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Ultrastructure of Kidney in the Freshwater Fish *Tilapia mossambica (Peters)*

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Abstract:

The kidney of fish develops from the pronephros and mesonephros. Besides filtering wastes from the blood, the basic function of kidney is osmoregulation Fish kidney possesses structure of a typical vertebrate. In sections of kidney, glomeruli with Bowman's capsule, neck region, proximal and distal ends of a uniferous tubule, collecting tubules are distinguishable. Sections of neck region show circular configuration, small diameter and small tubular lumen. They are characterized by a large number of cilia. Collecting tubules are differentiated by their large size and irregular oval shape (often curved) outline. Presence of a ciliated brush border over the inner margin of the epithelial cells lining the proximal ends are seen whereas the epithelial cells lining the distal ends of the tubules show cilia but brush border is not distinct. Diameter of the lumen is bigger at the distal ends. Since light microscopy studies often fail to establish ultrastructural details, this study was undertaken to establish the various region of the uriniferous tubules in Tilapia mossambica (Peters).

Key words: *Tilapia mossambica,* kidney, uriniferous tubule, ultrastructure

Introduction:

Fishes like most aquatic animals release nitrogenous wastes as ammonia. Some are filtered by the kidneys. The head kidney of fish is peculiar since it is also known to possess endocrine, haematopoietic and lymphatic tissue besides the excretory tissue (Roberts 1989). The head kidney in teleost is also known to be a major antibody producing and antigen trapping organ (Chiller *et al.*,., 1969, Rijker *et al.,* 1981, Kaattari & Irwin, 1985; Lamers *et al.,* 1985). Although, the ultrastructure of the teleost head kidney has been investigated by some authors, they have focused on the morphological features of the renal tubules (Anderson *et al.,* 1975; Brown 1985, Tytler, 1988); endocrine cells (Youson 1976, Yoakin *et al.,* 1980) or haemapoietic cells (Zuasti *et al.,* 1988, 1989; Meseguer *et al.,* 1990). Ultrastructural features have also been reported to differ in different species (Bulger Ruth Ellen *et al.,* 1968). These features vary not only with the species but also with the habits and habitats of the animal. These features have also been reported to vary with the sex of the animal as has been observed in mature male Stickleback, *Gasterosteus aculeatus* (Hickman Cleveland *et al.*,, 1969). Hence it is necessary to establish various regions of the uriniferous tubule in the fish, *Tilapia mossambica (Peters)* which is being widely used for pollution studies.

Material & Methods:

Live fish were obtained from Masunda lake in Thane district in Maharashtra, India and kept for a fortnight for laboratory acclimatization. The kidney of 10 fish samples were fixed in 3% glutaraldehyde for 30 minutes at 4°C and processed for electron microscopy. Ultra thin sections were taken on LKB ultramicrotome and picked upon G-200 copper grids. They were stained for 1 hour with uranyl acetate and counter stained with

lead citrate. Grids were scanned under a Ziess EM 109 electron microscope & JEM Joel 100 'S' Japan make electron microscope.

Observations:

Electron microscope observations of kidney:

Sections of fish kidney when observed under a light microscope present a generalized picture of a typical vertebrate kidney. In sections, glomeruli with Bowman's capsule, proximal and distal ends of uriniferous tubules and collecting tubules are distinguishable. Besides connective tissue cells & the blood cells, wandering cells are peculiar cells found in the interstitial regions (Fig. 1).

The electron micrographs of the uriniferous tubule consist of the following parts: - Neck region, Proximal region, Central slender region, Distal region and the collecting tubule.

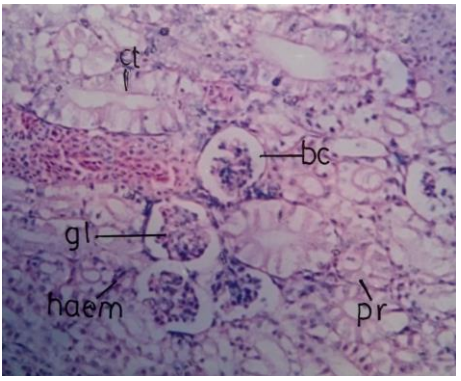

Fig 1 Light microscopic section of TS of Kidney (control) showing different regions of uriniferous tubules. Stain H/E

Key: Ct – collecting tubule; Bc – Bowman's capsule; gl – glomerulus; haem – haemopoietic region; pr – proximal region

Ultra structural details

(a) Neck region: The cells lining the neck region are cuboidal and are slightly narrower at their apical ends. The intercellular membranes being distinct, two adjacent cells can be easily differentiated. At the apical ends, junctional

complexes comprising of desmosomes can be seen distinctly. The basal ends are broader. The apical region is beset with closely placed microvilli which are comparatively short and extend into lumen. Cilia are many. The apical ends of cells occupy nearly one-third area of the cells. The region has small vesicles, dense granules and a profile of smooth endoplasmic reticulum. Mitochondria are characteristically lacking in the apical region.

The basal region of the cell occupies two-thirds of the cell. This region is characterized by the presence of a nucleus and a large number of mitochondria. Mitochondria are either elongated or spherical. Cristae are many and they run parallel to each other. The matrix is full of dense material. The outer region is covered by a distinct basal membrane. Plasmalemma gives rise to invaginations which are prominently seen in the vicinity of intercellular membranes. The cytoplasm is characterized by the presence of smooth tubules, dense granules and a few occasional lysosomal vesicles. The tubules are often dilated to give a vesicular look. Golgi body is situated in the vicinity of the nucleus. Nucleus is spherical or irregular in outline and is situated more towards the base. Nuclear membrane is distinct. Chromatin material is clumped at several regions. Usually a single nucleolus is present within nucleus (Fig 2 and 3).

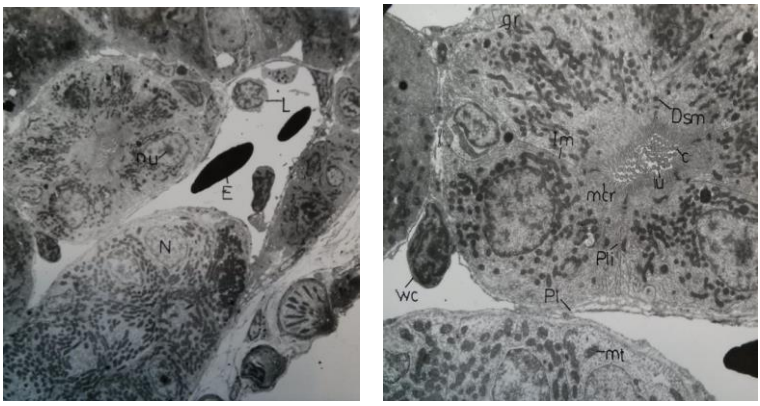

Fig 2 and 3 – Electron micrographs of the neck region of the uriniferous tubule (control) and its magnified version

Key: L – Lymphocytes; E – Erythrocyte; N – Nucleus; gr – granules; Dsm – desmosomes; c – cilia; lu – lumen; mcr – microvilli; Im – intercellular membrane; Pli – Plasmalemma invagination; Pl – Plasmalemma; wc – wandering cell; mt – mitochondria

(b) Proximal region: Proximal region too is circular in outline and has a distinct tubular lumen. The cells are cuboidal with their apical ends slightly narrower than their basal ends. The outer margin of the tubule is lined by a thin basal membrane. Intercellular membranes are extremely thin, thus it becomes little difficult to distinguish boundaries of individual cells. The adjacent cells have junctional complexes at the apical ends. Apical region of cells have closely placed microvilli. The microvilli are tubular and have a considerable length. Cilia are many and in sections, several cut portions of cilia can be seen. The cytoplasm of the apical end is full of dense granules, smooth tubules, ribosome and a few vesicles. The basal region of a cell encloses a spherical or oval nucleus. The perinuclear region is characterised by abundant number of mitochondria most of which are elongated. Cristae are slender and are transversely placed. The cytoplasm of the basal region has several of smooth tubules. The tubules which are towards the plasmlemma often run parallel to the latter. Plasmalemma invaginations are few and indistinct. Golgi body is prominent and is situated anterior or lateral to the nucleus. Nucleus is prominent and has a distinct nuclear membrane. Chromatin is of dispersive type and usually there will be a single nucleolus (Fig.4 and 5).

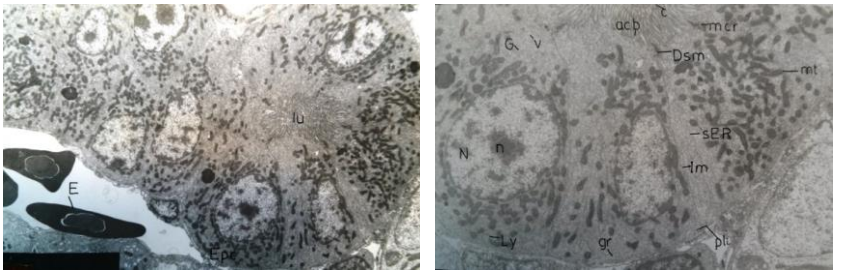

Fig 4 and 5: Electron micrograph of the proximal region of the uriniferous tubule (control) and its magnified version

Key: lu – lumen; Epc – Epithelial cell; E – Erythrocyte; wc – wandering cell; acp – apical ciliated border; c – Cilia; Dsm – desmosomes; mcr – microvilli; mt – mitochondria; gr – granules; v – vesicles; sER – smooth endoplasmic reticulum; Im – intercellular membrane; N – Nucleus; n – nucleolus; Ly – lysosomes; Pli – Plasmalemma invaginations

(c) **Central slender region:** This region is characterized by the presence of tall cuboidal cells. Apical end of the cell is very distinct. There is no distinct brush border but the luminal end of the cell is irregular and is thrown into pseudopodia like processes. The presence of these processes reduces the lumen to a great extent and makes it more irregular. There are no microvilli. Cilia are few and they arise from the pits between the two adjacent cells at the apical extremity. Intercellular membranes are extremely thin and cannot be easily differentiated. Desmosomes situated at the apical end however are quite prominent.

Apical region of the cell occupies nearly half the portion of the cell. The cytoplasm is full of membrane bound vesicles, which are of varying shapes and sizes. The region has a profile of smooth endoplasmic reticulum. In addition there are dense granules and ribosomes. The basal region is full of elongated mitochondria that are held at right angles to the plasmalemma. Mitochondria have several of elongated cristae that run obliquely. The cells have a prominent Golgi body which is situated close to the nucleus. Basal region is also characterized by plasmalemma invaginations which transverse within the cytoplasm to a considerable extent. The other cytoplasmic inclusions are the lysosomal vesicles and the autophagic vesicles. Nuclei are large, oval/irregular, each with distinct nuclear membrane. Chromatin material is of dispersive type (Fig. 6 and 7).

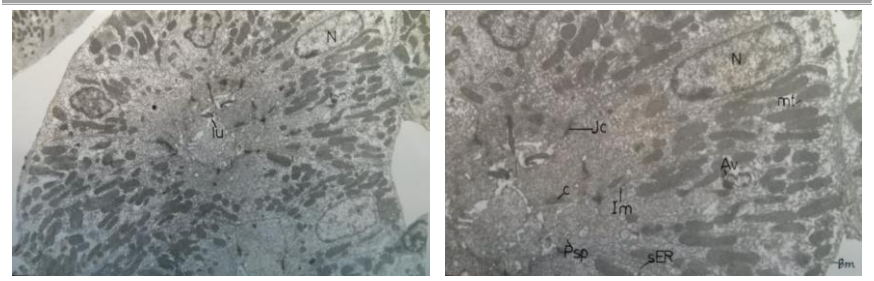

Fig 6 & 7 – Electron micrographs of TS of slender central region of the uriniferous tubule (Control) and its magnified version

Key: lu – lumen; N – Nucleus; mt – mitochondria; Jc – Junctional complex; c – cilia; Av – Autophagic vesicle; Im – Intercellular membrane; sER – smooth endoplasmic reticulum; Psp – pseudopodial processes; Bm – basal membrane

(d) **Distal end:** Sections are circular in outline with a distinct tubular lumen. The epithelial cells are columnar with distinct brush border formed of microvilli. Microvilli are closely placed and extend into the lumen up to considerable distance. Cilia are numerous.

Intercellular membranes are distinct. Thus the individual cells can be observed distinctly.

The cells have their apical ends which are totally devoid of mitochondria. Cytoplasm is represented by dense granules and vesicles are of moderate size. Endoplasmic reticulum profile is represented by both the smooth and the rough variety. Golgi is situated at the anterior extremity. The basal region of the cell is characterized by a large number of mitochondria which are small, round or spherical with distinct mitochondrial membranes. Cristae are numerous and placed transversely. The terminal ends of cristae often appear dark owing to the presence of dense material. Basal region is covered externally by a basement membrane. Plasmalemma gives rise to the invaginations which are situated close to intercellular membrane. These invaginations run parallel to the intercellular membrane and traverse two-third the length of the cell from basal extremity. Cytoplasm is traversed by a network of tubules which are dilated at various levels. This region is full of membrane bound vesicles of various shapes and sizes.

Cytoplasm also includes ribosomes, certain granules and a few small fibrils. Endoplasmic reticulum of both smooth and rough type is seen. Nucleus is irregularly spherical in outline an is situated more or less at the apical region of the cell. Chromatin is of dispersive type and no distinct nucleoli are seen (Fig 8).

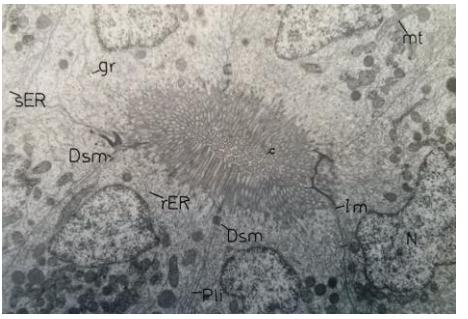

Fig 8 : Electron micrograph of TS of distal tubule (control)
Key: gr – granules; Im – Intercellular membrane; Dsm – desmosomes; N – Nucleus; sER – smooth endoplasmic reticulum; rER – Rough endoplasmic reticulum; Pli – Plasmalemmainvaginations; mt – mitochondria

(e) **Collecting tubule:** Collecting tubules are the large sized tubules with their lumen highly dilated. Tubules always remain flattened and hence never appear circular in sections. The cells have reduced heights. The apical region of the cell is slightly narrower than the basal one. However, the apical ends of the cells of these regions are wide as compared to the similar ends of the cells of other regions. The apical end is provided with microvilli that are closely placed. They are comparatively shorter. Cilia are few. The lumen is large and dilated. It is filled with some dense material. The region also encloses vacuoles, the sizes of which vary to a great extent. The inner surface of the collecting tubules is thrown into several of folds. These folds are large and spherical in outline and extend in to lumen. Owing to the presence of the folds, microvilli are cut in various planes. Such differentially cut microvilli are evident at various places along the border of the lumen. Microvilli invariably contain absorbedmaterial because of which they appear dark. Basal region occupies the lower half of the cell. The region is

characterized by the presence of centrally lodged spherical or oval nucleus and by the basally lodged mitochondria. Nucleus is fairly large and the nuclear membrane is distinct. Chromatin material forms clump at various places. Occasionally 1 or 2 nucleoli are seen. The cytoplasm is densely filled with granules at the basal extremity. Plasmalemma invaginations transverse into the cytoplasm. These invaginations run parallel to the intercellular membrane. At certain places, concentrically arranged endoplasmic reticulum tubules are seen. Such tubules are usually seen between the nucleus and the plasmalemma. Mitochondria are basally arranged. Most of these are held at right angles to plasmalemma and are situated between the plasmalemma invaginations. Mitochondria have several of cristae that are closely placed.

The region adjacent to the nucleus has a high profile of rough endoplasmic reticulum. The cytoplasm at the basal region also encloses certain autophagic vesicles. Golgi is usually situated lateral to the nucleus (Fig 9 and 10).

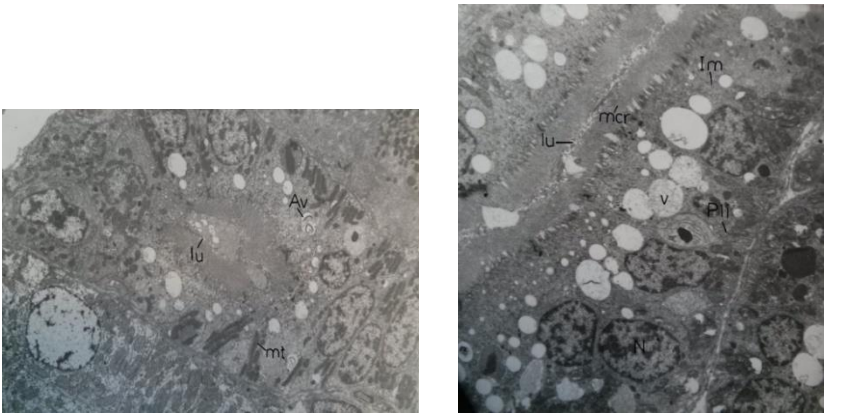

Fig 9 & 10: Electron micrograph of TS of collecting tubule (Control) and its magnified version

Key: Av – autophagic vesicle; lu – lumen; Im – Intercellular membrane; N – Nucleus; Pli – Plasmalemma invaginations; mt – mitochondria; v – vesicle; mcr–microvilli

Glomerulii

Glomerulii are seen distinctly. They are large and occupy more than half of the capsular lumen, Capillary wall and blood cells are easily distinguishable. Erythrocytic cells are oval shaped with deeply stained nuclei (Fig. 11).

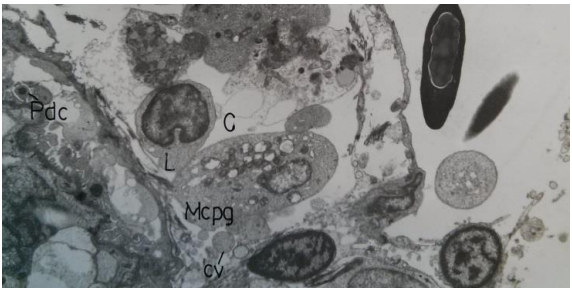

Fig 11: Electron micrograph of TS of kidney showing a single glomerulus capillary in the endothelial lining
Key: Pdc – Podocyte; cv – cytoplasmic vacuole; Mcpg– macrophage; L – lymphocyte; C – capillary lumen

Wandering Cells

In electron micrographs of the kidney sections peculiar cells called wandering cells have been seen besides connective tissue cells and the blood cells. These cells normally occupy the interlobular regions of the uriniferous tubules and have been identified as wandering cells (Trump Benjamin F. *et al.,* 1967; Bulger Ruth Ellen *et al.,*, 1968).

In *Tilapia mossambica (Peters)*the cells have been quite often found to be associated with the proximal tubule. The cells are either spindle shaped or triangular in outline. The cytoplasm is full of dark coloured granules and vesicles. Mitochondria are small, few and spherical. Ribosomes are seen in large numbers. Occasionally a few vesicles are also seen. The cytoplasm has a profile of rough endoplasmic reticulum. Nucleus is massive, triangular or V shaped and occupies most of the interior of the cell. Chromatin is dense and of pycnotic type (Fig 12).

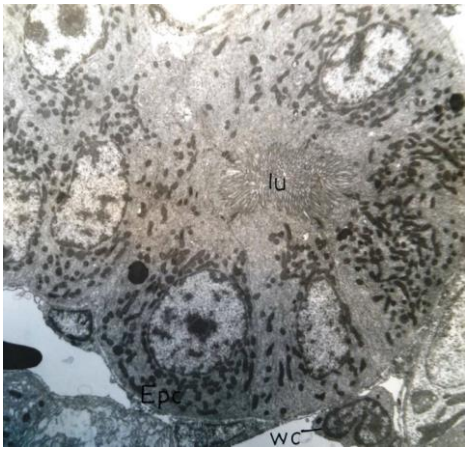

Fig 12: Electron micrograph of TS of the proximal region of the uriniferous tubule (Control) showing wandering cell
Key: lu– lumenEpc – epithelial cellwc – wandering cell

Results and Discussions:

Ultrastructural studies of control fish based on the observation of electron micrograph suggests the uriniferous tubule to consist of five different regions, viz., Neck region, Proximal region, Middle slender region, Distal region and Collecting tubule. Of the five regions, the first three regions have certain common characteristics like tubular lumen, high columnar epithelial cells with a narrow apical end and a broad basal end, basal nucleus and elongated large-sized mitochondria.

The fourth region viz. the distal one differs from the earlier three in having shorter epithelial cells with comparatively broader apical region; apical nucleus; small mitochondria occupying the region between the nucleus and the plasmalemma at the basal extremity and light staining cells with indistinguishable intercellular membranes.

The characteristics of the distal region are represented more or less uniformly in all the species of fishes (Hickman and Benjamin, 1969) and even in other groups of vertebrates (Ross Micheal *et al.*,, 1989) although different terms have been designated to the region by the different authors. The uniform

features of the region in all forms, therefore, makes the identification of the region much simpler.

The proximal region (often termed as proximal tubule) of *Tilapia mossambica (Peters)* has its resemblance to the first major segments described for the sole fish *Flounder* by Bulger Ruth Ellen and Trump Benjamin F. (1968). The most striking feature of the region being the presence of large, densely placed microvilli, which attributes a striated appearance to the inner border of the cells.

Differentiation of the neck region from that of median slender region poses some difficulty, more so because of the identical narrow dimensions of the two regions. Sections of the two regions however differ from each other in the following ways:

i. If the lumen is tubular in one it is irregular in the other owing to the presence of canals that extend laterally.
ii. The cells are furnished with microvilli in one while distinct microvilli are totally lacking in the other.
iii. Cilia are many in one while they are occasional in other.

These differences clearly indicate the two sections correspond to the two distinct regions. Following logical conclusions help in establishing these regions:

i. Neck region being short, probabilities of getting the sections of the region will be comparatively lesser than the median region.
ii. Neck region being the beginning of the uriniferous tubule, sections of this region should occur more in vicinity of glomerulii rather than to be away from those.
iii. The neck region being the beginning of the tubule it is logical to conclude that it is ideal to have a circular lumen rather than to have irregular one.

iv. Presence of large number of cilia will be ideal for the neck region to direct the capsular urine (i.e. filtrate) efficiently into the tubule.

v. Neck region being merely the region of conduction, absorptive type of epithelium is not expected.

Taking into considerations these logical aspects one can conclude that, the sections with the tubular lumen and with the distinct brush border corresponds to the neck region. The region thus identified as neck region in the present work resembles the similar region described for other vertebrates and more so with that of the sole fish described by Bulger Ruth Ellen and Trump Benjamin F. (1968). There is however one distinct difference, they have indicated the cells to have the cilia in cluster of twelve, in the present work nowhere cilia have been found to occur in clusters; nor there are any indications to that effect. The cilia on the contrary are seen to arise solitarily. This probably may be a species variation.

The region referred to as the middle slender region in the present work is unique. It is a narrow region situated between the proximal and the distal ends of uriniferous tubules and must be having a varying length in different nephrons. The cells have the same characteristics as those of proximal region but the apical region of the cells have no distinct brush border; instead the cells have irregular borders together with the pseudopodia like processes. The cilia are sparse and arise from the bases of apical pits that lie between the two adjacent cells.

The central slender region corresponds to the thin segment described by Ross Micheal H. *et al.,* (1989). According to the author, the region is likely to be represented in many species of fish and a detailed study is necessary to establish the region. The region however, is reported to be short and according to Ross Micheal H. *et al.,* (1989) the cells of the region differ in different species.

The electron microscopic study therefore establishes the existence of an additional region, the middle slender region, which is not detectable with the light microscopic studies. The regions referred to as second and third major segments of the nephrons described by Bulger Ruth Ellen and Trump Benjamin F. (1968) for the sole fish are not seen in *Tilapia mossambica (Peters).*

The ultrastructural details such as the presence of brush border and those of cilia at the apical extremity, so also the existence of apically placed tight junctions etc. are the characteristics which are represented in other vertebrates too and have been reported by several other authors. Similarly the existence of highly elongated mitochondria, the presence of plasmalemma invaginations etc. are also the characters that are attributed for the kidney cells by various workers.

The presence of wandering cells within the interstitial region is yet another characteristic which has been also reported by the earlier workers (Trump Benjamin F and Bulger Ruth Ellen 1967; Bulger Ruth Ellen and Trump Benjamin F., 1968). So the ultrastructural patterns of the different regions of nephrons of *Tilapia mossambica (Peters)* are similar to those described for various other vertebrates in general. The following three characteristics can be considered unique and probably may be the species differences:

a) Solitary non-clustered origin of cilia in the neck region
b) Presence of a distinct median slender region which differs from other region
c) Presence of cilia all along the length of uriniferous tubules although its abundance varies with the regions.

The study also covers the ultrastructural details of the collecting tubules. These details agree with those put forth by various authors with the following difference:

1) The vesicles and vacuoles which are observed at the apical extremities of the cell wall are much more as compared to the similar structures observed in other species.
2) Some of the earlier authors (Sandborn Edmund B., 1970; Ross Micheal H. *et al.*,, 1989) have indicated the existence of two types of cells, termed 'light' and 'dark' cells, lining the collecting tubules. In the present study such a differentiation has not been observed and all cells appear dark.
3) In electron micrographs of the collecting tubules one can occasionally observe cut portions of cilia indicating their presence in the region. These cut ends probably may be corresponding to the cilia of the neighbouring region.
4) In electron micrograph of collecting tubules, the microvilli appear to be cut in different planes at the apical extremities of the cell suggesting the possible uneven nature of the inner surface of the apical lining. Presence of a similar condition has also been reported by Ross Micheal H. *et al.,* (1989) and the structures have been termed as "lamellopodia".

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