MORPHOLOGY AND ULTRASTRUCTURE OF THE EXCRETORY ORGANS IN LARVAE OF *ISCHNURA ELEGANS* VANDER LINDEN (ZYGOPTERA: COENAGRIONIDAE)

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The production of urine in larvae of *Ischnura elegans* involves both Malpighian tubules and transport epithelia in the ileum. In final instar larvae 12 groups of mainly 5 Malpighian tubules occur. They discharge their contents into the intestine just anterior to the sphincter which separates midgut and hindgut. The Malpighian tubules show neither in their morphology nor in their ultrastructure any clear differentiation into segments as is found in many other insects. The luminal side of the tubule cells has an elaborated microvillous border. The basal cell membrane shows infoldings. Many mitochondria, sometimes concentrated just beneath the microvilli, occur in the tubule cells. The ultrastructure of the cells suddenly changes anterior to the confluence into a common duct (also called bursa or receptacle). The microvillous border disappears and the cells, containing fewer mitochondria, are filled with numerous vesicles and are typical mucus cells. The ileum has two types of epithelia; one is thin, the other is much thicker and has the features of a transport epithelium. Apically the cell membrane is much infolded. The basal membrane has many infoldings closely related to mitochondria. The presence of such epithelia suggests that reabsorption of electrolytes from the fluid produced by the Malpighian tubules occurs here.

INTRODUCTION

In insects the production of urine or excretion is a complex process, which involves different organs. The Malpighian tubules produce a fluid which is discharged into the hindgut. In many insects, this fluid is isotonic to the haemolymph and contains many useful products such as water and inorganic ions. In

terrestrial insects most of these ions and water are reabsorbed by the rectal papillae. Because they live in fresh water, larvae of Odonata do not need to reabsorb water; however, the retention of salts is very important in osmoregulation. In these larvae the rectum is filled mainly with water, and thus reabsorption takes place in the first segment of the hindgut, the ileum.

Although different authors (DUFOUR, 1852; GRIFFITHS, 1888; SADONES, 1896; KAISER, 1938; GAGNEPAIN, 1956a; LAUNAY & RAZET, 1983) have published on the morphology and microscopic structure of the Malpighian tubules in larvae of dragonflies, our knowledge is still incomplete. The principal aim of this study is to fill this gap.

MATERIALS AND METHODS

Larvae (mostly final instar) of *Ischnura elegans* v.d.L. were taken from a pond on the university campus in Diepenbeek, Belgium. The animals were starved for at least two days in pond water at room temperature (18°to 20°C). The morphological study used a Philips SEM 501 scanning electron microscope. Pieces of the gut were fixed and post-fixed as for the ultrastructural study (see below); after dehydration in a graded series of ethanol they were transferred to acetone. Critical point drying was applied with a Polaron E 3100 Jumbo. The tissue was mounted on a stub and coated with a 10 nm film of Au-Pd in a Polaron E 5100 Sputter Coater.

For the ultrastructural study the tissue was fixed in 2% glutaraldehyde in 0.05 M cacodylate + 0.15 M saccharose at pH 7.3, and 420 mosm. Postfixation was done in 2% osmium tetroxide in the same buffer. The tissue was embedded in Araldite after dehydration in acetone. Semi-thin sections (0.3 μm) and ultra-thin sections (60 nm) were made on an Ultracut Reichert. The ultra-thin sections were viewed with a Philips EM 400 electron microscope.

RESULTS AND DISCUSSION

GENERAL STRUCTURE AND MORPHOLOGY OF THE EXCRETORY ORGANS

The Malpighian Tubules

The Malpighian tubules discharge into the gut at the junction between the midgut (mesenteron) and the hindgut (proctodeum). Observations of freshly dissected animals show that the tubules are stretched, most of them being directed backwards. They are very mobile. After fixation they contract to form a dense mass (Figs. 1 and 2). All the tubules then show an helicoidal structure

(Fig. 3). The mobility as well as the helicoidal contraction is caused by a muscle which is attached to the outside of the tubule and has a helicoidal course. The muscle shows segmentation with an alternation of short and long segments (Fig. 4). The external diameter of the tubules ranges from 10 to 25 μm; the diameter of the lumen is 5 μm (Fig. 5). In contrast to many observations in other insects it is impossible to distinguish different segments in the Malpighian tubules of these larvae. The maximum length of the tubules is 5 mm. The number of tubules in 10 final instar larvae ranged from 60 to 68. The tubules do not discharge separately into the gut; there are 12 common ducts (receptacles or bursae) in which 3 to 8 tubules join together.

The Ileum

The ileum is a sac-like, enlarged first segment of the hindgut. At both ends, towards the midgut and the rectum, a sphincter is present. The ileum is enveloped by longitudinal and circular muscles (Figs. 2 and 3). After postfixation in osmium tetroxide and embedding in araldite, three special epithelial pads are visible in the thin-walled transparent ileum (Fig. 1).

MICROSCOPIC OBSERVATIONS

The Malpighian Tubules

A typical cross-section (Fig. 6) shows the following features. The wall of the tubule is composed of 4 or 5 cells, each 8 to 10 μm high, surrounding the lumen. An elaborated basal membrane is present, which shows numerous infoldings up to 2 μm long. The most conspicuous characteristic is the great number of mitochondria which suggests intensive activity in these organs. The cells contain numerous small vesicles and rough endoplasmic reticulum. The nucleus is situated in the centre of the cell; its longest diameter is 5.5μm. At their apices (facing the lumen of the tubule) the cells are lined with microvilli 1.5 to 2.0 μm high. Observations of many sections, made in different directions, indicate that these microvilli are cristae or plate-like foldings of the apical cell membrane rather than tubular as described in other insects. No evidence for finger-like or tube-like microvilli was found in the Malpighian tubules. The walls of adjacent cells do not show any interdigitations. Different types of cell junctions can be distinguished: a zonula occludens occurs immediately beneath the microvillar border followed by a gap junction, and the next third of the lateral cell membrane shows features of a septate junction.

If numerous sections of Malpighian tubules are studied it becomes clear that ultrastructural differences occur. At present it is impossible to conclude whether such differences represent discrete segments, as suggested by GAGNEPAIN (1956a) or simply reflect a cyclic activity of the tubules or both. The most important and striking ultrastructural variations are as follows. The foldings of the basal membrane are most elaborated in the middle region of the tubules (Figs.

8 and 11). Sometimes the intermembrane space of these foldings is enlarged; numerous small vesicles close to these foldings suggest pinocytosis (Fig. 7). Both the number and distribution of mitochondria vary. In some sections they are not as numerous as in others, and concentrated in the apical region of the cell, just beneath the microvillous border (Figs. 9 and 11). The height of the micro villi ranges from 1 to 2 μm (Figs. 9 and 10), and often the endings are enlarged and contain numerous small vesicles. The diameter of the lumen of the tubules varies from 2 to 8 μm. Striking differences are found in the kind and the amount of secretion accumulated in the lumen of the tubules (Figs. 8 to 11). In some sections the lumen appears empty, but often the tubules are filled with a substance containing membrane sacs or even multilamellar bodies (Fig. 11).

Just before the tubules join, their ultrastructure changes radically (Figs. 12 and 15). The microvillous border disappears and there are no more foldings of the basal membrane. The cells are filled with numerous vesicles with different electron density, and they show all the typical features of mucus cells. The presence of a mucous segment in the Malpighian tubules of dragonfly larvae was first demonstrated by GAGNEPAIN (1956b) at the light microscopic level. The basal region of mucus cells contains rough endoplasmic reticulum and Golgi complexes occur mostly in the basal region (Figs. 13 and 14). The wall of the common duct consists mainly of mucus cells (Fig. 15). This observation does not agree with that of GAGNEPAIN (1956b) who states that the wall of the common duct consists of non-mucus cells in larvae of *Libellula.*

The Ileum

The ileum contains three special epithelial pads; their ultrastructure suggests they are ion-transporting epithelia (Fig. 16). The basal membrane is highly folded, penetrating almost two thirds of the cell. These foldings, often containing tracheoles, are closely associated with numerous mitochondria, thereby forming a basal labyrinth. The apex of the cell is coated with a multi-layered cuticle, while the cell membrane is much folded, forming numerous micro villi directed to the base of the cells. The lateral cell membranes are highly folded, the interdigitations being closely associated with mitochondria.

CONCLUSIONS

Neither external morphology nor ultrastructure provides evidence of regional differentiation in the Malpighian tubules of *Ischnura elegans.* This is in contrast to many observations of other insects (BERKALOFF, 1960; EICHELBERG & WESSING, 1975; WALL *et al.*, 1975). Further investigations are needed to determine whether the observed ultrastructural differences reflect a morphological and functional segmentation or a cyclic activity or both. The presence of

special transporting epithelial pads in the ileum of these larvae agrees with observations in anisopteran larvae (MOENS, 1980).

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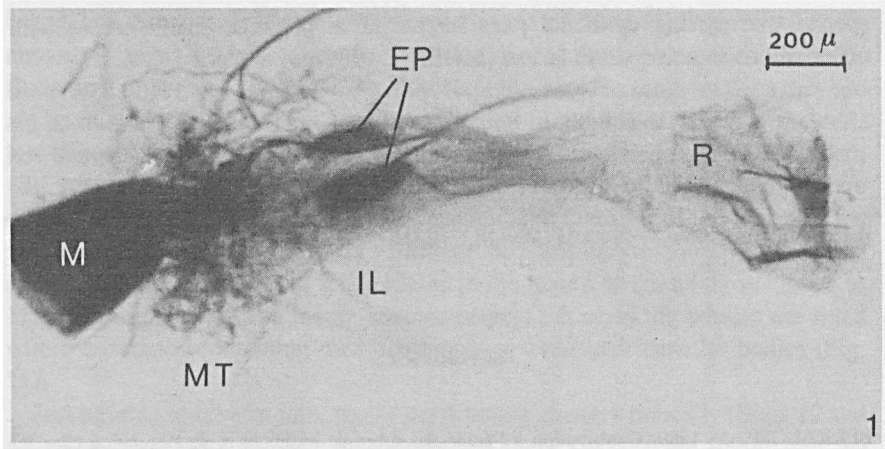


Fig. 1. A portion of the intestine after embedding in araldite. EP, epithelial pads; IL, ileum; M, mesenteron; MT, Malpighian tubules; R, rectum.

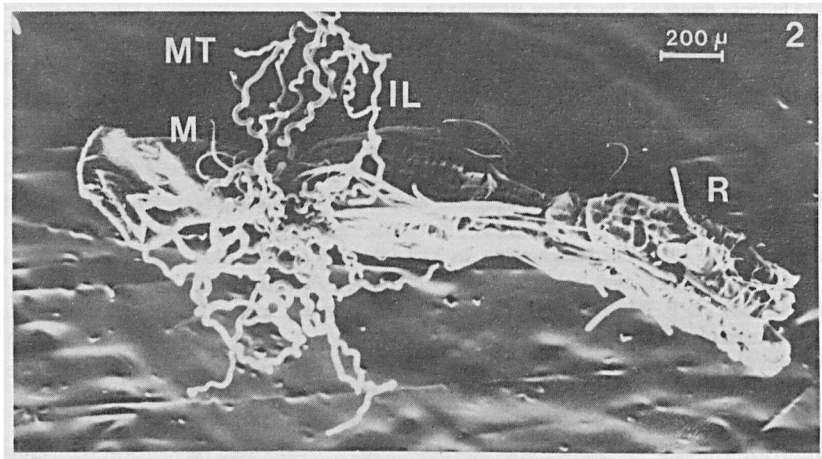
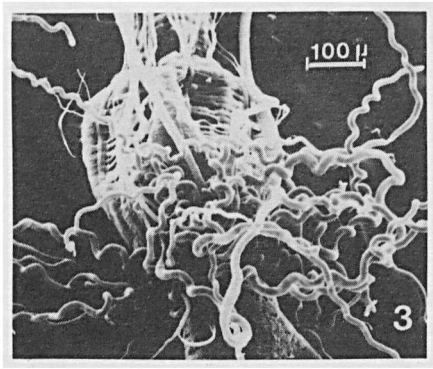


Fig. 2. A portion of the intestine viewed by a scanning electron microscope. Abbreviations as in Figure 1.



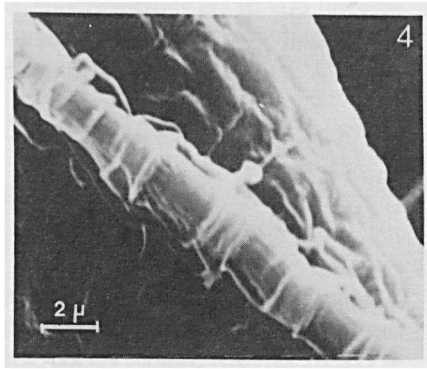
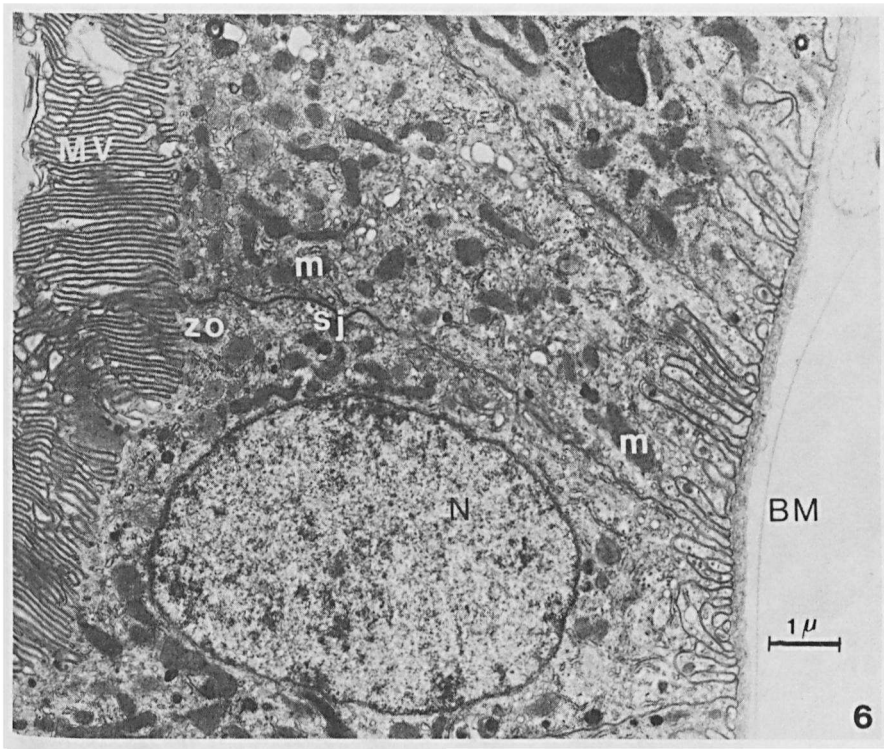
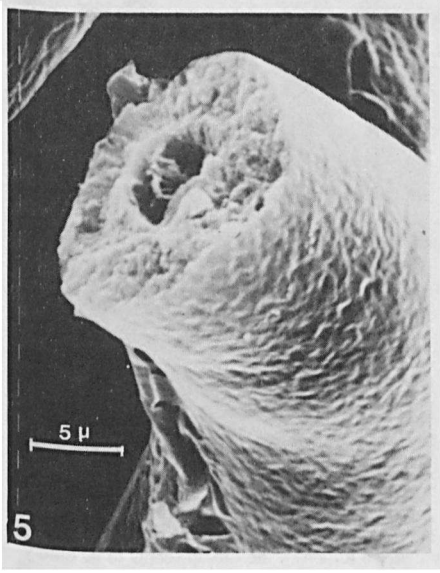
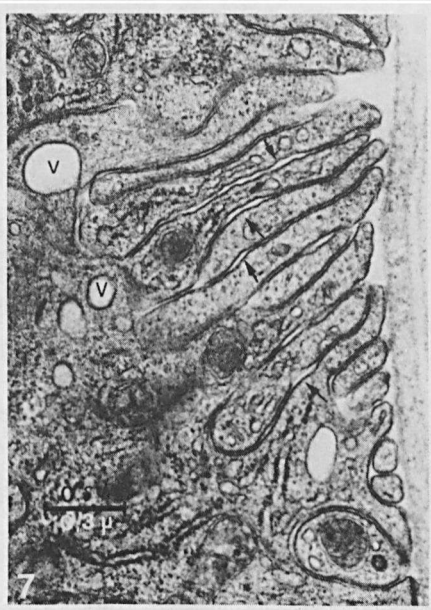


Fig. 3. Scanning electron microscopic view of the Malpighian tubules and ileum.

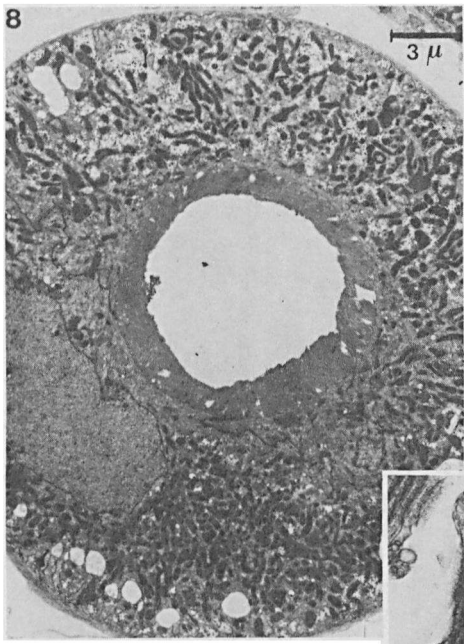
Fig. 4. Detail of the outer surface of a Malpighian tubule showing the muscle attached to the tubule.

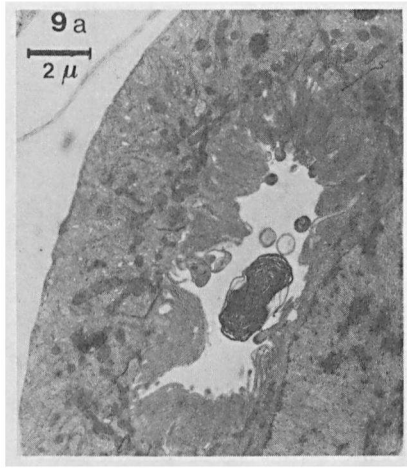


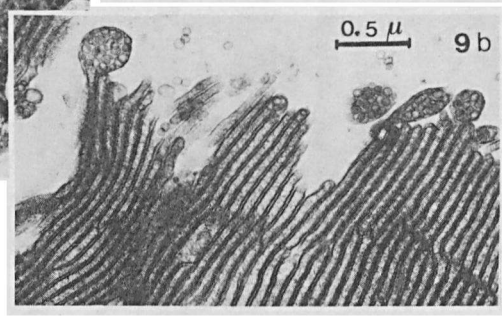


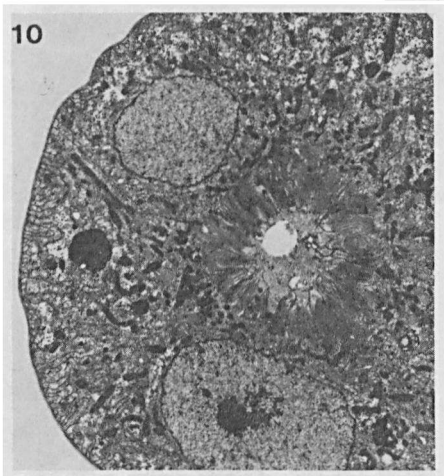


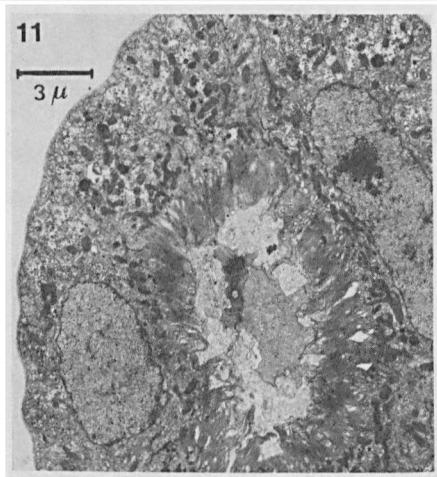
Figs. 5-7. (5) SEM view of a broken Malpighian tubule. (6) Longitudinal section of the tubular epithelium of the Malpighian tubule. MV, microvilli; N, nucleus; m, mitochondrium; BM, basement membrane; sj, septate junction; zo, zonula occludens. (7) Detailed view of the infoldings of the basal membrane of the Malpighian tubule epithelium. The intermembrane space is locally enlarged (arrows) and some vesicles (v) are connected with the basal membrane, suggesting pinocytosis.



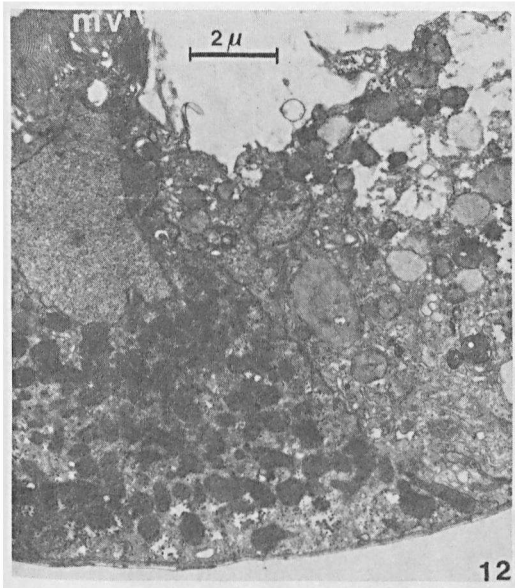


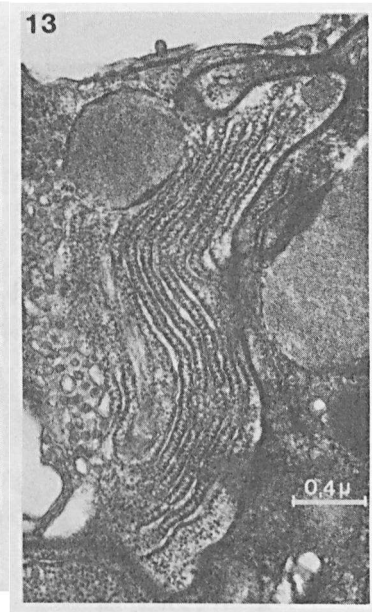


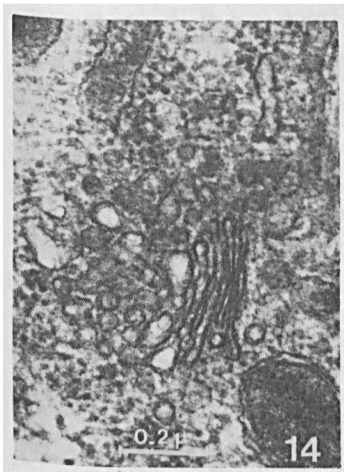


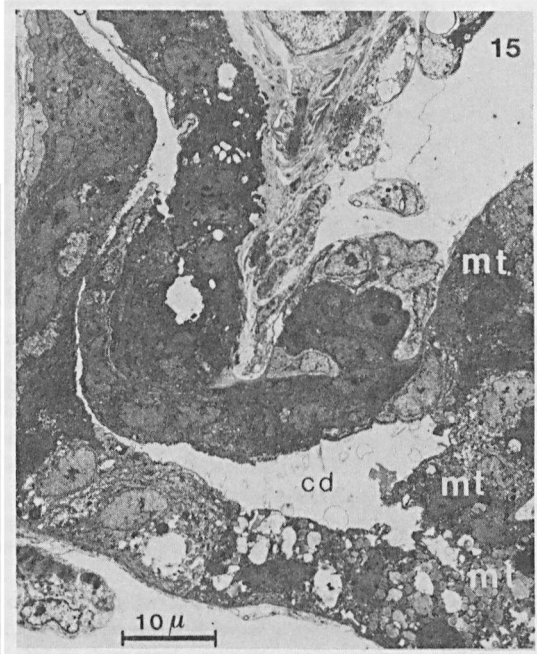


Figs. 8-11. Cross sections of different Malpighian tubules. (8) The numerous mitochondria are rather equally dispersed. The microvillar border is narrow and the lumen of the tubule seems to be empty. Large vesicles are present in the basal region of the cells. (9a) The mitochondria are concentrated beneath the microvilli. Elements, such as a multilamellar body are visible in the lumen. (9b) Detailed view of the apices of the microvilli to show enlargements containing small membrane vesicles. (10) The concentration of mitochondria just beneath the microvilli is not as clear as in Fig. 9a. The lumen of the tubule is very small and filled with a heterogenous substance. (11) Mitochondria are more concentrated just beneath the microvilli; the lumen is larger and filled with a heterogenous substance containing many membranes.









Figs. 12-15. (12) Transition to the mucous region in the Malpighian tubules. The cell on the left side shows the microvilli (mv) and many mitochondria. The adjacent cell (right) has a very different structure, the most striking difference being the presence of numerous vesicles and the absence of microvilli. (13) A portion of the elaborated rough endoplasmic reticulum in a mucus cell of a Malpighian tubule. (14) A Golgi complex from a mucus cell of a Malpighian tubule. (15) Longitudinal section through a common duct opening into the gut. At least 3 Malpighian tubules (mt) join together to form a common duct (cd) which is partially lined by mucus cells. The narrow channel from the common duct to the gut can be seen. The mesenteron is on the right side, the hindgut on the left side. The epithelium of the hindgut is lined with cuticle (c).

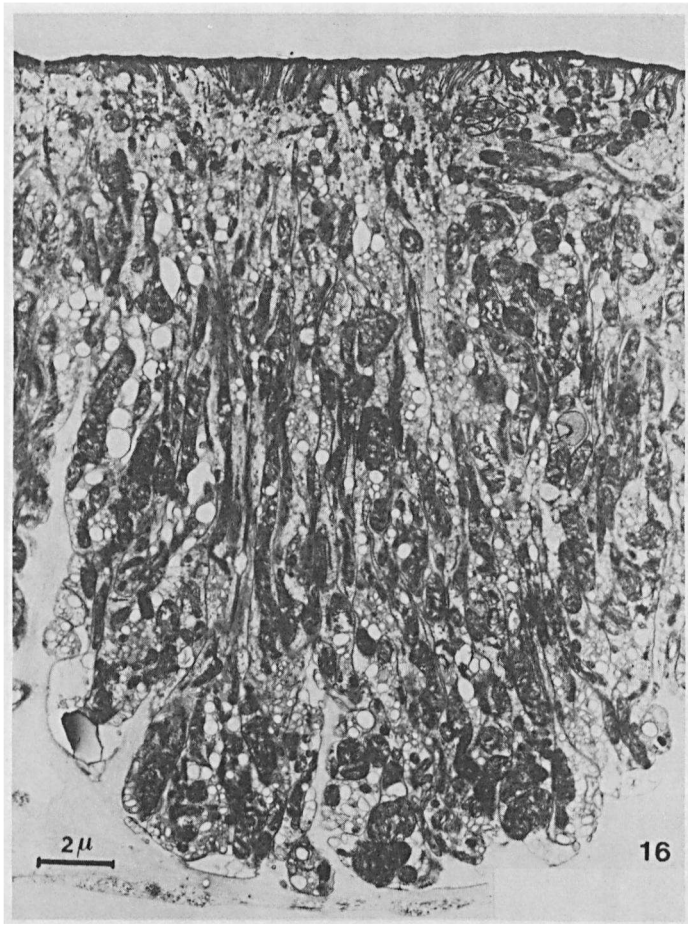


Fig. 16. General view of a special epithelial pad in the ileum. At the apex the cells are lined with a cuticle which covers the microvillar foldings of the apical cell membrane. The basal membrane shows many elaborated infoldings closely associated with mitochondria.