ELECTRON MICROSCOPE STUDIES ON THE CELLS OF THE MALPIGHIAN TUBULES OF THE GRASSHOPPER (ORTHOPTERA, ACRIDIDAE)*

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PLATES 55 TO 60

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The cytology of the cells from the kidney tubule has long been a subject of interest to biologists. With the advent of the electron microscope and ultrathin sectioning techniques, renewed efforts have been made toward a further analysis of the structure of these cells, particularly the basal striations, mitochondria, and brush borders (Pease and Baker (17), Dalton (6), Sjöstrand and Rhodin (22), and Rhodin (19)).

Since the aforementioned research has been limited to the vertebrate kidney tubules, it seemed of interest to attempt a similar study of the relatively large cells of the Malpighian tubules of the grasshopper, especially since they, too, possess pronounced basal striations, mitochondria, and brush borders. The results of this study, particularly as regards the behavior of the mitochondria, seem of sufficient interest for publication here.

Material and Methods

Malpighian tubules from the last instar nymphs and young adults of the grasshopper, *Melanoplus differentialis differentialis* (Thomas), were fixed for ½ to 1 hour in 2 per cent buffered osmium tetroxide (pH 7.25) using the method of Palade (15). They were subsequently washed, dehydrated, and infiltrated with equal parts of ethyl and *n*-butyl methacrylate. Polymerization was accomplished by means of ultraviolet light. Sections approximately 0.025 μ thick were cut by means of an International microtome. The sections were studied with the aid of an RCA model EMU electron microscope.

OBSERVATIONS

The Malpighian tubules of the grasshopper are relatively long, flexible, hollow tubules that end blindly in the body cavity and open into the intestine immediately behind the stomach (23). They vary in number from approximately 200 to 300 and are reported to have an excretory function (23, 24). Each tubule is accompanied by a tracheole and band of muscle fibers that are arranged spirally and in a longitudinal direction. The latter permits alter-

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nate contraction and extension of the Malpighian tubules within the body cavity.

In cross-section the Malpighian tubules often show 4 to 6 relatively large epithelial cells. These rest on a thin basement membrane and, for purposes of discussion, may be divided into basal, intermediate, and apical zones (Fig. 1 (*B*),(*I*),(*A*)).

Basal Zone.—Perhaps the most striking feature of this zone is that the cell membrane at the base appears to be thrown into a series of deep parallel folds that are oriented perpendicularly to the basement membrane (Figs. 1 (*L*), 3 (*L*), and 4). In Fig. 3 the relatively straight lateral cell membrane (*CM*) seems to be continuous with the infolded membrane at the base of the cell (*L*). This intricate infolding of the membrane at the base of the cell partitions the cytoplasm into many compartments (Figs. 1 (*IC*), 3, and 4).

The total average width of the two adjacent layers of the folded membrane is approximately 23 mμ. Each layer measures about 5 mμ in width, and the space between the adjacent layers is approximately 13 mμ in width.

The intracellular compartments, formed by the folded membrane, range in width from 38 to 160 mμ, and they contain mitochondria that are often arranged in rows (Figs. 1 (*M*), 2 (*M*), and 3). In addition to the mitochondria, smaller bodies of unknown nature are also present (Figs. 3 (*X*) and 4 (*X*)). These range in diameter from about 25 to 150 mμ, and they possess a relatively dense cortical zone which surrounds a less dense medullary zone. Some cells show, in addition to mitochondria and the small unidentified bodies, numerous vacuoles (Fig. 2).

Intermediate Zone.—Included in the intermediate zone is a relatively large, spherical or oval nucleus, with a well defined nuclear membrane (Fig. 1). Also, the cytoplasm contains short rod or filamentous mitochondria. These vary in number and length from cell to cell. In Figs. 12 and 13 the internal mitochondrial organization is displayed as a series of double membranes, arranged at various angles to the longitudinal axes. This condition is especially true of those mitochondria that appear to be moving into the protoplasmic processes of the brush border (Figs. 6, 7, 9 to 11). Other mitochondria show rounded or circular elements of approximately the same width and density as the double membranes (Fig. 14 (*T*)). The fact that these elements show a light core limited by a dense outline seems to indicate that they are actually small tubules.

Apical Zone.—In this zone, which includes the free border of the cell, a prominent brush border is observed. This is composed of a multitude of vertically arranged parallel protoplasmic processes which vary from 3 to 4 μ in length and are about 1 μ in width (Figs. 1 (*PP*), 5 to 11). These processes may be considerably wider than indicated, when filled with mitochondria. Each one is bounded by the cell membrane (Figs. 5, 6 (*CM*)).

Perhaps the most important characteristic of the border is the elongation and apparent movement of the mitochondria from the cell body into the protoplasmic processes (Figs. 5 to 11). As this occurs, their internal double membranes become oriented more or less at random with respect to the long axes of the mitochondria. Further extension of the mitochondria into and through the protoplasmic processes continues until they eventually reach the blind ends. In this position the distal portions of the filamentous mitochondria round up to produce bulbous ends on the cell extensions (Figs. 5 to 10). This process presumably continues until the ends of the protoplasmic processes are pinched off, thus eliminating portions of the mitochondria and the cell membrane covering the ends of the protoplasmic processes.

Sometimes a single filamentous mitochondrion may "flow" into two or more protoplasmic processes (Figs. 8 and 9). Fig. 8 also shows a cross-section of the protoplasmic processes, some of which contain mitochondria.

DISCUSSION

The elaborate infolding of the cell membrane at the base seems characteristic for certain cells of the excretory organs of vertebrates (6, 9, 17, 19, 22). It has been demonstrated in this paper that a similar infolding of the cell membrane occurs in the Malpighian tubules of the grasshopper. This is also true for the cells of the distal nephron tubules of the crayfish (Beams, Anderson, and Press, data unpublished).

The complicated arrangement of the intracellular membranes has been interpreted by Sjöstrand and Rhodin (22) and especially Rhodin (19) in the mouse kidney as formed by an elaborate infolding of the cell membrane adjacent to the basement membrane. This condition gives rise to many intracellular compartments of the cytoplasm which appear as digit-like extensions protruding from the intermediate cell zone. In the frog kidney Fawcett (9) also reported an elaborate infolding of the cell membrane at the base, dividing the cytoplasm into numerous compartments. In addition, he noted in some of the adjacent cells a complex interlocking of their lateral margins. Just how much, if any, the apparent intracellular membranes in the cells of the Malpighian tubules of the grasshopper are due to sections through interdigitating membranes of adjacent cells, is unknown.

We wish to point out that although we have interpreted certain of the images of fixed and sectioned mitochondria in the protoplasmic processes as stages in their elimination from the cell, this is obviously an hypothesis. Final proof that the mitochondria are actually extruded would seem to depend upon observations based on living cells. Yet, it should be emphasized that we have never observed cells that were free of mitochondria, which suggests that they may be renewed as rapidly as they are presumably lost. In this connection it should be mentioned that there are other observations

on mitochondria which indicate that they may be expendable. For example, Bensley (3) has warned against elevating the mitochondria to the status of cell organelles. He has pointed out that they may appear and disappear under certain experimental conditions. A similar situation has been observed by Lewis and Lewis (13) in tissue culture cells and by Harvey (12) in the centrifuged quarters of *Arbacia* eggs. Recently, Frederic (10) has claimed to demonstrate a *de novo* origin for mitochondria. Ehret and Powers (8) have shown structural identity between young nucleoli and mitochondria at the time of nucleolar extrusion. However, if the elimination of mitochondria from grasshopper Malpighian cells be true as suggested here it is difficult to harmonize this unusual behavior with their important cellular functions (20).

As it is known, many investigators favor the view, reemphasized by Palade (16), that the mitochondria should have the status of cell organelles, the implication being that they are perpetuated by division. In addition, evidence is rapidly accumulating to show that mitochondria may act as possible passageways in the cell for the active transport of certain substances (ions) (7). If this be true the transport substances may be completely removed from the cells of the Malpighian tubules of the grasshopper in association with the mitochondria, thus obviating the necessity of their diffusion through its membrane.

Noël and Tahir (14) observed mitochondria within the elements of the brush border of the Malpighian tubules of *Bombyx mori*. Bradfield (4), in a preliminary electron microscope study of the Malpighian tubules in the wax-moth caterpillar (*Galleria*), saw what he interpreted as cytoplasmic double membranes present in the basal region of the cell. In the apical region, the brush border was described as being composed of numerous rods, or filaments, of variable length. It was noted that the inner (proximal) ends of the rods sometimes connected with mitochondria. The mitochondria in question often showed internal double membranes or irregular circles which were interpreted as oblique sections through small tubules. No mention was made of the possibility suggested in this paper that the mitochondria may, at times, be extruded from the cell through the ends of the protoplasmic processes of the brush border.

The ultrastructure of mitochondria in the Malpighian tubules of the grasshopper is difficult to determine: some show structure similar to that interpreted as cristae by Palade (16), or double membranes by Sjöstrand and Rhodin (22) and Rhodin (19); others appear to be composed of many small tubules comparable to those recently described for the mitochondria in *Paramecium* by Powers, Ehret, and Roth (18) (see also Palade (16)). In view of the apparent variation in structure of the mitochondria studied here, it seems unwise to attempt to draw final conclusions concerning this point until further work is completed. In this connection it is interesting to note that addi-

tional evidence is accumulating to show that not all mitochondria have the same type of internal structure (1, 2, 5, 8, 11, 18, 21).

SUMMARY

For purposes of description, the cells of the Malpighian tubules of the grasshopper may be divided into basal, intermediate, and apical zones.

The basal zone of the cell contains what appears to be an elaborate infolding of the cell membrane at the base. This condition results in the basal cell cytoplasm being divided into many compartments. The compartments contain mitochondria that are often arranged in rows. Other small bodies which possess relatively dense outer borders and less dense cores were observed within the compartments. These bodies are unidentified.

The brush border of the apical zone contains a multitude of vertically arranged protoplasmic processes. Stages were found which suggest that the filamentous mitochondria migrate from the cell body to the base of the protoplasmic processes, where they enter them and move apically. Some of the mitochondria were observed at the very tips of the processes where they enlarge producing an accompanying bulging of the tips. This condition is interpreted as a stage in the pinching off of the mitochondria-laden tips of the protoplasmic processes.

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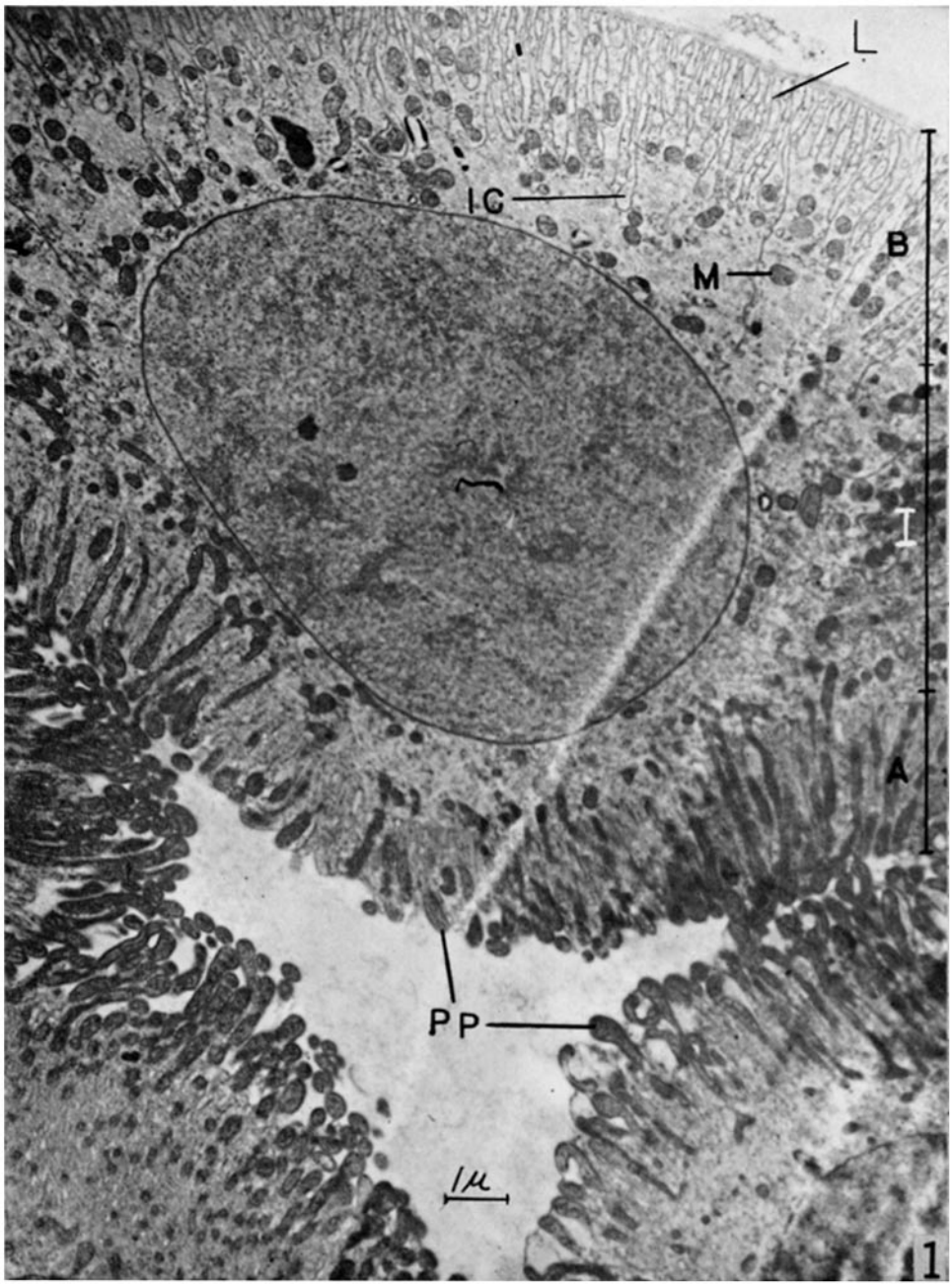
EXPLANATION OF PLATES

PLATE 55

FIG. 1. Section of Malpighian tubule showing distribution of cellular elements. Within the basal zone (*B*) are seen numerous infoldings of the cell membrane (*L*), intracellular compartments (*IC*), and mitochondria (*M*). The intermediate zone (*I*) contains the nucleus and mitochondria. The apical zone (*A*) shows the protoplasmic processes of the brush border (*PP*), many of which possess mitochondria. × 8,000.

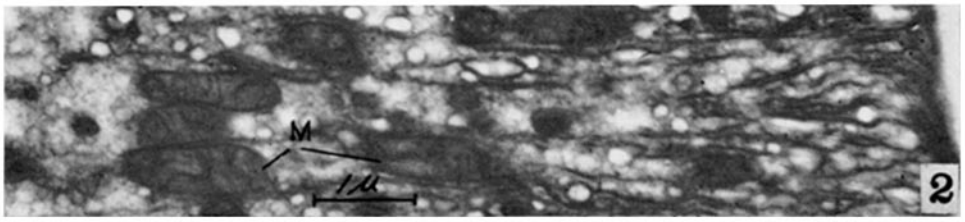


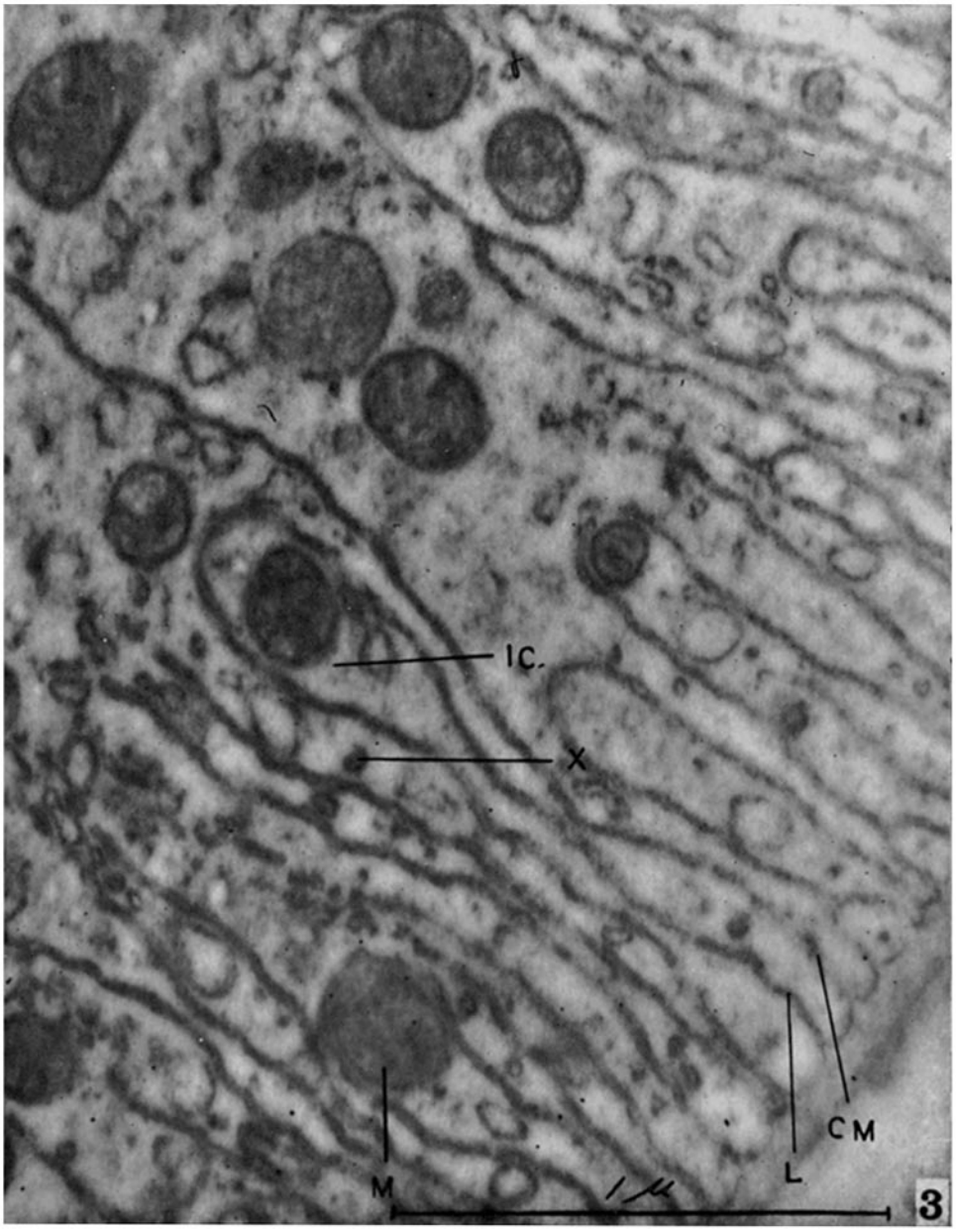
(Beams *et al.*: Electron microscope studies of Malpighian tubules)

PLATE 56

FIG. 2. Section through basal zone revealing mitochondria (*M*) and vacuoles within the intracellular compartments. × 13,000.

FIG. 3. More highly magnified section through basal zone. Note continuity of the relatively straight lateral cell membrane (*CM*) with the infolded cell membrane at the base (*L*). Some of the mitochondria (*M*) are seen within the intracellular compartments (*IC*). Small unidentified bodies are seen at *X*. × 66,000.

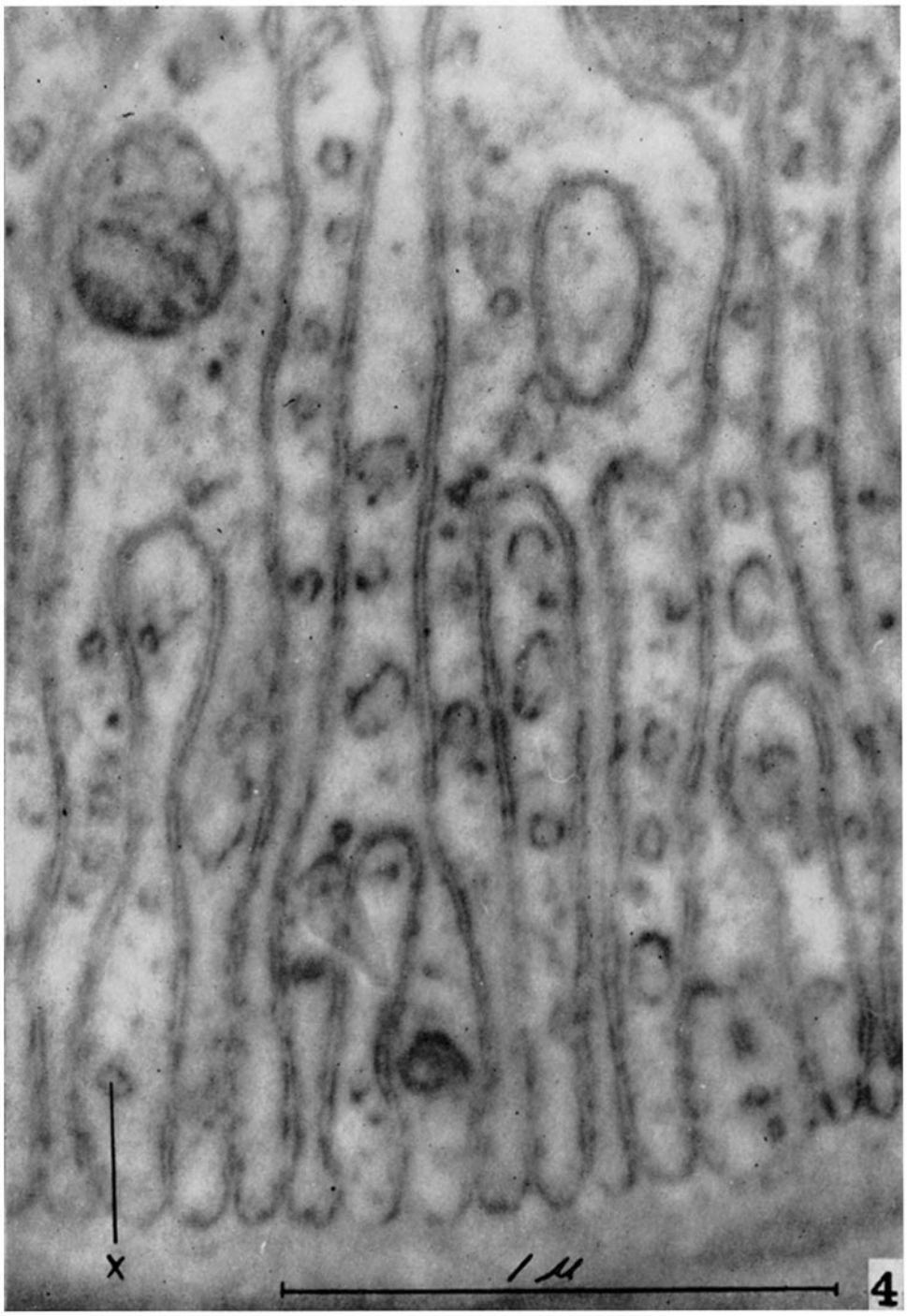




(Beams *et al.*: Electron microscope studies of Malpighian tubules)

PLATE 57

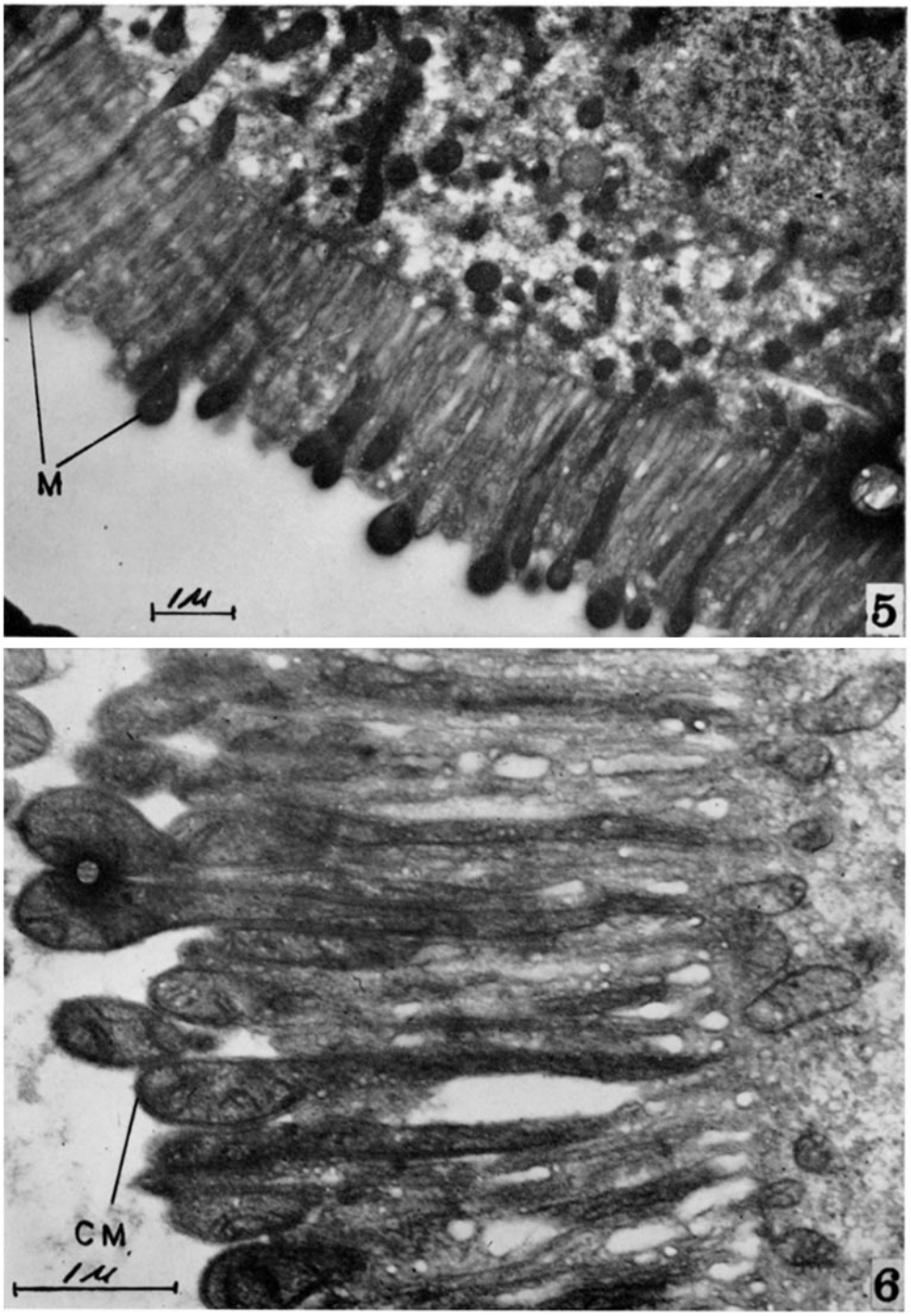
FIG. 4. Similar to Fig. 3, except that the doubled membrane of the fold is clearly demonstrated. Note also the unidentified body at *X*. × 79,000.



(Beams *et al.*: Electron microscope studies of Malpighian tubules)

PLATE 58

FIGS. 5 and 6. Apical zone showing protoplasmic processes of the brush border. Various stages in the apparent movement of mitochondria (*M*) from the intermediate zone into and through the protoplasmic processes are shown. Note bulb-like enlargement of the ends of protoplasmic processes bearing mitochondria. Internal mitochondrial structure may be seen in Fig. 6. Note also the cell membrane (*CM*) covering the ends of the protoplasmic processes. × 12,000; × 23,000 respectively.

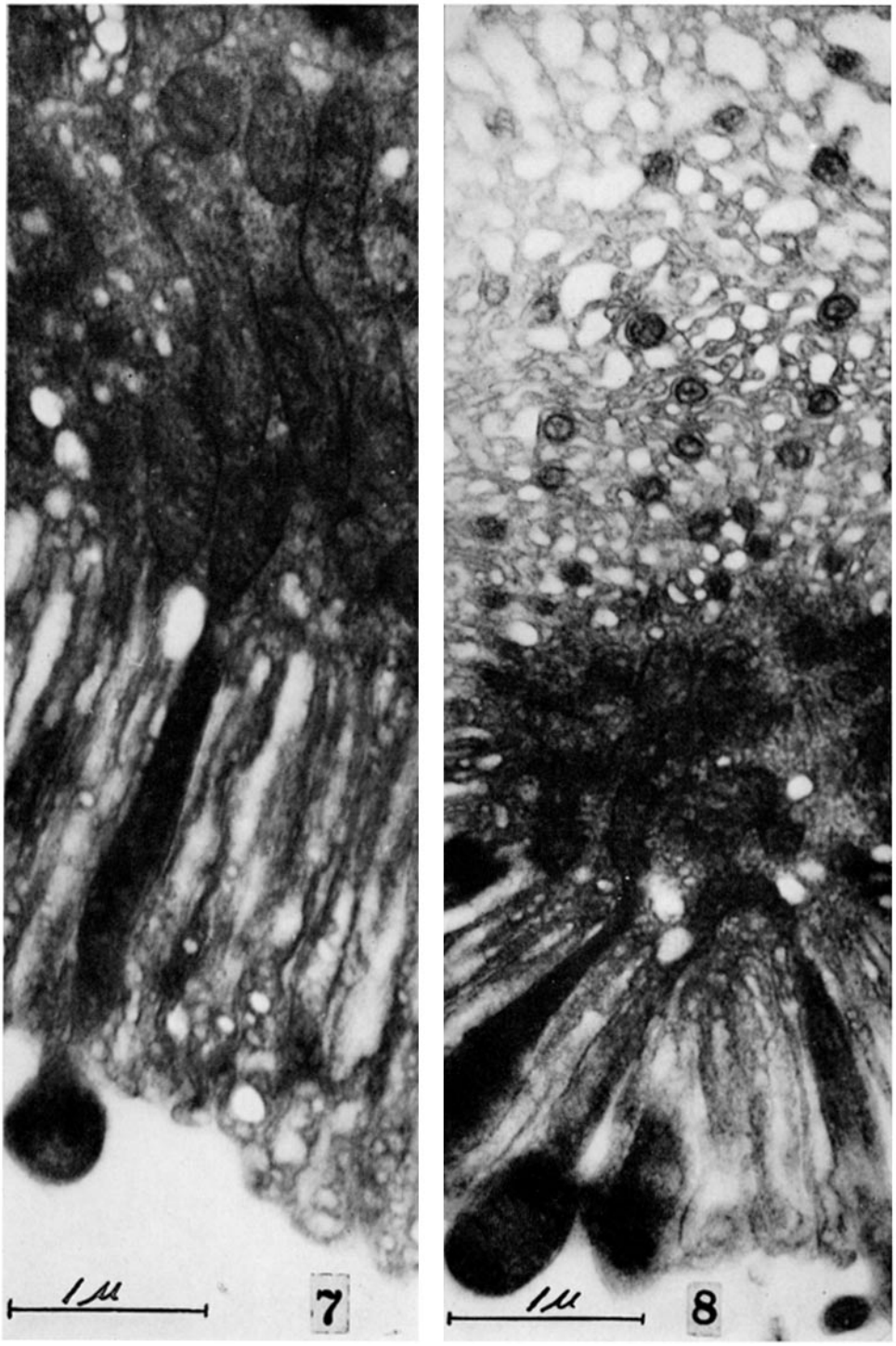


(Beams *et al.*: Electron microscope studies of Malpighian tubules)

PLATE 59

FIG. 7. Mitochondrion extending from cell body into and filling a protoplasmic process. End of process shows bulb-like enlargement which is thought to be a stage in the extrusion of mitochondrial material. × 29,000.

FIG. 8. Tangential section of brush border. In upper part of figure protoplasmic processes bearing mitochondria have been cut in cross-section. In lower portion of figure is seen a mitochondrion that is entering two different protoplasmic processes. × 28,000.



(Beams *et al.*: Electron microscope studies of Malpighian tubules)

PLATE 60

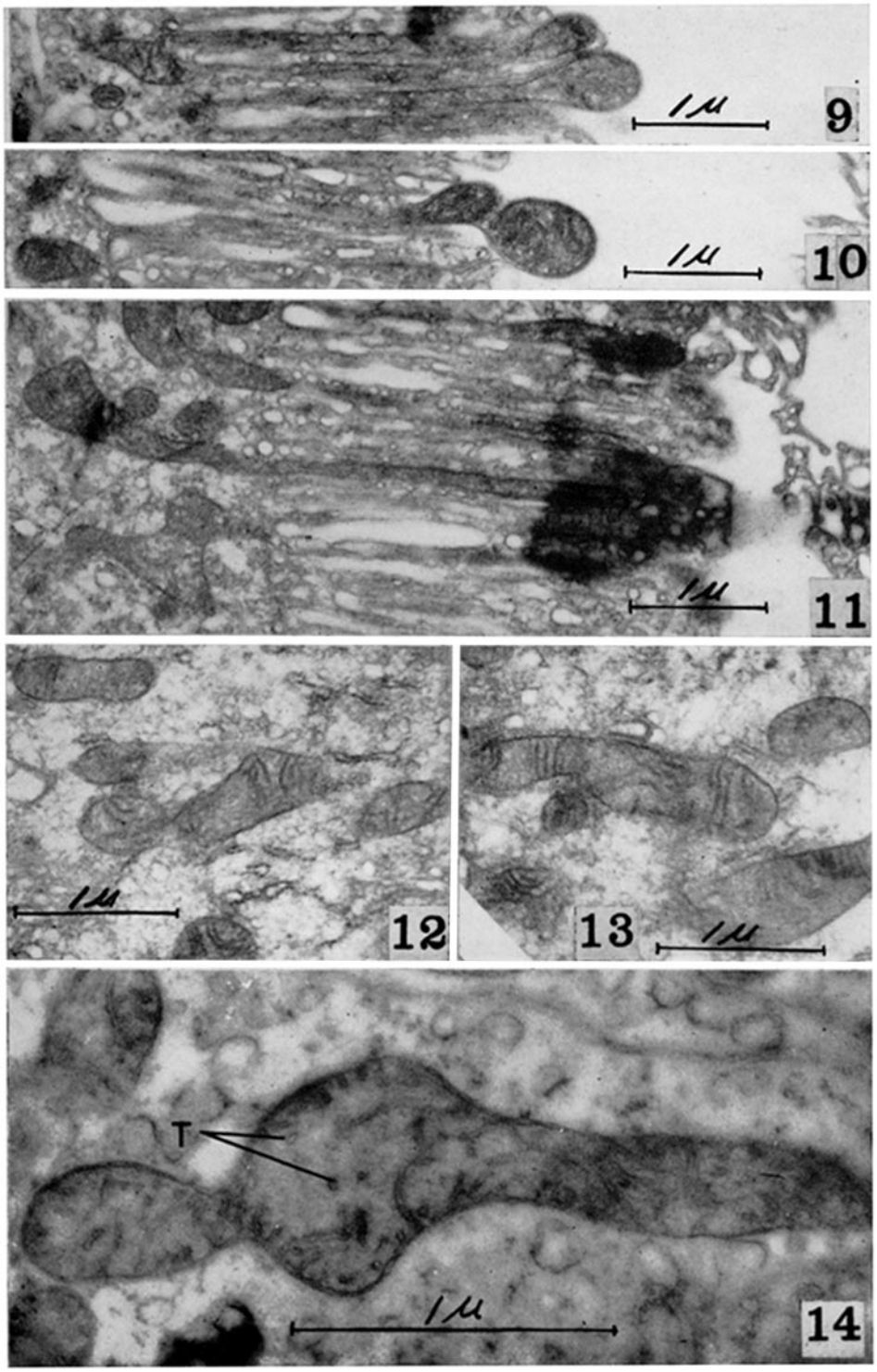
FIG. 9. Filamentous mitochondrion apparently moving into and filling two protoplasmic processes. × 19,000.

FIG. 10. End of mitochondria-laden process about to be pinched off. Note the presence of double internal mitochondrial membranes. × 19,000.

FIG. 11. Mitochondria penetrating protoplasmic processes of cell. × 19,000.

FIGS. 12 and 13. Mitochondria showing variously arranged internal membranes. Some are directed transversely and some are parallel to the long axes of the mitochondria. × 24,000.

FIG. 14. Mitochondrion showing rounded or oval elements (*T*) which may represent the cross-section of small tubules. × 46,000.



(Beams *et al.*: Electron microscope studies of Malpighian tubules)