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Everett Anderson
Howard University

H. W. Beams
State University of Iowa

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The Fine Structure of the Malpighian Tubules of *Dytiscus* Sp.: Preliminary Communication¹

By EVERETT ANDERSON² AND H. W. BEAMS

INTRODUCTION

An extensive literature has accumulated dealing with the cytology of Malpighian tubules of insects as revealed by the light microscope, but relatively few papers have dealt with a study of their ultrastructure (Bradfield, 1953; Beams, Tahmisian and Devine, 1955). Shiwago (1915) called attention to the behavior of the mitochondria in the Malpighian tubules of the cockroach. He observed that they apparently migrate from the cell body into the striated border and hence, into the lumen of the tubule. Beams, Tahmisian and Devine (1955) have, in essence, confirmed Shiwago's results and they have been able to reveal, because of the high resolution of the electron microscope, that the striated border is composed of microvilli, instead of cilia as claimed by some (Davis, 1927). In addition, they describe in detail how the mitochondria migrate from the cell body into the microvilli and how the tips of the mitochondria-laden villi are eventually pinched off into the lumen of the tubule.

Since this type of mitochondrial behavior is unusual, even to the extent of their presence in the microvilli, it was thought important that a further study of them be made in other species of insects to determine how widespread this condition is.

MATERIALS AND METHOD

The Malpighian tubules of *Dytiscus* were dissected and fixed in a 1% solution of osmium tetroxide, pH 7.3. After fixation they were washed, rapidly dehydrated, infiltrated and embedded in methacrylate. Sections were made with a Porter-Blum ultrathin sectioning microtome and studied with a Phillips electron microscope (EM100 A).

OBSERVATIONS

The cells of the Malpighian tubules show a distinct brush border at their apical region. This border, in thin sections is composed of

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²Present address: Department of Anatomy, Howard University, Washington, D. C.

numerous filamentous protoplasmic processes (microvilli) (figure 1, bb.), which are extensions of the apical cytoplasm, arranged perpendicularly to the lumen. In figure 1 numerous mitochondria may be seen aggregated at the bases of the protoplasmic processes. Of interest in this connection, in addition to the aggregation of mitochondria, is the fact that a few may be seen extending into the filaments of the brush border. At m_e in figure 1, portions of one mitochondrion may be seen occupying the bases of two filaments.

Scattered irregularly in the matrix of the ground substance a number of dense particles (figure 1, dp) may be observed which presumably correspond to the particulate associated with the endoplasmic reticulum as described by Palade (1955).

The region between the base of the cell and the apical end may be designated as the middle or intermediate zone. It consists of a nucleus (not shown in present micrographs), and a number of mitochondria (figure 2, m) with no preferred orientation, limited by a double membrane, and displaying an internal structure composed of cristae (Palade, 1955). In addition to the mitochondria a number of profiles of the endoplasmic reticulum are seen at *er*, without granules associated with their membranes, which perhaps belong to the smooth surface variety (Palade, 1956).

The basal portion of the cell displays an infolded membrane, oriented perpendicularly to the basement membrane (figure 3, pm). This infolding has been interpreted as an infolding of the plasma membrane and has been described for many tissues, particularly those having a secretory function (Rhodin, 1954; Pease, 1955, 1956; Beams, Anderson and Press, 1956; Anderson and Beams, 1956, and others). In figure 3 the infolded plasma membrane may be seen resting on what appears to be a granular component (gc) of the basement membrane. In addition to the granular layer of the basement membrane, there appears to be a homogeneous layer (hc) of low density.

Surrounding the Malpighian tubules is an outer serosa composed of epithelial cells; a portion of one cell is seen in figure 3 just below the homogeneous layer of the basement membrane. In this section profiles of endoplasmic reticulum of the smooth variety (*er*) and mitochondria (*m*) may be observed. In addition to these components, profiles of intracellular tracheoles, limited by a dense intima, may be observed displaying their spatial relationship to the other cellular components. These intracellular tracheoles are similar to those found in cells composing the light organ of *Photinus pyralis* (Beams and Anderson, 1955).

DISCUSSION

What significance, if any, can be attributed to the finding of mitochondria in the filaments of the brush border to the function

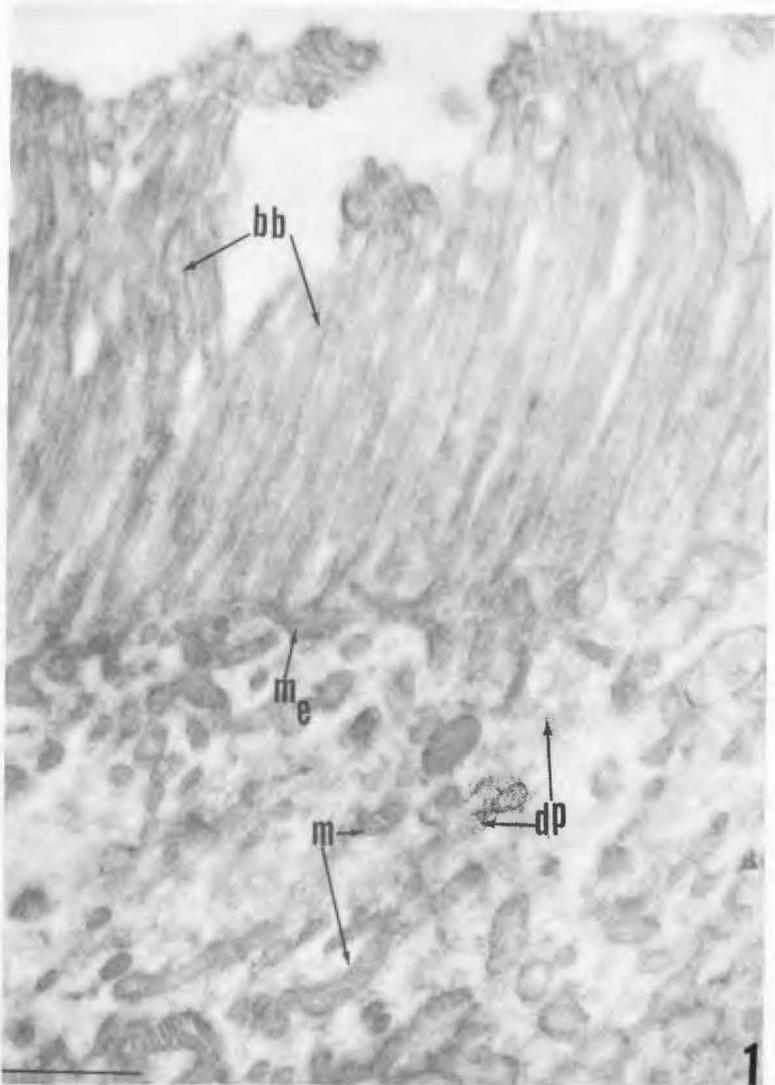


PLATE I

Figure 1. Section of the apical portion of a Malpighian tubule cell showing filamentous processes (microvilli) of the brush border (bb), dense particles in the cytoplasm (dp), and mitochondria (m). Note the mitochondrion at *m_e* occupying the bases of two processes of the brush border. 30,000 X.

PLATE II

Figure 2. Small field of the middle or intermediate zone showing mitochondria (m), profiles of the endoplasmic reticulum (er) and two dense inclusion bodies (ib). 30,000 X.

Figure 3. Basal portion of the cell showing the infolded plasma membrane (pm), and a mitochondrion between two folds at *m*. The granular component of the basement membrane may be seen at *gc* and the homogeneous component at *hc*.

of the Malpighian tubules is a matter of conjecture. However, it is known from the time-lapse cine-photographic studies of cells grown in tissue culture that mitochondria undergo extensive movements. Gey, Shapras and Borysko (1954) suggested that this movement is due to passive streaming of the cytoplasm. A few investigators envision the movement as being due not only to passive cytoplasmic streaming but also to metabolic exchange between mitochondria and cytoplasm (Frederic and Chèvremont, 1951; Frederic, 1954; Tobioka and Biesele, 1956 and others).

The Malpighian tubules of *Dytiscus* do not show, at least, in the animals here studied, the pinching off process of the tips of the microvilli which is so characteristic of the microvilli in the grasshopper Malpighian tubule cells. The condition in *Dytiscus* may represent an intermediate stage between: (1) where the mitochondria never enter the microvilli (Beams and Anderson, 1957) and (2) where they are apparently pinched off from the cells at the tips of the microvilli (Beams, Tahmisian and Devine, 1955).

The physiological significance of the mitochondria in the microvilli of the striated border is not clear; it may be that they act as an intracellular transport system between the cytoplasm and the membrane of the cell.

SUMMARY

The cells of the Malpighian tubules of *Dytiscus* were investigated with the aid of the electron microscope. The apical end of the cell consists of a prominent brush border composed of protoplasmic filaments (microvilli) which are extensions of the cytoplasm. At the bases of the protoplasmic filaments numerous mitochondria were observed and a few were seen extending into these filaments. The middle or intermediate zone contains a nucleus and many mitochondria, and the basal portion of the cell displays an infolded plasma membrane oriented perpendicularly to the basement membrane.

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DEPARTMENT OF ZOOLOGY
STATE UNIVERSITY OF IOWA
IOWA CITY, IOWA