftlet's nephron focusing on (proximal and distal convoluted tubule, intermediate segment and collecting duct). The functional significance of these parts of the nephron could be better understood when their morphologies are examined at the ultrastructural level

1.2 HYPOTHESIS

From the above objective it is hypothesized that the swiftlet's nephron is structurally simple but highly efficient in its function.

2.0 LITERATURE REVIEW

2.1 BEHAVIOR OF SWIFTLET

Swiftlets are a family of highly aerial birds. The family name, Apodidae, is derived from Greek, meaning "footless", referring to the small, weak legs of these most aerial of birds (Bourton, 2010). Thus, the swiftlet are always in constant flight from dawn when it leaves it nest to forage on insects in the upper strata of the atmosphere until dusk when it return to its nest (Fullard and James, 1993).

While on a constant flight of duration of about 12 hours, the swiftlet have limited access to water. The swiftlet drinks while on flight. It glides down from the sky and skim skillfully over the water surface, dipping their open beak and sip up a few drops. Hence, it has to have a very efficient urinary system in order to conserve the ion and water in the body (Casotti and Braun, 2000).

2.2 HISTOLOGY OF SWIFTLET KIDNEY

The first and only, study on the histology of the swiftlet's kidney was reported by Raihan and Tengku Azmi (2015). Under the light microscope, the kidney appeared lobulated. Each lobule comprised of a vast cortex and limited medulla.

Contrary to the proximal tubules which is highly convoluted in most animal and avian species the proximal tubules in the swiftlets which occupied the major part of the cortex were mainly straight tubule except at the peripheral cortex. The proximal straight tubule were arranged in parallel rows transversing the thickness of the cortex. Similarly unlike the mammalian or kidney of chicken where the renal

corpuscle are distributed throughout the cortex the renal corpuscle in swiftlet are arranged in a row above the cortico-medullary junction.

In the same study by Raihan and Tengku Azmi (2015) it was also reported that the descending and ascending limbs of the loop of Henle were absent in the nephron of the swiftlet; no tubules lined by simple squamous epithelium which normally line the descending and ascending limbs of the loop of Henle could be found in the medulla of the kidney of this avian species.

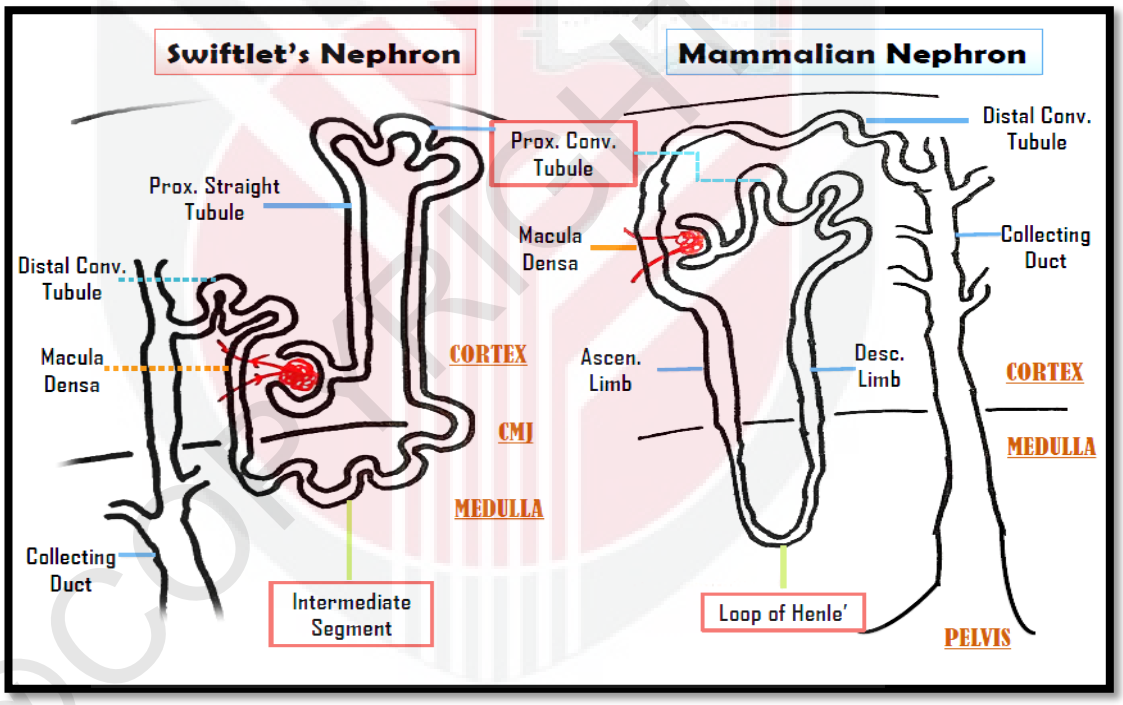

Figure 1 Structural organization of swiftlet nephron (left) and the mammalian nephron for comparison. The descending and ascending limbs of the loop of Henle are absent in the swiftlet kidney resembling that of the reptilian type

3.0 MATERIAL AND METHOD

Live swiftlet were obtained from the bird houses in Kluang district and in the town of Kota Bharu. Following Muslim slaughter, the kidney samples were fixed in 4% glutaraldehyde in 0.1M sodium caccodylate buffer for 16 – 18 hrs. The samples were then washed thrice in the caccodylate buffer and post fixed in 1% osmium tetroxide (OsO_4) for 1 hour.

Following post-fixation, the samples were dehydrated in graded series of concentrations of alcohol (30%, 50%, 70%, 80%, 90% and 100%) with 10 minutes interval for each concentration. Then, the samples were infiltrated in the acetone and resin (50%: 50%) mixture. The samples were then embedded in 100% resin and polymerized overnight. Ultrathin section ($1A^{o}$) was obtained using the ultramicrotome and stained with a saturated solution of uranyl acetate and lead citrate in 50% alcohol. Finally, the stained samples were examined under the transmission electron microscope (TEM).

4.0 RESULTS AND DISCUSSION

The proximal tubules in the nephron of the swiftlet were lined by simple cuboidal epithelium as evidenced by the position of their nuclei which are located in the centre of the epithelial cells (Fig. 2). At the apical surface of the epithelial cells were densely packed with microvilli (Fig. 3) while the basal cell membrane appeared in the form of extensive indentations and infoldings (Fig. 4). Numerous mitochondria were present in the cytoplasm of the epithelial cells and at the base of the epithelium were a number of peritubular capillaries. The densely packed microvilli and numerous mitochondria could be an indication of a highly active reabsorption of the glomerular filtrate while the extensive basal infoldings facilitate efficient release of the reabsorbed filtrate into the peritubular capillary.

An interesting observation in the lumina of the proximal tubules was the presence of a large number of lipid droplets. The high incidence of lipid droplets in the tubular nephron in all probability could be attributed to the feed of the swiftlet which is principally insects foraged in the upper strata of the atmosphere. Insects' body mass is known to contain a high percentage of lipids in some as high as 60% (Ronald, 2012).

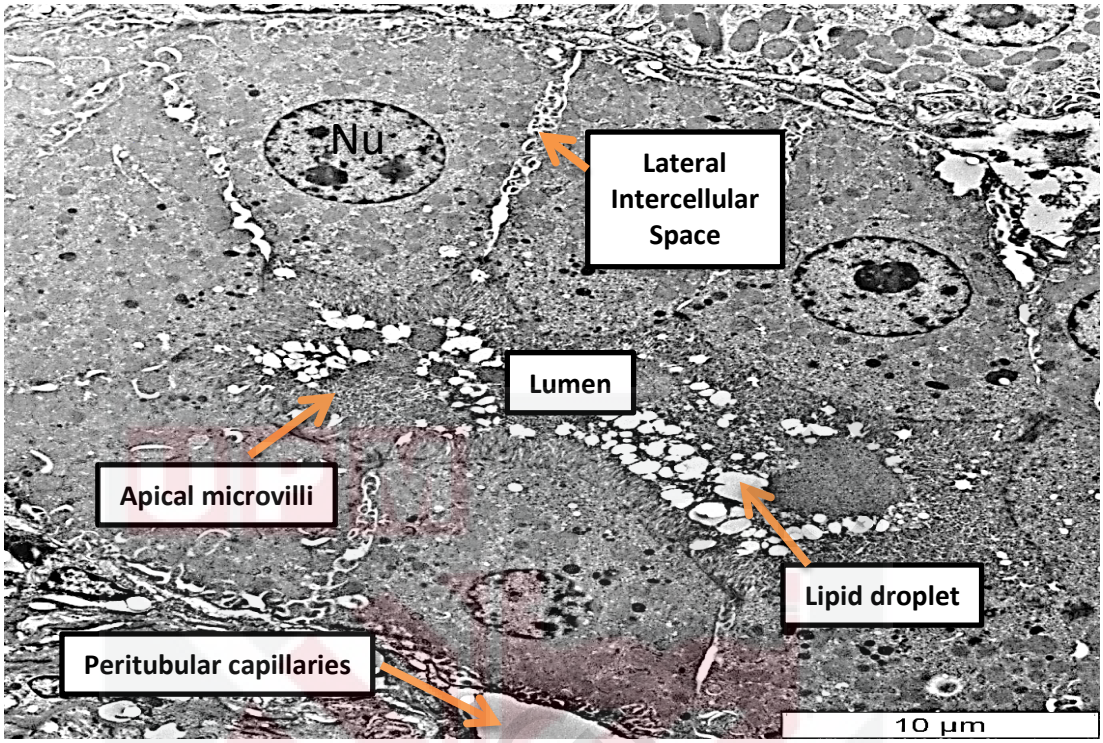

Figure 2 EM of proximal tubule lined by cuboidal epithelium cells. The proximal tubule reveals profuse tall microvilli constituting the brush border. The cytoplasm consists abundant of mitochondria. 3000x.

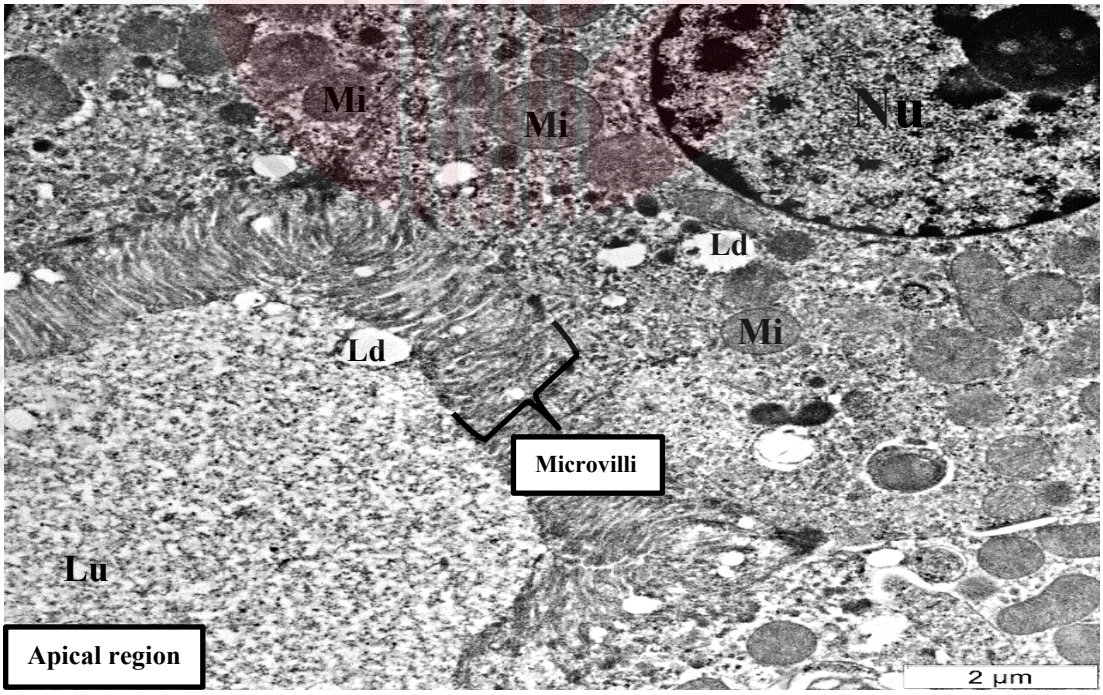

Figure 3 EM at the apical surface of proximal convoluted tubule. Microvilli at the apex of epithelial cell lining the proximal tubule with high incidence of mitochondria in supranuclear cytoplasm. Mitochondria (Mi), lipid droplet (Ld), nucleus (Nu), lumen (Lu). 10000x.

Figure 4. The basal part of the epithelial cell lining the proximal tubule. There is extensive indentation and infoldings of the basal plasma membrane. 8000x.

Raihan and Tengku Azmi (2015) had reported that the nephron of the swiftlet is the reptilian type being devoid of the ascending and descending limbs of the loop of Henle. Examined under the electron microscope the cuboidal epithelial cells lining epithelium of the intermediate segment were provided with microvilli, not as densely packed but provided with dilated ends, extensive basal membrane indentation and infoldings and numerous mitochondria in the epithelial cell cytoplasm (Fig. 5). Lipid droplets were present both in the tubular lumen and within the cytoplasm of epithelial cells. With the presence of lipid droplets in the lumen of the intermediate segment it is tempting to speculate that the microvilli with the dilated ends could be specifically designed for the absorption of lipid droplets (Fig. 6).

Figure 5. The cross section of the intermediate segment made up of cuboidal or columnar epithelium with presence of numerous microvilli. 7000x.

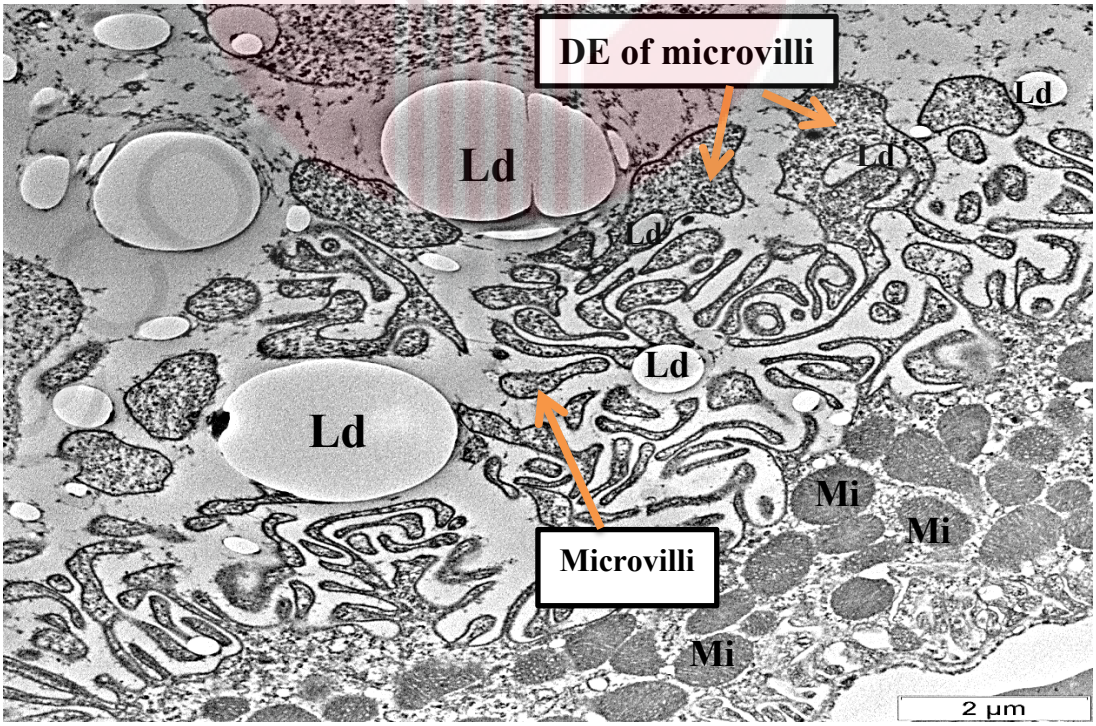

Figure 6.Higher magnification of the microvilli in the intermediate segment showing the dilated end (DE) at the microvilli.10000x.

In the distal convoluted tubules of the swiftlet nephron there was a marked reduction in the incidence of microvilli on the apical surface of epithelial cells (Fig. 7). However there was still a preponderance of mitochondria and basal infolding indicating to a certain degree there is still active reabsorption and release of substrate into peritubular vasculature in the distal convoluted tubule. Lipid droplets appear to persist in the tubular lumen and in the cytoplasm of epithelial cells.

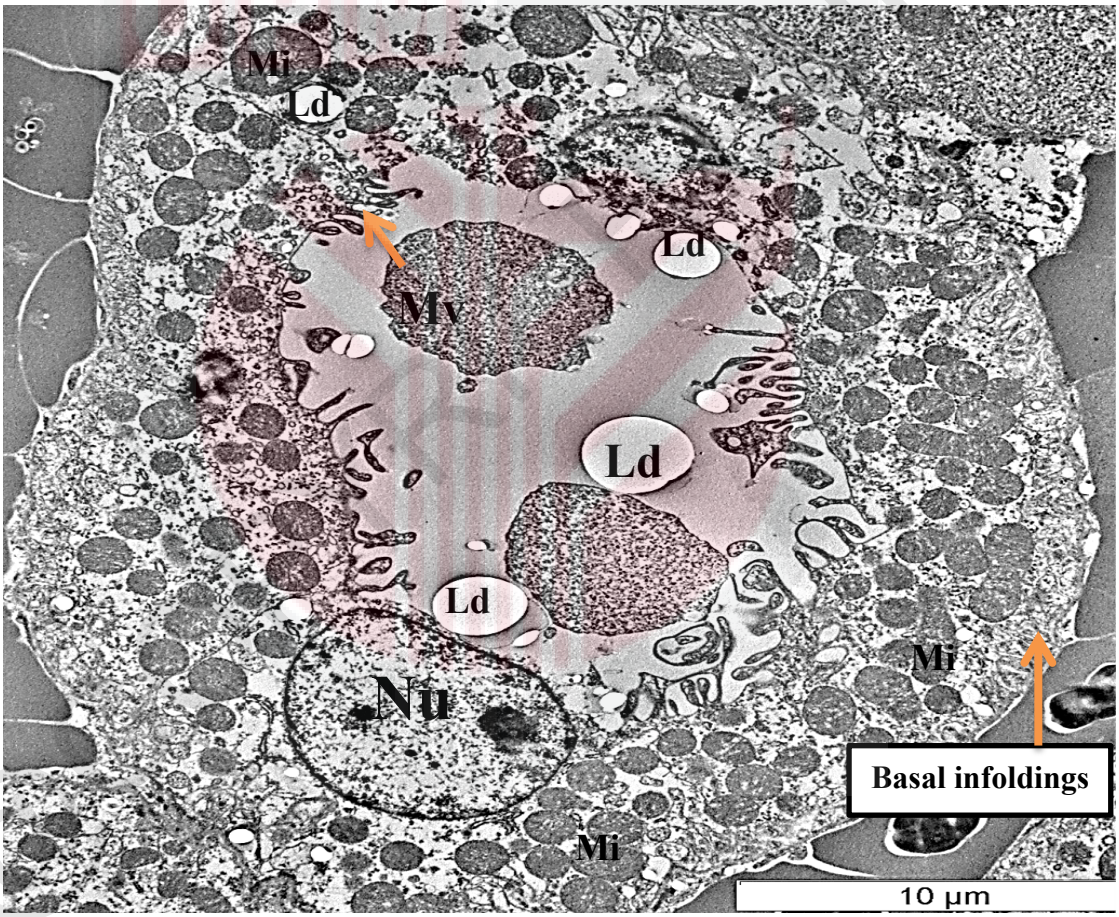

Figure 7 Cross section of the distal convoluted tubule made up of cuboidal epithelium with reduced presence of microvilli. Basal infoldings and large number of mitochondria are still present.4000x.

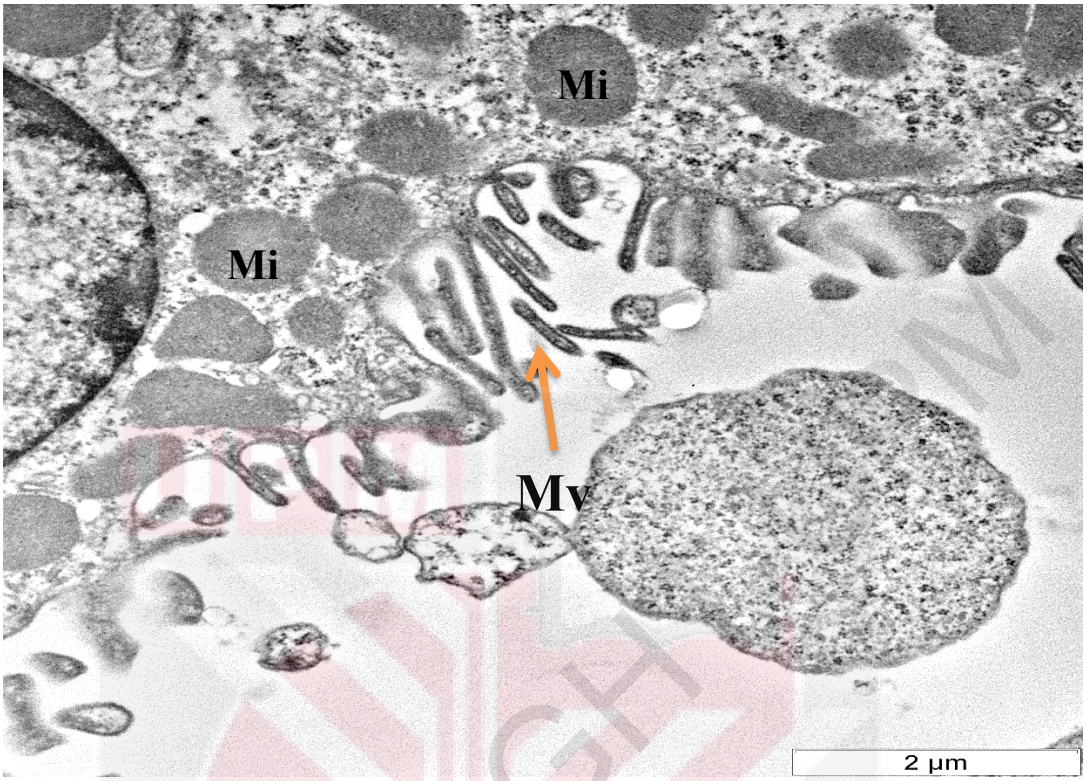

Figure 8. Showing the morphology of microvilli in the distal convoluted tubule which is different from that in the intermediate segment and proximal tubule. 15000x.

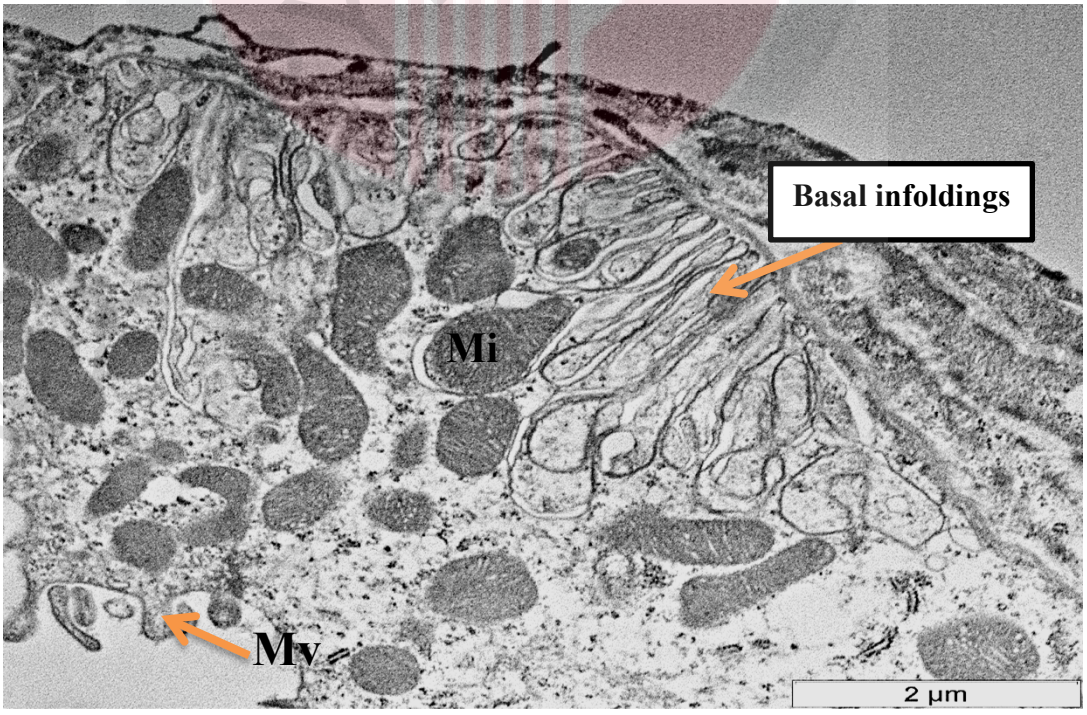

Figure 9. Showing the microvilli, numerous mitochondria and basal infoldings indicate that there are still active reabsorption of glomerular filtrate and release into peritubular capillaries in the distal convoluted tubule. 15000x.

With the exception of the presence of a large number of secretory granules in the supranuclear cytoplasm the ultrastructural features of the epithelium of the collecting duct of the swiftlet nephron resemble that of the distal convoluted tubule (Fig. 10 & Fig. 11). The functional significance of these granules is not known; however in reptilian species these granules are believed to secrete mucus to prevent the block of lumen by uric acid precipitation.

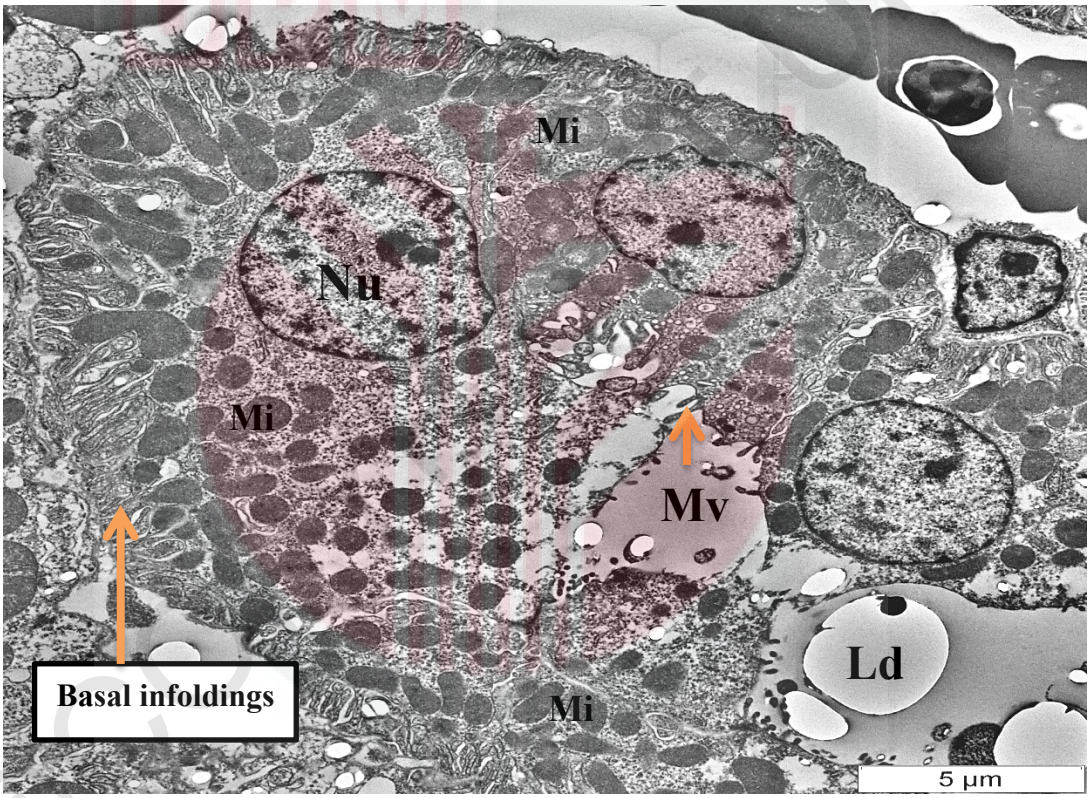

Figure 10 Cross section of the collecting duct made up of pyramidal epithelium. The apical surface of the collecting ducts are still provided with microvilli. 2500x.

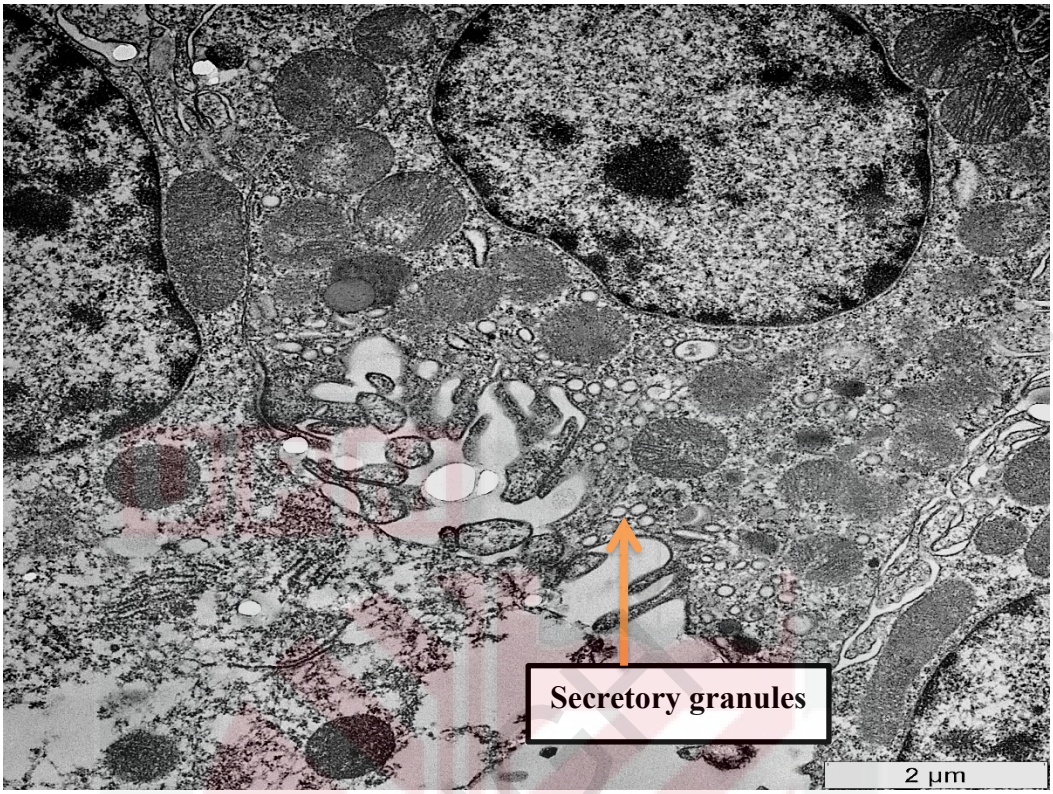

Figure 11. Higher magnification of the secretory granules in supranuclear cytoplasm of the epithelial cell of the collecting ducts.12000x

This present study hypothesized that swiftlet's nephron is simple yet efficient. Simple as it is loopless resembling that of the reptilian nephron in which there is absence of the descending limb and ascending limb of the loop of Henle. Being loopless, the entire lobule is principally made up of the cortex which is occupied mostly by the proximal tubule. The proximal tubules in the cortex are mainly straight tubules except at the peripheral cortex which are convoluted (Raihan and Tengku Azmi, 2015). The proximal straight tubules thus provide a very massive surface area for the reabsorption of the glomerular filtrate.

Besides, the efficiency of the swiftlet's nephron is further enhanced by the presence of the microvilli. The microvilli will provide a very extensive surface area for the reabsorption of the glomerular filtrate from the tubular lumen into the cell

cytoplasm. Microvilli were present not only in the proximal tubules but also in the intermediate segment, distal convoluted tubules and to a limited extend in the collecting ducts.

An interesting observation in this study is by the presence of the lipid droplets in the lumina of the proximal tubule, intermediate tubule, distal convoluted tubule and the collecting duct. The presence of lipid droplets in the nephron could be related to the feed of the swiftlet which are principally insects. The swiftlet forage in insect midair such as fruit fly, dragonfly, flying ants, bees and mosquitoes. Fruit fly and dragonfly contain approximately 60-70% lipid in their body mass (Ronald, 2012). However, at this juncture it is not possible to provide a rational explanation as to how the lipid could reach the kidney under normal physiological conditions, the lipids absorb by the lacteal in the small intestine would have been metabolized in the liver.

5.0 CONCLUSION

From the present study the following conclusion could be drawn:

1. The nephron of the swiftlet is simple in structure but highly efficient being provided with a very extensive area for reabsorption of the glomerular filtrate from the proximal tubule to the collecting duct.
2. The nephron of the swiftlet is also involved in the reabsorption of lipid in the glomerular filtrate.

For the future, we would recommend to undertake a study on the ultrastructure of the renal corpuscle to be undertaken to complete the study on the nephron in the kidney of the swiftlet. The renal corpuscle comprise of the structure of the glomerulus and juxta glomerulus apparatus (JGA) which in turn comprise of the juxta glomerular cells, the cells of the macula densa and the lacis cells. Hence, it requires a separate study as it will be too voluminous to be included in this present investigation.

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