Light and Electron Microscope Studies on the Cells of the Distal Portion of the Crayfish Nephron Tubule\textsuperscript{1,2}

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Electron microscope studies on the cells of the kidney tubule have, among other things, established the fact that the intracellular fibers long ago described by Heidenhain (1874) and Mislawsky (1913) are due in part to a great number of infoldings of the plasma membrane at the base of the cell (Pease and Baker, 1950; Dalton, 1951; Sjöstrand and Rhodin, 1953; Rhodin, 1954; Beams, Tahmasian and Devine, 1955; Pease, 1955). Such an arrangement of the plasma membrane results in a series of compartments within the basal cytoplasm and in an orientation of the mitochondria. The function of the folded plasma membrane, while probably significant, has not been revealed; it is not the same as the ergastoplasm, or the endoplasmic reticulum.

While examining slides for class work of fixed and stained cells from the distal portion of the crayfish nephron tubule, our attention was drawn to the markedly striated appearance of the basal zone of the cytoplasm and to the orientation of the mitochondria in these cells. Such observations suggested that electron microscope studies might reveal unknown detail of the intracellular structures in these cells. Furthermore, insofar as we know, electron microscope studies have not been made on the crayfish kidney tubule.

\textbf{Material and method}

Material for this study consists of the distal portion of the nephron tubule of the crayfish (\textit{Cambarus} sp.). Pieces of the green gland were fixed in buffered osmic acid (pH 7.25) for one-half to one hour. They were then washed, dehydrated and embedded in methacrylate. Sections were cut by a glass knife mounted on an International Minot microtome equipped with an 80 to 1 gear ratio, thus delivering sections of the order of .025 μ in thickness. Electron micrographs were made of sections both before and after removal of the plastic. The electron microscope used was an RCA model EMU-2B. Magnification of the electron micrographs is indicated by the one micron scale on the Figs.

For histological study green glands were fixed in Bouin’s, Champy’s and

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Regaud’s solutions and stained in Delafield’s hematoxylin and eosin or in Heidenhain’s hematoxylin. Following Champy’s fixation the sections were bleached in 1% potassium permanganate followed by treatment with 5% oxalic acid before staining in Heidenhain’s hematoxylin.

Description

Light microscope observations

The gross anatomy, histology and physiology of the crayfish kidney (green gland) have been the subject of extensive study of Peters, 1935 and Maluf, 1939, to whom the reader is referred for references. Each kidney consists, beginning with the proximal end, of a coelomosac, a labyrinth, a nephron tubule and a bladder. It is with the cells of the distal portion of the nephron tubule that we are here concerned.

Histologically, the epithelium consists of a highly involuted single layer of columnar cells which are in contact with a relatively thin basement membrane (Figs. 1, 2). These cells, for convenience of discussion, may be divided into basal, intermediate and apical regions. It will be noted that the basal surfaces of the cells are often extremely irregular due to local evaginations of this region (Figs. 1–6). Following Bouin’s fixation, the basal portions of the cells display fiber-like structures which are oriented perpendicular to the basement membrane and extend to the level of the position of the nucleus or slightly beyond (Figs. 1, 2). In Regaud’s and Champy’s fixed material, filamentous mitochondria, whose orientation is similar to that of the fibrous bodies seen in Figs. 1, 2, are observed in the basal regions of the cells (Figs. 3–6). It will be noted that the intermediate and apical zones of these cells display a remarkably empty appearance, which is probably associated with the existence here of a highly fluid cytoplasm (Figs. 1, 2). Note also the relatively few mitochondria in these regions (Figs. 3–6).

Electron microscope observations

1. Basal zone of the cell

Fig. 8 was included to show at relatively low power the general distribution of the cellular elements under the electron microscope. It will be noted that the basement membrane (BM) is uneven, thus conveying an irregular surface to the base of the cells. Extending from the region of the basement membrane (BM) to a position near the nucleus are seen many lamellae (L). In the cytoplasmic compartments between the lamellae (IC) are observed poorly preserved mitochondria (M) a condition which is due, in part, to the fact that the plastic was removed from the section before the electron micrograph was made. However, this section is useful to illustrate the topographical relationship existing between the cellular components and the associated underlying tissue (C). It is instructive to compare this Fig. with those shown in Plate IV.
Better resolution of the intracellular elements is seen in the portions of cells shown in Figs. 7, 9, and 10 in which the plastic was not removed. For example, the basement membrane (BM) seems to be composed of small fibers that are disposed parallel to the long axis of the membrane (Figs. 9 and 10 BM). In contact with, or cemented to the basement membrane are the proximal ends of numerous folds of the basement plasma membrane. These extend perpendicularly from the basement membrane (BM) as lamellae (Figs. 9 and 10 L). It is assumed that the lamellae are formed by an intricate infolding of the plasma membrane at the base of the cell. That is to say, the lamellae are the result of the plasma membrane at the base of the cell being thrown into extensive tightly arranged folds, thus imparting to the lamellae a double membrane appearance (Fig. 10, L). In fact, a partial separation of these membranes may sometimes occur (Fig. 7, SM) they may also show anastomosis (Fig. 7, AM). Occasionally, the proximal folded ends of the plasma membrane are seen to be artificially separated from their normally close association with the basement membrane (Fig. 7).

Between the lamellae are cytoplasmic compartments (IC) that contain oriented mitochondria (M). It will be observed that in the sections shown in Figs. 9 and 10 many of the distal ends of the intracellular compartments (IC) appear closed. Furthermore, some of the compartments seem to be enclosed or encased one within another. Such appearances are probably due to sections through secondary foldings of the inner surface of the primary folds. Since the intracellular compartments are composed of cytoplasm and mitochondria they are undoubtedly continuous with the remaining part of the cytoplasm at their distal extremities.

The mitochondria do not display a striking internal structure as occurs in certain other invertebrate and vertebrate tissues.

2. Intermediate and apical zones of the cell

A relatively large rounded or oval nucleus occupies the central portion of the intermediate zone (Figs. 8 and 9, N). Here also is observed the termination of the intracellular lamellae and compartments (Figs. 7 and 9). The surface of the cell appears smooth, that is, free of papillae. Occasionally we have observed relatively large vacuoles in the apex of the cell, causing a bulging of the cell membrane into the lumen of the gland. As previously mentioned, the intermediate and distal portions of these cells are relatively free of mitochondria.

Discussion

Evidence has been presented by early workers on the cytology of the kidney tubule which suggests that oriented intracellular fibers and mitochondria exist in the basal zone of these cells. Such a hypothesis has been in part substantiated by electron microscope studies of certain vertebrate kidney cells (Dalton, 1951; Sjöstrand and Rhodin, 1953; Rhodin, 1954; Pease, 1955).
A similar condition exists in the cells of the Malpighian tubules of the grasshopper (Beams, Tahmisian and Devine, 1955). From the results recorded in this paper we can now add the cells of the distal nephron tubule of the crayfish to the list of cells displaying folded basement plasma membranes.

Speculation as to the possible function connected with the lamellae is difficult because of the lack of knowledge concerning the detailed physiology of these cells. It has been suggested that they somehow aid in the selective absorption of water from the blood and thus contribute to the formation of hypotonic urine (Maluf 1939). However, it is difficult to understand how this specific function might be correlated with the presence of lamellae and compartments in the basal portion of the cell. Hence, it would seem that, until we have a more detailed knowledge of the function of these cells, we cannot hope to correlate their structure with their specific function. Accordingly, for the present, at least, it seems that, in general, cell function is enhanced by increased cellular surface areas such as are provided here by the lamellae.

**Summary**

Cells from the distal portion of the nephron tubule of the crayfish may be divided for purposes of description into three different regions, i.e., basal, intermediate and apical. Light microscope preparations of Bouin’s fixed and hematoxylin stained material show the basal portion of the cells deeply stained and to contain fiberous like structures, oriented perpendicular to, and in contact with, the basement membrane. This method displays the intermediate and apical portions of the cells as relatively free of distinctive structure. Champy’s and Regaud’s methods reveal the filamentous oriented mitochondria chiefly present in the basal zone with few, if any, in the apical zone of the cells.

Electron micrographs demonstrate in the basal zone of the cell a series of perpendicularly arranged lamellae extending from the region of the basement membrane to the intermediate zone of the cell. These lamellae are thought to be formed by the intricate infolding of the plasma membrane at the base of the cell. This arrangement gives rise in the spaces between the lamellae to a series of elongate cytoplasmic compartments which contain rows of short rod or filamentous mitochondria.

No specific correlation of function in these cells with the presence of lamellae in their basal zone can be made, other than to state that, physiological processes are probably enhanced by the increased membrane area provided by these structures.

**Literature cited**


Rhodin, J. 1954. Correlation of ultrastructural organization and function in normal and experimentally changed proximal convoluted tubule cells of the mouse kidney. Privately printed, Karolinska Institute, Stockholm.


Explanation of Plates IV and VI

Fig. 1 to 6 are photographs made through the light microscope. Figs. 7 to 10 are electron micrographs.

Plate IV

Figs. 1 and 2. Bouin's fixed and Delafield's hematoxylin and eosin stained. Note fibrous nature of the basal zone of the cells. The intermediate and apical zones of the cells show relatively little structure. Fig. 8, 800×; Fig. 9, 1,000×. Figs. 3, 4, 5 and 6. Fixed in Champy's, bleached and stained in Heidenhain's hematoxylin. Here the basal zone reveals filmentous mitochondria oriented perpendicular to the basement membrane. Evaginations of the basal portions of the cells are also obvious. 1200×.

Plate V

Fig. 7. Portion of base of a cell showing the lamellae (L) separated from the basement membrane (BM). A separation of the membranes composing the lamellae is seen at (SM), and an anastomosis of them occurs at (AM). Fig. 8. Portion of three cells showing lamallae (L) and compartments (IC). The intermediate and apical portions of the cell are relatively free of obvious structure. The nucleus is shown at N.

Plate VI

Fig. 9. Perpendicular section through basal portion of cell. Note lamellae (L), interlamellar compartments (IC), and mitochondria (M). The relationship between the folded lamellae at the base of the cell and the basement membrane (BM) is clearly shown. Fig. 10. Similar to Fig. 9 except that here the lamellae (L) are clearly seen to be composed of two membranes. In this preparation the basement membrane (BM) presents a somewhat fibrous appearance. The interlamellar compartments (IC) and mitochondria are clearly demonstrated.