Studies on the Strucutural Basis for Ion-and Water Transport by the Osmoregulatory Organs of Mosquitces, (2). Fine Structure of the Rectal Epithelium of Aedesalbopictus Larua.

Sci. Rep. Kanazawa Univ.,
Vol. 15 No. 1, pp 37—56
June 1970

Studies on the Structural Basis for Ion- and Water-Transport by the Osmoregulatory Organs of Mosquitoes.

(I). Fine Structure of the Rectal Epithelium of *Aëdes albopictus* Larva.

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ABSTRACT The rectal epithelium of the fresh-water mosquito larvae (*Aëdes albopictus*) is described by light and electron microscopy. It is composed of remarkably large cells arranged in a single layer, and is usually thrown into longitudinal folds. The epithelial cells have a thin cuticular lining or intima on the luminal surface. Under the light microscope, a well-marked striated border is observed on the apical border of the cells under the intima. In electron micrographs, the striated border consists of extensive infoldings of the plasma membrane which are in close contact with mitochondria. This apical infolded plasma membrane is characterized by a particulate coat on the cytoplasmic surface of the membrane. The basal surface of the epithelium is deeply infolded to form narrow grooves which branch off in an arborescent appearance in cross sections. The space of grooves is lined by the extension of the basement membrane. The basal plasma membrane facing the grooves as well as that fronting the haemocoel are again thrown into deep, narrow, fine, branching infoldings, but the infoldings are not connected with the apical ones. Numerous mitochondria are lodged in the compartments delimited by the infoldings. In contrast to the apical and basal plasma membranes, the lateral plasma membranes do not have any significant infoldings. Free ribosomes and vesicles of various sizes are found throughout the cytoplasmic matrix. Also found within the cytoplasm are rough ER, microtubules, Golgi complexes, multivesicular bodies and large masses of fine fibrillar material. The cellular morphology of the rectal epithelium correlates well with that seen in other ion-transporting tissues. From the fine structural point of view, it is expected that active ion transport processes occur at both the apical and the basal side of the epithelial cells of the rectum in fresh-water mosquito larvae. The possible roles of these infolded plasma membranes in the ion-transport by the rectum is discussed.

It is generally believed that the rectum in insects is concerned with the reabsorption of salts and water from the excretory fluid of Malpighian tubules, and plays an important part in the maintenance of salt and water balance of the body fluid (Edney, 1957 ; Shaw and Stobbert, 1963 ; Stobbert and Shaw 1964 ; Wigglesworth, 1965). The nature of the work involved in the reabsorption by rectum, however, is not the same in different external environments to which insects have adapted themselves. In the case of fresh-water mosquito larvae, which are not compelled to be so careful of their water supply as in insects of dry habitats and in salt-water larvae, the rectum is responsible for the reabsorption of some inorganic ions, such as potassium and sodium, and consequently for the hypotonicity of the urine (Ramsay, 1950, 1953 ; Clements, 1963), although some reabsorption of water can take place (Wigglesworth, 1933). Similar function of this organ has also been found in some fresh-water larvae of other insects (Boné and Koch, 1942 ; Shaw 1955 ; Sutcliffe, 1961),

The rectum (or colon) in mosquito larvae is used here after Clements (1963) as a dilated posterior portion of the hindgut which is defined as the portion of the alimentary canal between the opening of the Malpighian tubules and anus, being distinctively separated from the anterior portion referred to as the ileum or small intestine and from the posterior portion, the anal canal*. In the rectum of the fresh-water mosquito larvae, as was already mentioned by Christophers (1960) and Clements (1963), there are no structures corresponding to the rectal glands (rectal papillae or pads) which are seen in the adult of mosquitoes and many other insects and assumed to be concerned with an active uptake of ions and water from the rectal contents (Wigglesworth, 1932, 1965). According to the histological observations of Ramsay (1950) and Christophers (1960) on *Aëdes aegypti* larvae, the rectal epithelium is fairly deeply folded and composed of a single layer of relatively large epithelial cells with the thick well-marked striated border along the luminal surface.

Recent electron microscopic observations (Noirot and Noirot-Timothee, 1960 ; Baccetti, 1962 ; Gupta and Berridge, 1966a, b ; Oschman and Wall, 1967) on the rectum (including the rectal glands) of some isopteran, orthopteran and dipteran insects seem to confirm the earlier suggestion by Wigglesworth (1932) and the later found physiological evidences (Shaw and Stobbert, 1963) that this organ is responsible for the reabsorption of water and ions. Further, Berridge and Gupta (1967) has suggested

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* According to Christophers (1960), the dilated portion of the hindgut was referred to as the "colon", and the anal canal as the "rectum".

in the cortical epithelial cells of the rectal papillae of *Calliphora* that, in addition to the leaflets of the plasma membrane facing the lumen of rectum, the lateral plasma membranes, which are folded into stack and closely associated with mitochondria, may play an important role in the active transport of ions and water in the cells. On the rectum of aquatic insect larvae, however, the fine-structural study has not yet been reported.

It is the purpose of this paper to describe the fine structure of the epithelial cells of the rectum in *Aëdes albopictus* larvae as an approach to the problem of reabsorption mechanisms by the rectum.

Material and Methods

Larvae of *Aëdes albopictus*, which were reared from egg to fourth instar in distilled water added a small amount of dry yeast as sustenance, were used. There, the rectum of the larvae is presumed to be working nearly at the maximum to reabsorb salts from the excretory fluid.

A 2.5% solution of glutaraldehyde in 1/15 M phosphate buffer at pH 7.4 containing 0.025 M sucrose was used for fixing the tissue. The eighth abdominal segment including the rectum, or the entire rectum were dissected from the fourth instar larvae, transferred to the fixing solution and maintained at 4° C for one hour. Subsequently the tissue was washed in 1/15 M phosphate buffer solution containing 0.05 M sucrose and maintained at 4° C for one hour. It was then postosmicated for one hour in ice-cold 1% solution of osmium tetraoxide in veronal acetate buffer at pH 7.4 (Palade, 1952).

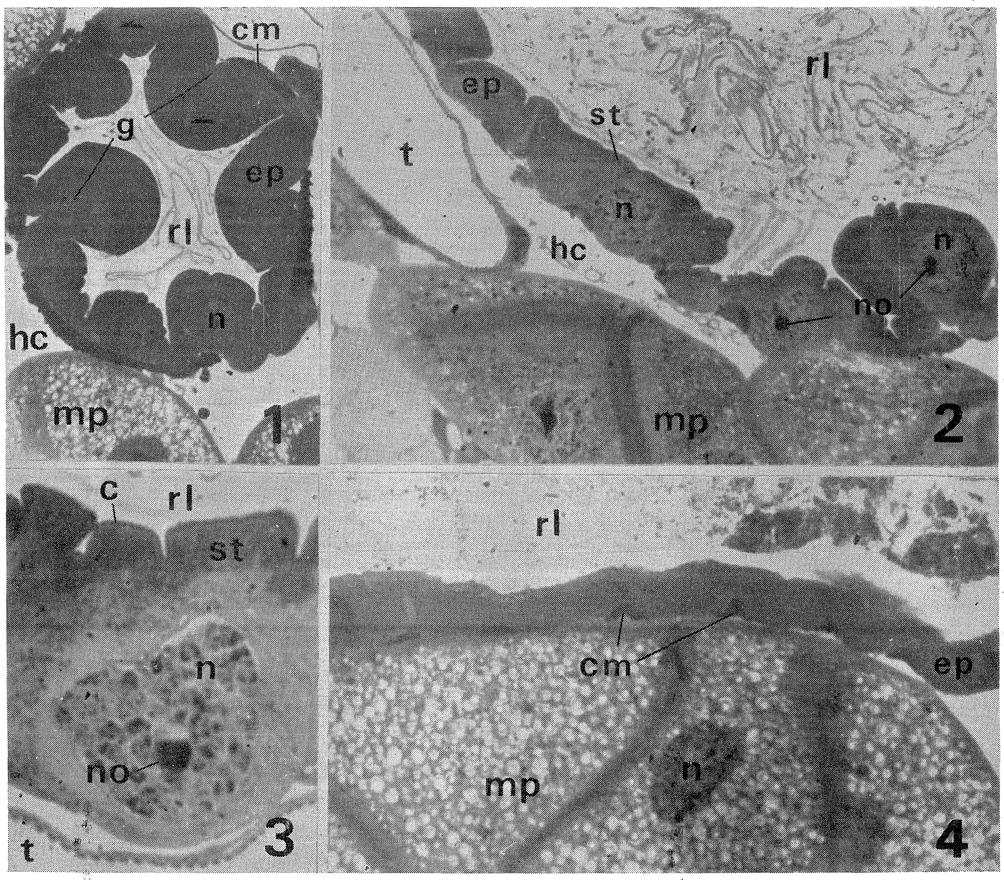
The tissue was rapidly dehydrated in graded acetones and embedded in Epon 812 (Luft, 1961). For electron microscopy, thin sections were stained in a saturated solution of uranyl acetate in distilled water followed by lead hydroxide (Millonig, 1961) and then examined with JEM-6A electron microscope, operated at 80 kv.

For light microscopy, 1μ thick sections of the material embedded in Epon 812 were stained with 1% solution of toluidine blue buffered to pH 7.4 with phosphate buffer (Yamamoto, 1963). For paraffin embedding, the rectum was fixed for 24 hours in Zenker-formol solution and dehydrated in ethanol. Paraffin sections were cut at 8μ and stained with Delafield's haematoxylin and eosin.

Results

Light microscopic observation

The rectum of *A. albopictus* larvae extends from the posterior portion of the seventh abdominal segment to the eighth segment. It is a dilated portion of the hindgut and extremely thinned at the anterior and posterior ends attaching to the small intestine and the anal canal, respectively. The rectal epithelium with a thin



Figs. 1–4 are photomicrographs of 1μ thick sections stained with toluidine blue.

Fig. 1. A cross-section through the rectum showing the longitudinal folds of the epithelium (ep). The basal surface of the epithelium on the coelomic side is deeply infolded to form grooves (g). Arrows point light-staining areas within the epithelial cell cytoplasm. rl : rectal lumen, hc : haemocoel, cm : circular muscle fiber, n : epithelial cell nucleus, mp : Malpighian tubule. ×280.

Fig. 2. A section through the rectum showing that the epithelium (ep) is not deeply folded. The epithelial cell has a large nucleus (n) with a prominent nucleolus (no) and the striated border (st) located along the luminal surface. rl : rectal lumen, hc : haemocoel, mp : Malpighian tubule, t : trachea. ×420.

Fig. 3. Enlargement of the photomicrograph of the rectal epithelial cell showing the thin cuticular lining (c) on the luminal surface, the well-marked striated border (st), the large nucleus (n) with a nucleolus (no) and dark-staining granules within the cytoplasm. t :. trachea × 1,050.

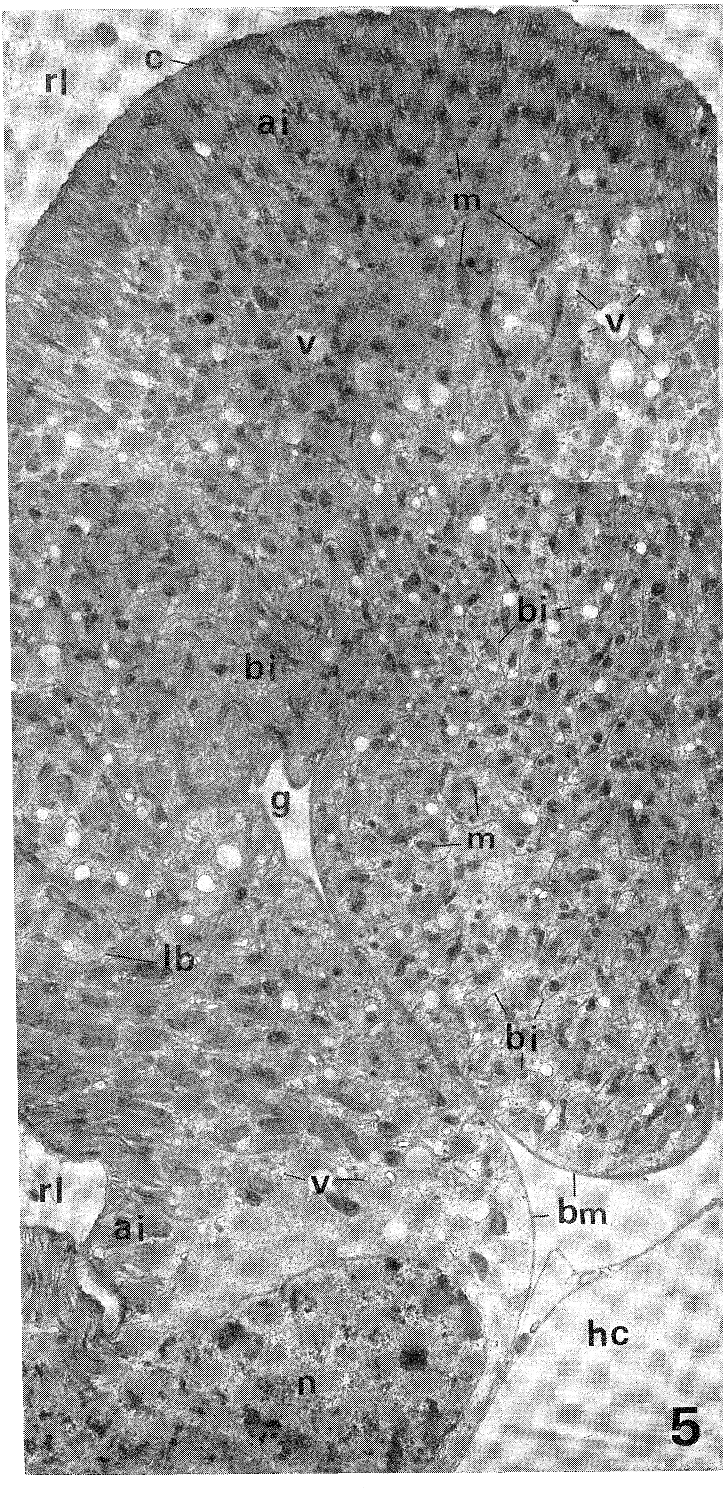
Fig. 4. A longitudinal section through the rectum showing the circular muscle fibres (cm) spaced at short intervals and the Malpighian tubule (mp) placed in contact with the rectum. rl : rectal lumen, ep : rectal epithelium, n : nucleus of Malpighian tubule cell. ×420.

cuticular lining on the luminal surface is usually thrown into longitudinal folds (Fig. 1), but, if the rectum contains large amounts of excreta, it is rather flattened and not folded (Fig. 2). In cross sections the folds are seen as the finger-like projections protruding into the rectal lumen. On the coelomic side of the projections, the surface of the epithelium is deeply infolded to form narrow grooves which extend to the apex of the projections and are oriented in parallel to the longitudinal folds (Fig. 1). The epithelium is composed of a single layer of remarkably large epithelial cells, each of which possesses a prominent oval nucleus usually containing a dark-staining nucleolus (Figs. 2, 3). The cells have the well-marked thick and dense striated border which is continuous along the luminal surface (Figs. 2, 3). In 1μ sections, numerous dark-staining granules are found throughout the cytoplasm and sometimes light-staining areas are also seen (Figs. 1, 3). The basal surface of the epithelial cells is separated from the haemocoel by the basement membrane. External to the basement membrane are circular muscle fibres, spaced at short intervals (Figs. 1, 4). The distal parts of Malpighian tubules are placed in contact with the basal surface of rectum (Figs. 1, 2 and 3), and the tracheae are also distributed with close contact to the surface (Figs. 2, 3).

Electron microscopic observation

The surface of the epithelium facing the rectal lumen is covered with a cuticular intima with two distinct layers (Figs. 7, 14), viz. an outer, very dense layer (ca. 30 $m\mu$ wide), and an inner, much less dense layer (ca. 150 $m\mu$ wide). The plasma membrane of the epithelial cells underlying the intima, or the apical plasma membrane is thrown into an extensive array of deep, narrow, parallel folds that are perpendicular to the long axis of the rectum (Figs. 5, 7 and 14). The infoldings of the apical plasma membrane extend about one-third to one-sixth of the way to the base of cell. The numerous infoldings divide the apical cytoplasm into many compartments of various sizes. In cross sections these compartments resemble the profiles of microvilli (Figs. 5, 7), but, seeing from the sections cut normal to the long axes of them, they are evidently leaflets with the sheet-like nature (Fig. 6). The large compartments are characterized by elongated mitochondria with closely packed distinct cristae and sometimes with fine particles (about 100 Å in diameter) in the mitochondrial matrix (Fig. 7). These mitochondria are in intimate contact with the infolded plasma membranes (Figs. 6, 7 and 8). Cisternae of rough endoplasmic reticulum, small vesicles and microtubules are also contained in the compartments (Figs. 6, 7). Relatively narrow compartments usually contain all of the organelles mentioned above except for mitochondria, but in very narrow ones nearly no organelles are recognized (figs. 6, 7). The apical infoldings combined with mitochondria probably correspond to the apical striated border observed with the light microscope.

The space separating the paired plasma membranes of apical infoldings has a fairly constant width of about 100 to 150 Å but the tips or edges of the infoldings are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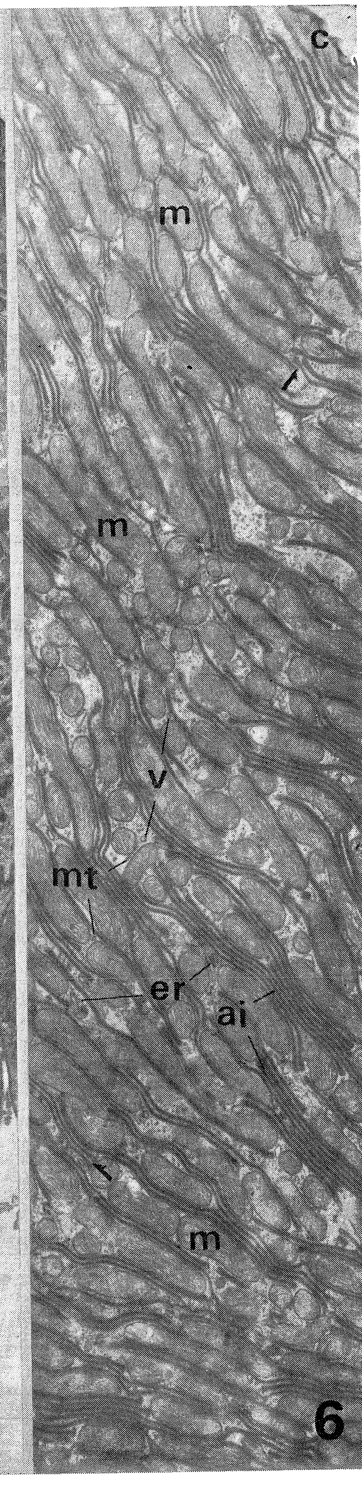
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slightly expanded, and it is composed of a material of the moderate electron opacity (Figs. 6, 7 and 8). In some cases, the paired plasma membranes are very closely attached to each other (Fig. 6)

The cytoplasmic surface of the apical infolded plasma membranes is covered with an electron-dense layer of very uniform width, about 150 Å, which is further resolved into particulate subunits (Figs. 6, 7 and 8). The exact shape of these subunits is yet difficult to determine. The particulate coat is confined to the apical plasma membrane and is not observed on any other membranes in the present material.

The basal surface of the cells is covered with a thin basement membrane which has a fairly constant width of about 80 mμ and consists of a felt-work of fine fibrillar material (Figs. 5, 12 and 13). The grooves, mentioned in the light microscopic description, often branch off to narrower grooves, extending to the apical and lateral regions of cytoplasm, and thus present an arborescent aspect in cross sections (Figs. 5, 9 and 11). These grooves are lined by the extension of the basement membrane underlying the basal plasma membrane (Fig. 12) and sometimes contain tracheal branches in them (Fig. 11). The basal plasma membrane facing the grooves turns inward to form deep, narrow, branching infoldings that extend for variable distances into the basal region of cells (Figs. 5, 9, 11 and 12). The basal plasma membrane facing the innermost recesses of the grooves is usually again infolded to extend more deeply into the apical cytoplasm, but they are not connected with the apical plasma membrane (Figs. 5, 9 and 11). The basal plasma membrane, except for the part facing the grooves, is also thrown into deep infoldings, but these infoldings appear to be relatively few in number (Fig. 10). The basal infoldings are not lined with the basement membrane unlike the grooves. The numerous infoldings of the basal plasma membrane divide the cytoplasm into numerous compartments of various sizes and shapes. Some of the compartments, in sections, are completely enclosed by a

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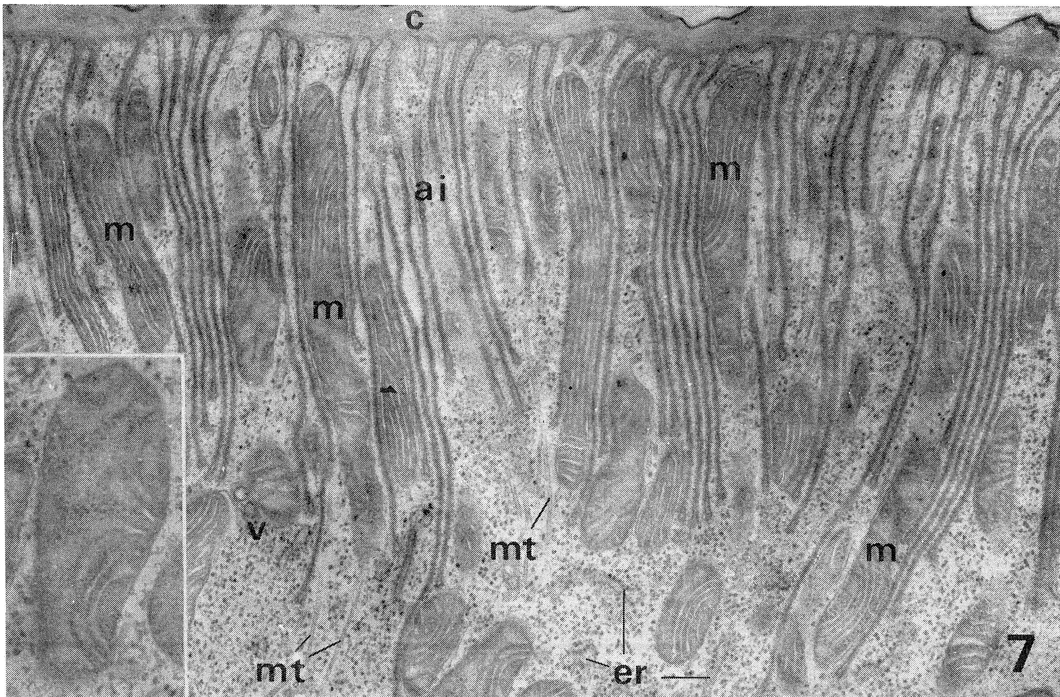
Fig. 5. A low magnification electron micrograph showing a longitudinal fold of the rectal epithelium. Note the infoldings (ai) of the apical plasma membrane underlying the thin cuticular intima (c) facing the rectal lumen (rl). The basal surface of cells facing the haemocoel (hc) is deeply infolded to form a groove (g) which is lined with the extension of the basement membrane (bm). Note the infoldings (bi) of the basal plasma membrane facing the groove. Numerous mitochondria (m), which appear as electron dense bodies, lie between the apical infoldings and in the cytoplasmic compartments delimited by the basal infoldings, and they are also dispersed in the rest of the cytoplasm. Abundant ribosomes and vesicles (v) of various sizes are seen throughout the cytoplasm. The nucleus (n) of the cell contains some masses of chromatin material. lb : lateral boundary of adjoining epithelial cells. ×3,200.

Fig. 6. A section through the apical region of the cell showing the apical infoldings (ai) to form leaflets, and numerous mitochondria (m) intimately associated with them. Marginal ends of the apical infoldings are slightly expanded (arrows). Cisternae of rough ER (er), sectioned microtubules (mt) and small vesicles (v) are found within the cytoplasmic compartments. c: cuticular intima. ×12,000.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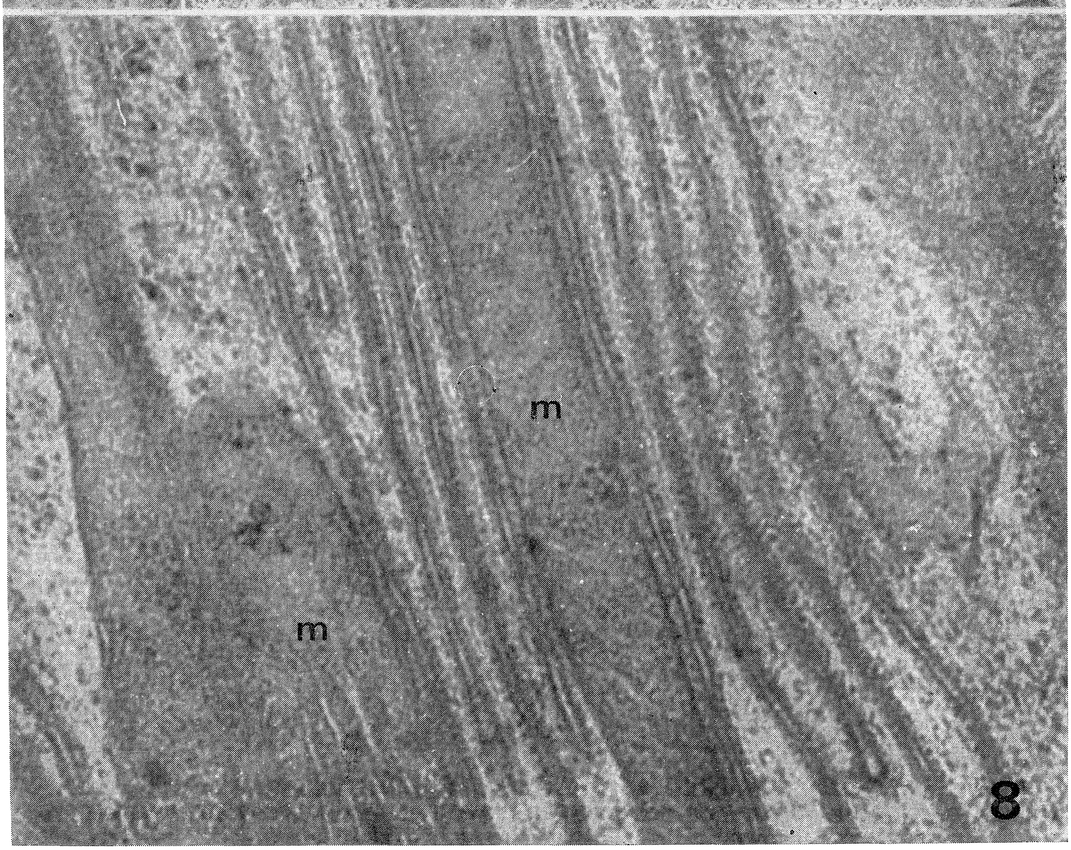
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continuous infolding that can be traced arround them and backs again to the basal surface (Fig. 11). The compartments contain mitochondria with distinct tightly packed cristae, in company with ribosomes, vesicles of various sizes, cisternae of rough endoplasmic reticulum and microtubules (Figs. 5, 9, 11, 12 and 13).

The space separating the paired plasma membranes of basal infoldings has a relatively constant width of about 200 to 350 Å, and is composed of a material of the low electron opacity (Figs. 12, 13). The cytoplasmic surface of these membranes is covered with felt-like material (Fig. 13).

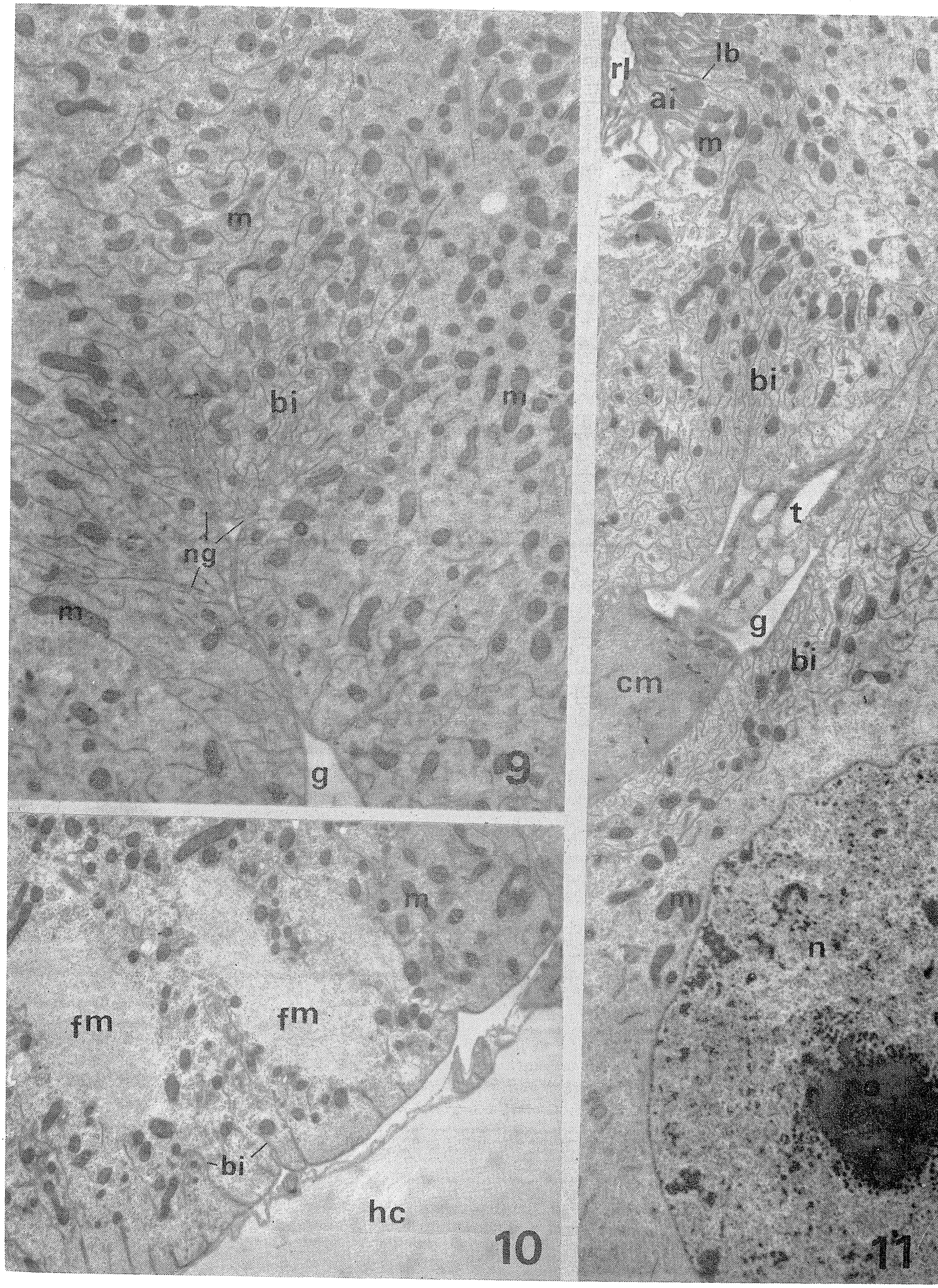
The cytoplasmic surface of the basal plasma membrane is often studded with dome-shaped dense material resembling hemidesmosome. This is also seen on the plasma membrane facing the grooves (Fig. 12).

The lateral boundary between the neighboring cells is clearly recognized in the electron micrograph (Figs. 5, 11). The lateral plasma membranes are relatively straight and apposed closely to the neighboring one, with a fairly constant width (about 250 to 350 Å) (Fig. 15). Near the cuticular surface the lateral plasma membranes form a terminal bar and a septate desmosome, being sometimes followed by an area of close apposition, probably a ***zonula occludens*** (Fig. 14). The lateral plasma membranes are devoid of any notable infoldings in contrast to the apical and basal plasma membranes.

An abundance of both free ribosomes and vesicles of various sizes is found within the cytoplasmic matrix of the cells (Figs. 5, 7, 12 and 13). Also scattered within the cytoplasm are cisternae of rough endoplasmic reticulum (Figs. 7, 12 and 13). The relatively small Golgi complexes, being found in the perinuclear region as a rule, are composed of three to five cisternae, usually with vesicles at the free ends (Figs. 12, 13). Microtubules, approximately 200 to 250 Å in diameter, and multivesicular bodies (Fig. 16) are found throughout the cytoplasm. Mitochondria may be distributed throughout the cytoplasm but their dense population is always observed in the apical cytoplasm and the compartments of the basal cytoplasm of the cells. Sometimes the cytoplasm contains some large masses of fine fibrillar material (Fig. 10), which probably correspond to the light-staining areas observed with the light microscope. Infrequently, one sees in the cytoplasm of the cells membrane-bound granular bodies containing fibrillar material (Fig. 17).

Fig. 7. A field from the apical region of the cell showing the cuticular intima (c), the apical infoldings (ai) closely associated with mitochondria (m), small vesicles (v), rough ER (er) and microtubules (mt). The cytoplasmic surface of the apical infoldings is uniformly covered with an electron-dense particulate coat. Some mitochondria contain fine particles (arrow) within their matrix. ×25,600. Inset shows a mitochondrion with fine particles at higher magnification. ×40,000.

Fig. 8. A small portion of the apical infoldings, shown at higher maginfication to reveal that the membranes are covered with a regular array of particulate subunits on the cytoplasmic surface. m: mitochondria. ×80,000.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The nucleus is oval in shape, having sometimes indentations (Figs. 5, 11 and 12). The nuclear membrane is double, and the outer element facing the cytoplasm is frequently studded with ribosomes. The membranes are separated by a space of low eletron opacity and of a fairly constant width (about 200 to 350 Å). The nucleus has many nuclear pores, about 450 Å in diameter. The nucleoplasm is granular, in which are suspended many masses of chromatin material. The nucleolus is composed of very tightly packed, electron-dense, round granules (Fig. 11).

Discussion

The fine structure of the epithelial cells of the rectum of *A. albopictus* larvae resembles in many respects the picture found in other types of cells regarded as transport cells. The most prominent feature of the cells is the deep infoldings of apical and basal plasma membranes closely associated with mitochondria. This feature is very suggestive of the modifications at the base of renal tubular cells in mammals, described by Sjöstrand and Rhodin (1953), Pease (1955) and Rhodin (1958). Similar specializations of the plasma membrane with close mitochondrial association have been described in various ion- and water-transporting tissues, such as renal tubules of frog (Karnovsky, 1961) and of marine teleost with glomerular kidney (Steen Olsen, 1966), nephridial tubules of crayfishes (Beem, et al., 1956 ; Miyawaki and Ukeshima, 1967), nasal glands of marine birds (Doyle, 1960 ; Komnick, 1963), rectal glands of dogfish (Bulger, 1963), striated ducts of parotid and submaxillary glands of mammals (Scott and Pease, 1959 ; Rutberg, 1961), and Malpighian tubules (Beem, et al., 1955 ; Smith and Littau, 1960 ; Wigglesworth and Salpeter, 1962), gastric caeca (Jones and Zeve, 1968), midgut (Anderson and Harvey, 1966) and anal papillae (Copeland, 1964 ; Sohal and Copeland, 1966 ; Mashiko and Asakura, 1968) of insects. The present author (MS) has also observed such a close association between infolded plasma membranes and mitochondria in both the apical and the basal region of rectal epithelial cells in other fresh-water mosquito larvae, such as *Aëdes hatorii, Armigeres obturbans* and *Culex pipiens pallens.*

In general, it is believed that the infoldings of the plasma membrane intimately

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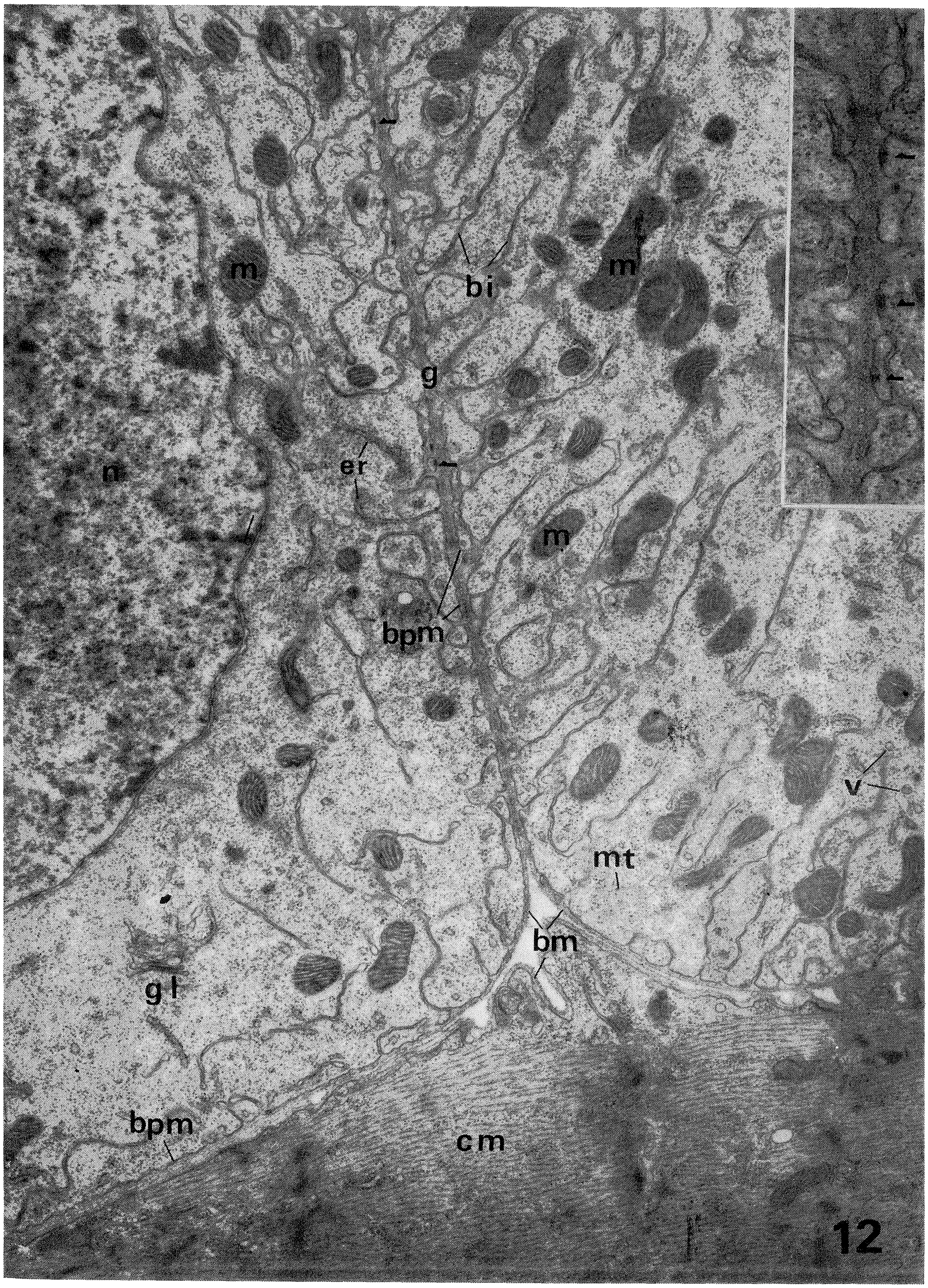
Fig. 9. A section through the basal region of the cell showing the groove (g) which branches off to narrower grooves (ng), infoldings (bi) of the basal plasma membrane facing the grooves, and mitochondria (m) lodged in the compartments delimited by the infoldings. ×6,000.

Fig. 10. A section through the basal region of the cell showing a few infoldings of the basal plasma membrane facing the haemocoel (hc). Large masses of fine fibrillar material (fm) are seen within the cytoplasm. m : mitochondria. ×4,500.

Fig. 11. A micrograph showing the numerous infoldings (bi) of basal plasma membrane, a large nucleus (n) with a prominent nucleolus (no) and a tracheal branch (t) in the groove (g). rl : rectal lumen, ai : apical infoldings, lb : lateral boundary, m : mitochondria, cm : circular muscle fiber. ×4,500.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associated with mitochondria play an important role in the transport of ions (FAWCETT, 1962 ; SCHMIDT-NIELSEN, 1965 ; LATTA, et al., 1967). The studies of CLARK (1957) on the development of basal infoldings in renal tubules, the findings of RHODIN (1958) and STEEN OLSEN (1966) on fine-structural differences in the proximal tubules between glomerular and aglomerular fishes, and those of SOHAL and COPELAND (1966) on ultrastructural variations in the anal papillae of mosquito larvae at different environmental salinities indicate that the infoldings of the plasma membrane of these cells are of importance for reabsorptive or absorptive processes. Recent studies by electron microscopic histochemistry show that in renal tubules and in rectal papillae the ATP-ase activity is found to be associated with the infolded plasma membrane (GOLDFISCHER, et al., 1964 ; BERRIDGE and GUPTA, 1968). The localization of this enzyme suggests that these membranes are the site of active transport, presumably of sodium or potassium which may be the main substances actively transported by these tissues. This view, at least in the renal tubules, is in accordance with the physiological evidences, which indicate that the active transport pump for the uphill movement of sodium ion is localized in the basal membrane (PITTS, 1963).

As previously mentioned by FAWCETT (1962), the infoldings of the plasma membrane may contribute to a magnification of surface area available for the transport, and many mitochondria lying close to the infoldings probably provide the energy for the active transport of ions across the membrane. In the case of rectal epithelium of freshwater mosquito larvae, therefore, active processes can be expected to occur at both the apical and the basal side of the cells.

The true role of the apical infoldings in the ion transport by rectum is not yet quite clear. The close contact of the apical plasma membrane with mitochondria similar to the present case was also found in the hindgut of *Macrosteles fascifrons* (SMITH and LITTAU, 1960), in the rectum of *Acheta domestica* and termites (NOIROT and NOIROT-TIMOTHEE, 1960) and in the rectal pads of *Periplaneta americana* (OSCHMAN and WALL, 1967).

The coat of particulate subunits on the cytoplasmic surface is another feature of the apical plasma membrane. The particulate coat with a close resemblance to this has been described in the rectal papillae of *Calliphora erythrocephala* by GUPTA and BERRIDGE (1966a), and of *P. americana* by OSCHMAN and WALL (1967). In the rectum of salt-water *Aëdes togoi* larvae, the present author (MS) has also observed a similar

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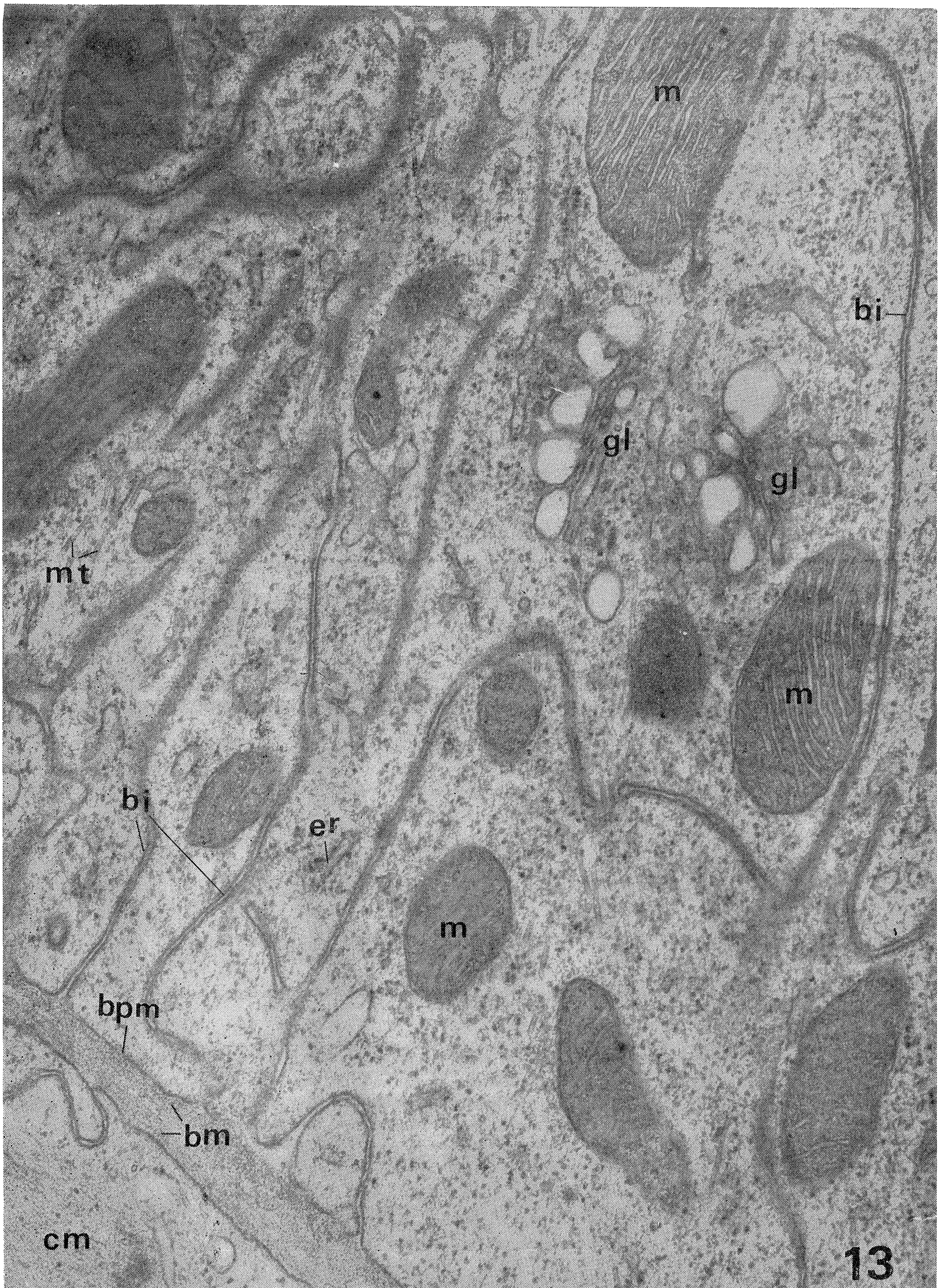
Fig. 12. A small field from the basal region of the cell showing the numerous infoldings (bi) of the basal plasma membrane (bpm) facing the narrow groove (g) lined with the extension of the basement membrane (bm), mitochondria (m) associated with the infoldings, Golgi complex (gl) near the nucleus (n), rough ER (er), small vesicles (v) and microtubules (mt). Free ribosomes are seen within the cytoplasm. Note the dome-shaped dense material (arrows) on the cytoplasmic surface of the basal plasma membrane. cm : circular muscle fiber. ×15,000. Inset shows the dense material (arrows), resembling the hemidesmosomes, on the cytoplasmic surface of the basal plasma membrane at higher magnification. ×28,000.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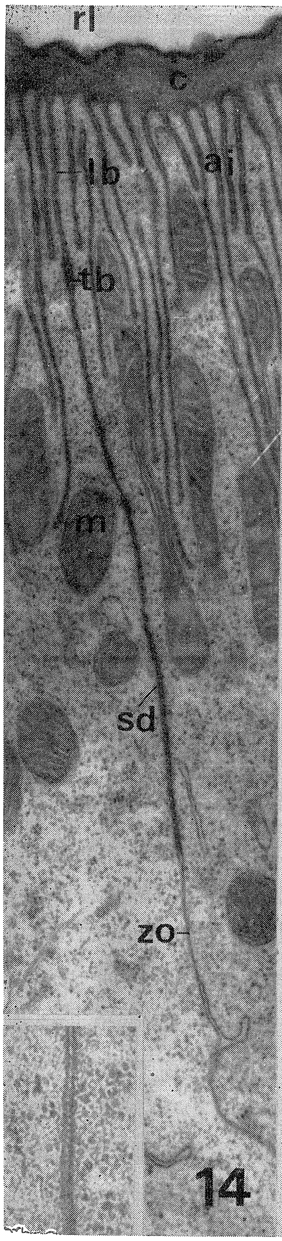
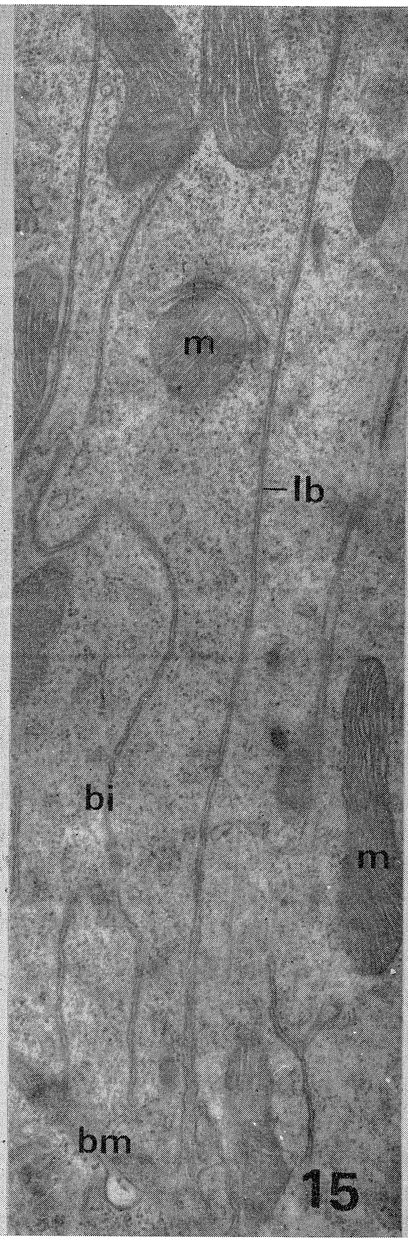
coat on the apical infolded plasma membrane with close mitochondrial association. Such a similarity in fine structure seems to indicate that some common ion-transport mechanism across this membrane may exist in different insects of different environments. Schmidt-Nielsen (1965) suggested that the apical infoldings might be concerned with the secretion of ions into the rectal lumen. According to Gupta and Berridge (1966a), one of the physiological functions localized in the apical plasma membrane of rectal papilla cells, may be an active secretion of hydrogen ions either alone or as a part of the exchange diffusion mechanism for the uptake of potassium, resulting in acidification of the rectal fluid. Further, they mentioned that the particulate subunits on the membrane might be also involved in some of these transport processes. In the rectum of *A. aegypti* larvae, the secretion of hydrogen ions was already suggested by Ramsay (1950, 1956) who found that, in the larvae allowed to ingest phenol red, the intestinal fluid became acid about one minute after reaching the rectum. Ramsay (1953) also found that potassium is the predominant cation in the excretory fluid of Malpighian tubules, and that in the larvae kept in distilled water most of the ions is reabsorbed by the rectum.

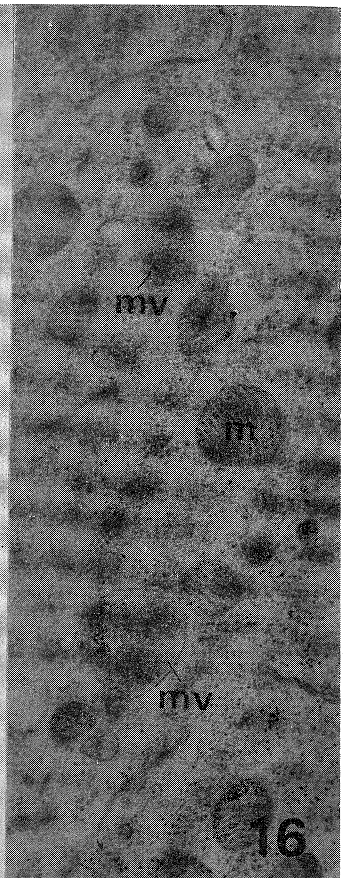
The basal infoldings with close mitochondrial association are one of the characteristic structures in the rectal epithelial cells of fresh-water mosquito larvae. They may play a part in the transport of sodium by the rectum, as in those of renal tubular cells which are believed to be the site of the active sodium pump in the cells (Schmidt-Nielsen, 1965 ; Latta, et al., 1967). According to the physiological study of Ramsay (1953) on *A. aegypti* larvae, sodium is almost completely reabsorbed by the rectum of the larvae kept in distilled water, although the concentration of sodium in the excretory fluid from Malpighian tubules is much lower as compared with that of potassium. He found that this reabsorption takes place against a concentration gradient between rectal fluid and haemolymph. In the latter the sodium concentration is over three times higher than that in the former.

The extensive infoldings of the lateral plasma membranes closely associated with mitochondria as described in the rectal papillae and pads of some insects (Gupta and Berridge, 1966a, b ; Berridge and Gupta, 1967 ; Oschman and Wall, 1967), are not found in the present study. This difference may be attributable to the role of the rectum in the reabsorption of ions and water, for the rectum of fresh-water mosquito larvae does not possess the ability to elaborate the hypertonic urine, unlike terrestrial insects as well as salt-water larvae (Ramsay, 1950 ; Edney, 1957 ; Clements, 1963 ; Shaw and Stobbert, 1963 ; Stobbert and Shaw, 1964).

Fig. 13. A small field from the basal region of the cell showing the basal infoldings (bi) covered with felt-like material on the cytoplasmic surface of the membrane, mitochondria (m) closely associated with the infoldings, Golgi complexes (gl), microtubules (mt), rough ER (er) and abundant ribosomes within the cytoplasm. bm : basement membrane, cm : circular muscle fiber. ×40,000.







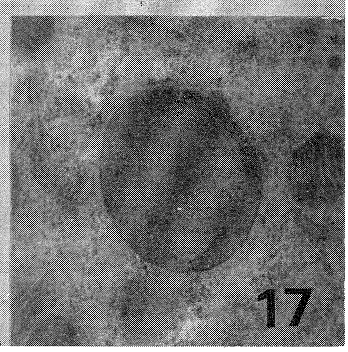


Fig. 14. A section through the apical region of the cells showing the apposed lateral plasma membranes (lb) with a terminal bar (tb), a septate desmosome (sd) and a *zonula occludens* (zo). ai : apical infoldings, m : mitochondria. × 16,800. Inset shows a septate desmosome at higher magnification. ×44,800.

Fig. 15. A section through the basal region of the cells showing the apposed lateral plasma membranes without any notable infoldings. bm : basement membrane, m : mitochondria, bi : basal infoldings. ×24,000.

Fig. 16. A portion of the cytoplasm of the cell showing multivesicular bodies (mv). m : mitochondria. ×16,800.

Fig. 17. A micrograph showing a cytoplasmic inclusion. ×15,000.

Acknowledgement

The author wishes to express his sincere thanks to Dr. Kikuya MASHIKO, Professor of Zoology at the University of Kanazawa, for many helpful discussions and suggestions during this work and for improvement of the manuscript. He is also indebted to Assistant Professor Kenji JOZUKA, University of Kanazawa, for helpful discussions, and to Dr. Yoshikazu KISHIDA, University of Kanazawa, who gave him valuable advice on electron microscopical techniques.

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