

Ultrastructure of Malpighian Tubules and Rectal Epithelia of the Locust *Poekilocerus bufonius* Klug (Orthoptera)

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ABSTRACT. The Malpighian tubules and rectum of the locust *Poekilocerus bufonius* were prepared for transmission electron microscopy (TEM). In both the Malpighian tubules and rectal epithelial cells, apical and basal modifications of the plasma membrane and mitochondria in between were demonstrated. Such arrangement may play a role in osmoregulatory function and increase surface area for reabsorption of water and recovery of mineral constituents. This morphological information would substantiate biochemical and physiological methods used to elucidate resorptive and excretory functions of these organs in *P. bufonius* feeding on a *Calotropis procera* and synthesize calotropin glucoside.

The fine structure of Malpighian tubule cells of the grasshopper has been studied by Beams *et al.* (1955), Berkaloff (1958, 1959, 1960), Tsubo and Brandt (1962), Wiggelsworth and Salpeter (1962) and by Anstee (1977) and Charnley (1982). The rectum in *Calliphora erythrocephala* has been examined by Gupta and Berridge (1966) and Bacetti (1962).

The present study deals with the fine structure of Malpighian tubules and the rectum of *Poekilocerus bufonius* in order to provide a basis for further histochemical and biochemical investigations.

Materials and Methods

Malpighian tubules and rectal tissues of 5 *P. bufonius* were dissected, fixed for TEM in 4% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.3) for 3 hrs

washed in cold buffered at 4°C. Postfixation was conducted for 2 hrs by immersing the tissues in ice cold 1% OSO_4 maintained at pH 7.3 with cacodylate buffer, dehydrated in acetone and embedded in Epon 812.

Thick sections (1μ) were stained with toluidine blue, whereas thin sections were stained with lead citrate and uranyl acetate for 30 min, prior to their examination with Jeo1100 CX TEM.

Results

Malpighian Tubules

The Malpighian tubules of *P. bufonius* were observed in profiles after sectioning. The epithelial cells were simple cuboids containing round or oval nuclei with well distributed chromatin granules in the karyoplams (Fig. 1). The tubule epithelium rested on a basement membrane surrounded by connective tissue containing collagen fibrils, tracheoles (Fig. 4), nerve endings and infrequent muscle fibers. The apical portion of the Malpighian tubule cells had a striated border consisting of thin, long and regularly packed microvilli (Figs. 2, 6 and 8). The microvilli were of variable length and separated by small intervillous spaces equal to or greater than their own diameters. Mitochondria from the luminal cell cytoplasm could be seen extending into the lumen of the microvilli (Figs. 1,2 and 8). Electron dense pinocytotic vesicles were observed in close contact with microvilli. Mitochondria were abundant and usually cylindrical in shape and few rounded profiles were encountered (Figs. 3,7 and 8). Internally, the mitochondria demonstrated few cristae and tubules in a matrix of moderate density (Figs. 3 and 7). The mitochondria were distributed throughout the cytoplasm with the exception of the basal and apical portion of the cells which showed a higher concentration of mitochondria and some appeared to be streaming through the microvilli (Figs. 2 and 8). The mid-part of the cell contained rounded nucleus (Figs. 1 and 5), PER, free ribosomes and glycogen granules. The basal plasma membrane of Malpighian tubule cells demonstrated infoldings with mitochondria in between (Fig. 3).

Rectum

The rectal epithelial cells were simple, cuboidal and covered with thick, colorless laminated cuticle (Figs. 8 and 14). The nuclei were oval to round, irregular in outline with large (heterochromatin) and fine (euochromatin) chromatin granules distributed in the nuclei with a very thin chromatin rim attached to the inner nuclear membrane (Figs. 9 and 14). The epithelial cytoplasm contained mitochondria and cisternae of rough endoplasmic reticulum (Figs. 11 and 14). Many of the mitochondria were closely associated with the folded plasma membrane (Figs. 13 and 15). The apical plasma membrane was characterized by

extensive invaginations in the form of leaflets (Figs. 10 and 13) extending within the luminal part of the epithelial cells. Mitochondria were also seen between these invaginations. At the basal region of epithelial cells mitochondria were encountered associated with the invaginated basal plasma membrane. The mitochondria throughout the rectal epithelial cell are of moderate electron density and showed few cristae and tubules. A well defined basement membrane, collagen fibrils, striated muscles, tracheoles (Figs. 11 and 12), nerve endings and fibroblasts were found outside the epithelial cells. Close to the apical surface of the cells the lateral plasma membranes were firmly attached by a series of septate desmosomes (Figs. 14 and 15). In the luminal portion of some rectal epithelial cells, endocytotic vesicles were numerous near the cuticle (Fig. 16).

Discussion

In both the Malpighian tubules and rectal epithelial cells of *P. bufonius*, folded plasma membranes were found with mitochondria closely associated with those infoldings. Similar observations have been reported in the epithelial cells of Malpighian tubules in *Calliphora erythrocephala* (Berridge and Oschman 1969), in *Carausius morosus* (Taylor 1971), in *Periplaneta americana* (Wall *et al.* 1975), in *Jamaicana flava* (Peacock and Anstee (1977b) and in *Locusta migratoria* (Bell and Anstee 1977 and Charnley 1982).

Infoldings of the rectal epithelial cells plasma membranes and the association of mitochondria with such membranes have been also recorded in other species such as *Calliphora erythrocephala* (Gupta and Berridge 1966, Berridge and Gupta 1967, Oschman and Wall 1969) and *Jamaicana, flava* (Peakcock and Anstee 1977a).

Gupta and Berridge (1966) believed that such arrangements play a vital part in osmoregulatory function. Mitochondria from the luminal cytoplasm of the Malpighian tubule cells extending into the core of microvilli are essentially the same as described in other insects. The apical and basal elaborations of Malpighian tubules, the association between mitochondria and the membranes, and the extending of mitochondria into the core of microvilli indicate that these regions are of high energy demand. A number of functions have been assigned to the membrane elaborations, but many physiologists regard the geometry of the elaboration as being of particular significance in understanding the mechanism whereby fluid moves from haemolymph across the epithelial cells into the lumen of the tubule (Peakcock and Anstee 1977 a&b). According to Lehninger (1956), the most fundamental function of mitochondria are electron transport with its coupled phosphorylation and secretory activity. This involved the property of actively accumulating or secreting water, electrolytes and presumably other substances

against a gradient. It is also suggested that the microvilli filaments could be derived from mitochondria.

Based on results obtained in the present investigation, the rectal epithelial cells of *P. bufonius* showed profound inflexions of the plasma membrane of the neighbouring cells at the area of contact between the cells, particularly the apical 1/3 of the cell. The presence of septate desmosomes at the lateral plasma membranes of the rectal epithelial cells of *P. bufonius* may provide high resistance to intercellular movement of ions between the inside and outside the rectal epithelial cells and may facilitate lateral transfer of these ions and molecules from one cell to the next along the epithelium.

The rectal cells of *P. bufonius* were characterized by extensive invaginations of the apical plasma membrane (Leaflets) extending into the body of the cell with a large number of mitochondria in between. This is contrary to the Malpighian tubule cells which demonstrated extensive microvilli projecting into the lumen and mitochondria streaming through these microvilli. The infolding of the plasma membrane may serve to increase the surface area of the cell (Pease 1956). The study by Phillips (1964a, 1964b and 1964c) on metal absorption in the desert Locust, *Schistocerca gregaria*, points out that the water is actively absorbed from the lumen of the rectum against an osmotic gradient and in the absence of significant net flux of solute. Phillips (1964a) suggested the possibility of more rapid ion exchange across the epithelial membranes. The basal plasma membranes were infolded in both the Malpighian tubules and rectal epithelial cells of *P. bufonius*. Rhodin (1958) suggests that the laminated cytoplasm was necessary for resorptive processes possibly with the intercellular space serving as pathway for fluid resorption on the brush border.

Berridge and Oschman (1972), Maddrell (1980) and Phillips (1980) have surveyed the physiology and ultrastructure of various transporting epithelial cells and pointed out how the local osmosis and standing gradient (Diamond and Bossert 1968) can help to explain the mechanism of fluid transport. A number of models have been proposed to explain ion and fluid secretion across epithelial cells (Berridge and Oschman 1969, Maddrell 1971, 1977, and Phillips 1980, 1981). Almost all the proposed models require the occurrence of active ion transport across the basal and apical cell membrane, and that K or Na⁺ are transported into the lumen by apical electrogenic cation pumps (Anstee *et al.* 1979, 1986). It is known that (Na⁺ - K⁺) - ATPase is involved in translocation of Na⁺ to the outside and K⁺ to the inside of the cell membrane.

The Locust *P. bufonius* feeds and consumes *Calotropis procera*, a plant which contains cardiac glycosides (Duffy 1980). These compounds are specific inhibitors of (Na⁺ - K⁺)-ATPase and according to a study by Anstee *et al.* (1986) concerning on (Na⁺ - K⁺)-ATPase from Malpighian tubules of *Locusta migratoria*, an estimation of the total number of binding sites per cell is 1.15×10^7 . The study of

Vaughan and Jungreis (1977) and Jungreis and Voughan (1977) found that the neuronal-(Na⁺-K⁺)-ATPases of the milkweed - feeding monarch, *Danaus plexippus* are 300 times less sensitive than those of *Manduca sexta* and *Hyaphora acropia*, neither of which feed on cardiac glycoside containing plants. It has been found that the isolated Malpighian tubules of *Zonocerus variegatus*, a species that feeds on toxic plants and sequesters toxins, excreted cardiac glycosides more efficiently than those of *Locusta migratoria*, which does not feed on toxic plants. (Rafaeli-Bernstein and Mordue 1978). In this connection the characterization of (Na⁺ - K⁺)-ATPase of *P. bufonius* is under consideration.

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(Received 24/03/1987;
in revised form 28/10/1987)

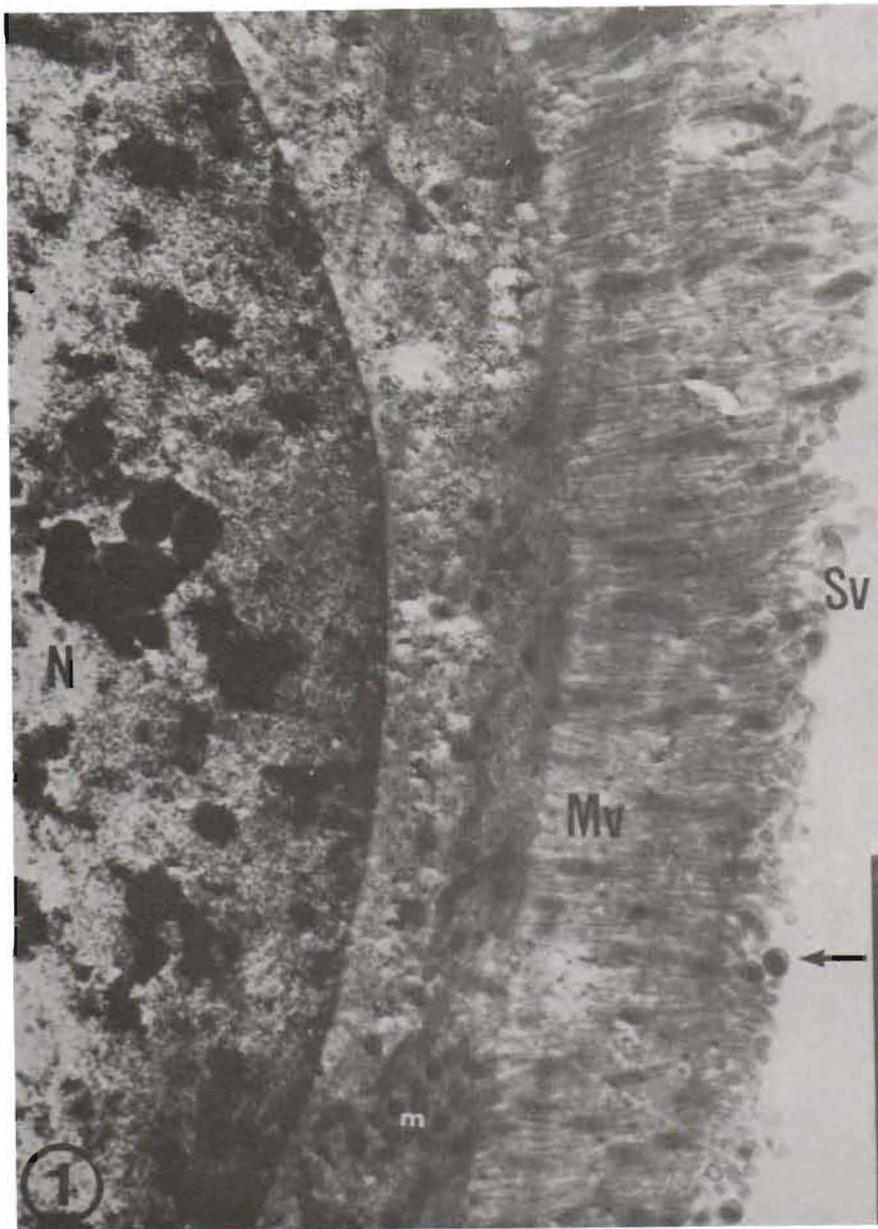


Fig. 1. Malpighian tubule cell of *P. bufonius*. The border of microvilli (Mv) is well developed within which mitochondria are present. Mitochondria (m) are abundant in the apical cytoplasm. Nucleus is rounded (N) with chromatin granules widely distributed. Secretory vesicle (Sv, arrow) are demonstrated associated with the microvilli in the lumen; X 5,890.

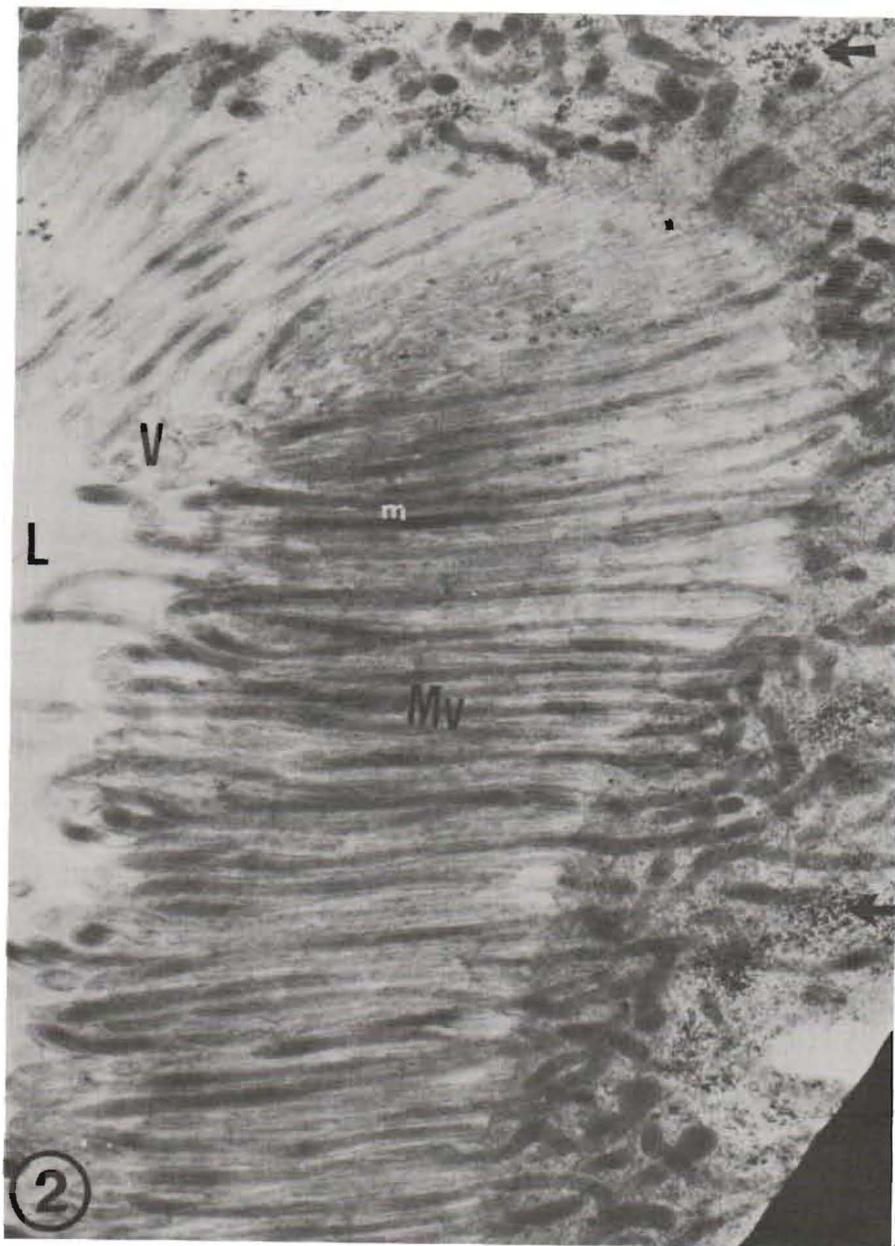


Fig. 2. Apical surface of Malpighian tubule cells. Note the long cylindrical microvilli (Mv) on the luminal surface and mitochondria (m) streaming through the microvilli. Glycogen granules (arrow), Vesicular elements (V) are demonstrated in the lumen (L); X 14,684.



Fig. 3. Basal area of Malpighian tubule cells. The basal membrane (Pm) is infolded in association with mitochondria (M); X 24,749.

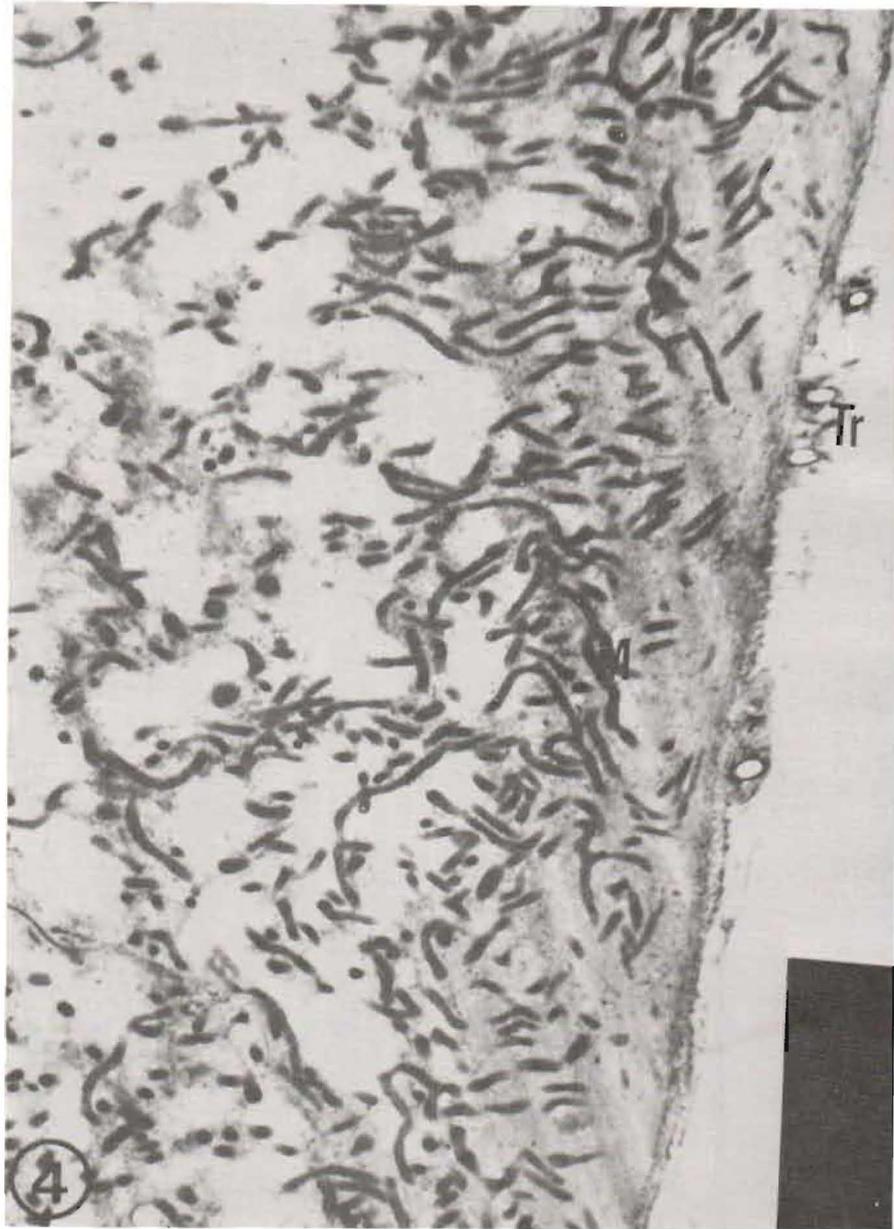


Fig. 4. Malpighian tubule cells showing concentration of branched mitochondria at the basal portion of the cell. Small tracheoles (Tr) are demonstrated in the peritubular tissue. X 9,750.

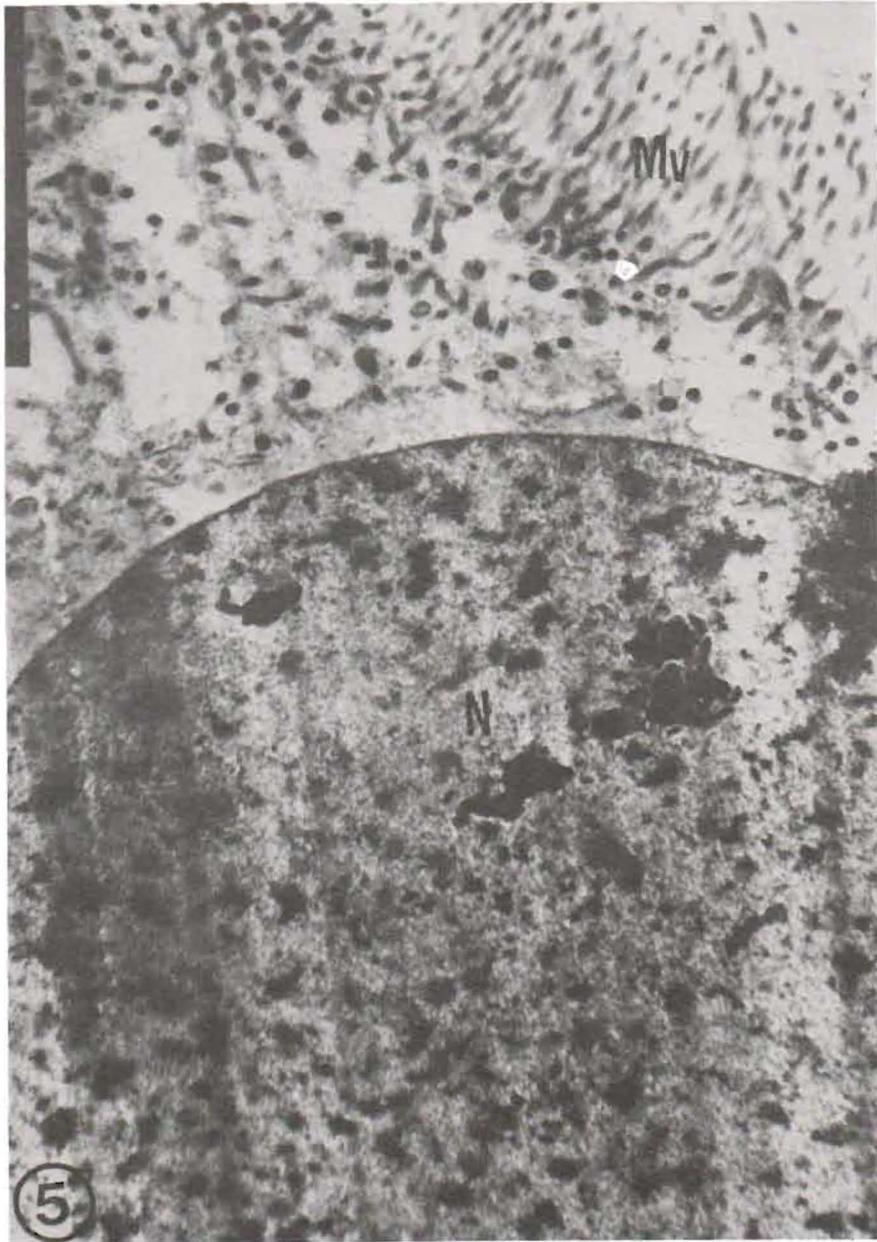


Fig. 5. Low power electron micrograph of Malpighian tubule cell. Round nucleus (N), mitochondria in different profiles, apically streaming into the microvilli (Mv); X 9,750.

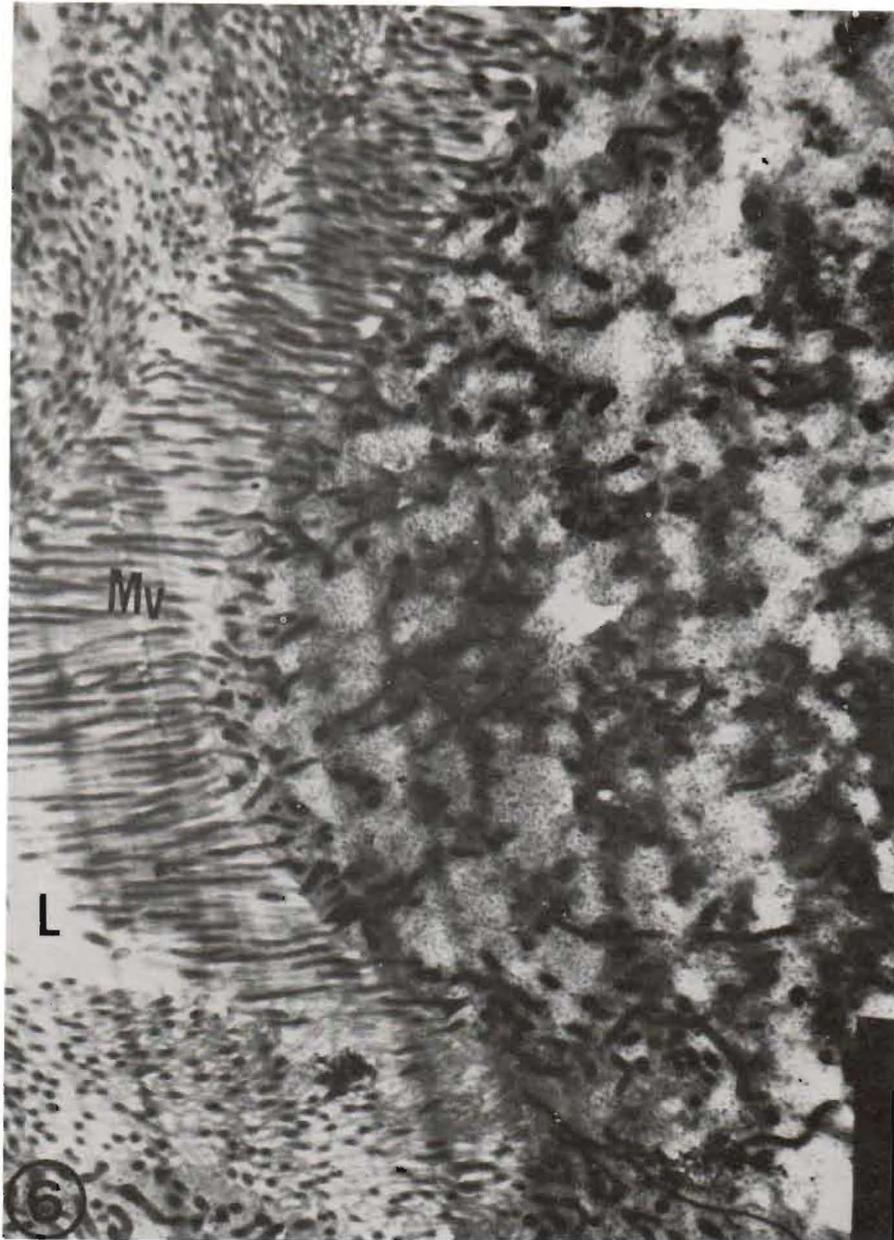


Fig. 6. Malpighian tubule cells with abundant microvilli (Mv) projecting into the lumen (L). The electron dense structures in the cytoplasm and microvilli are mitochondria; X 9,750.

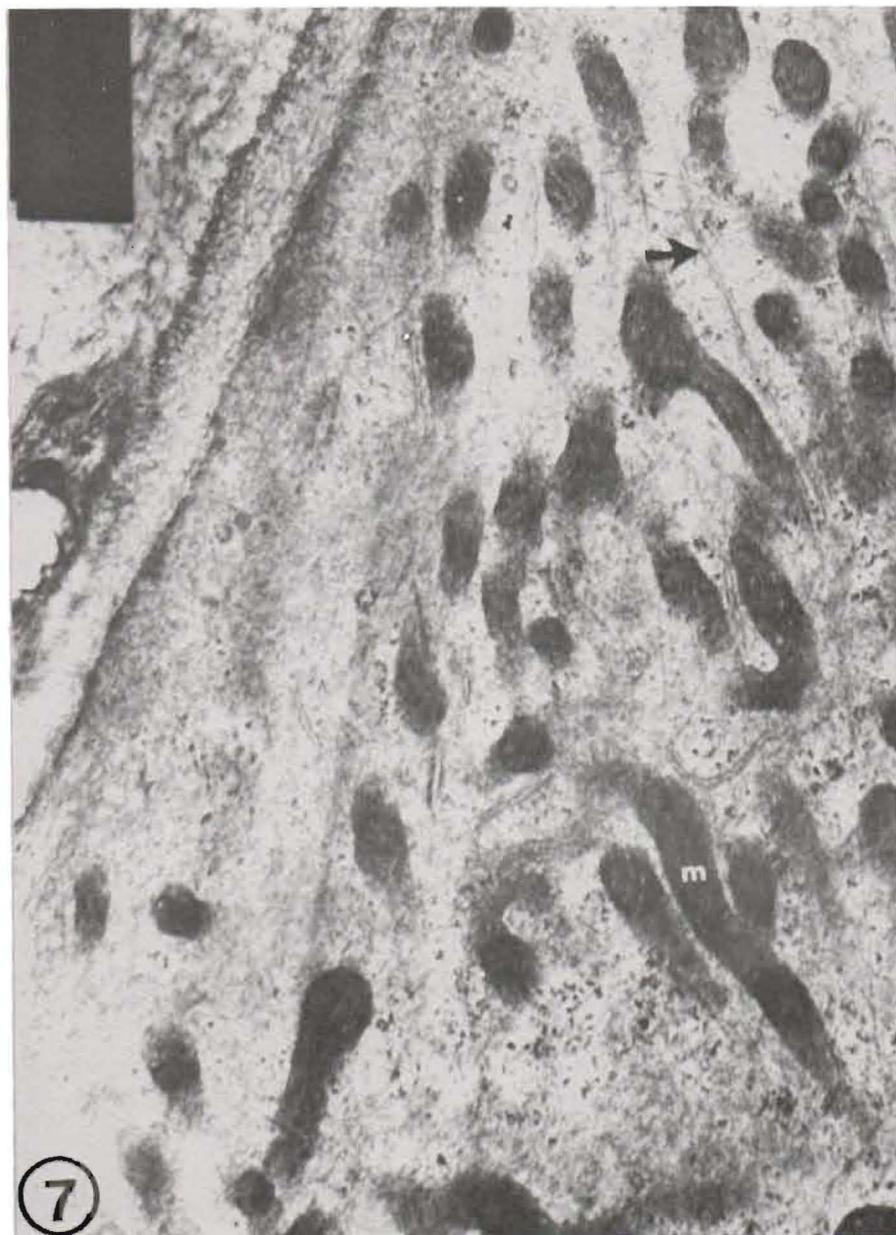


Fig. 7. Basal portion of Malpighian tubule showing mitochondria (m) in association with plasma membrane infoldings (arrow); X 24,749.

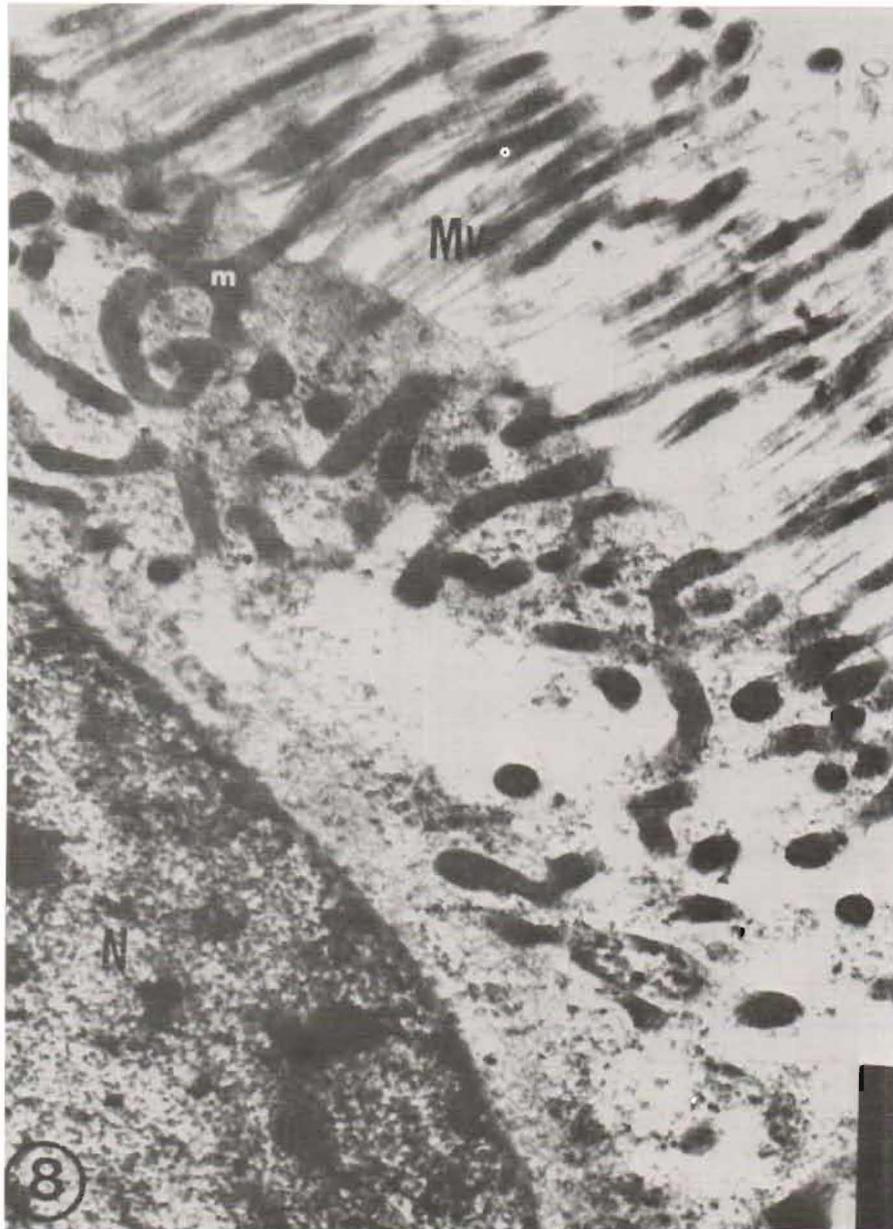


Fig. 8. High power electron micrograph of apical portion of Malpighian tubule cell. Nucleus (N) and mitochondria (m) entering the microvilli (Mv) are shown; X 19,560.

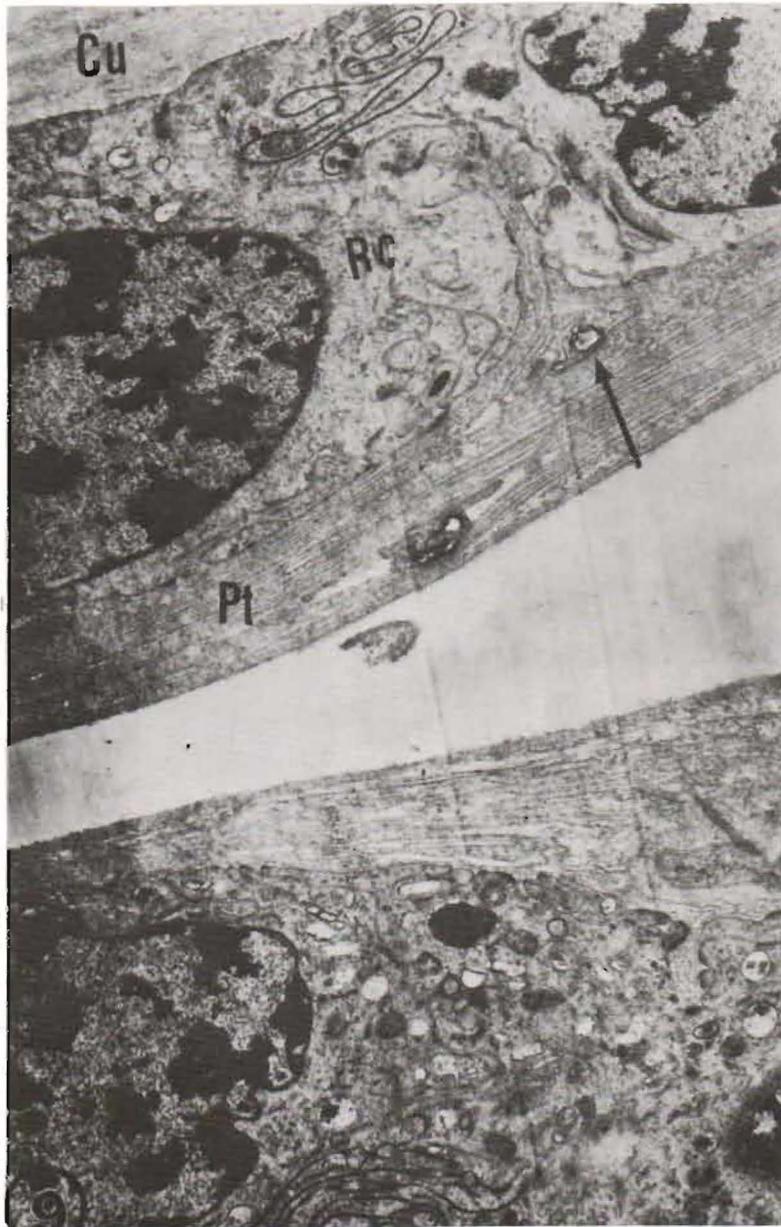


Fig. 9. Rectal epithelial cells (Rc) of *P. bufonius* covered with laminated cuticle (Cu), peritubular tissue (Pt) containing fibrils and tracheoles (arrow); X 9,750.

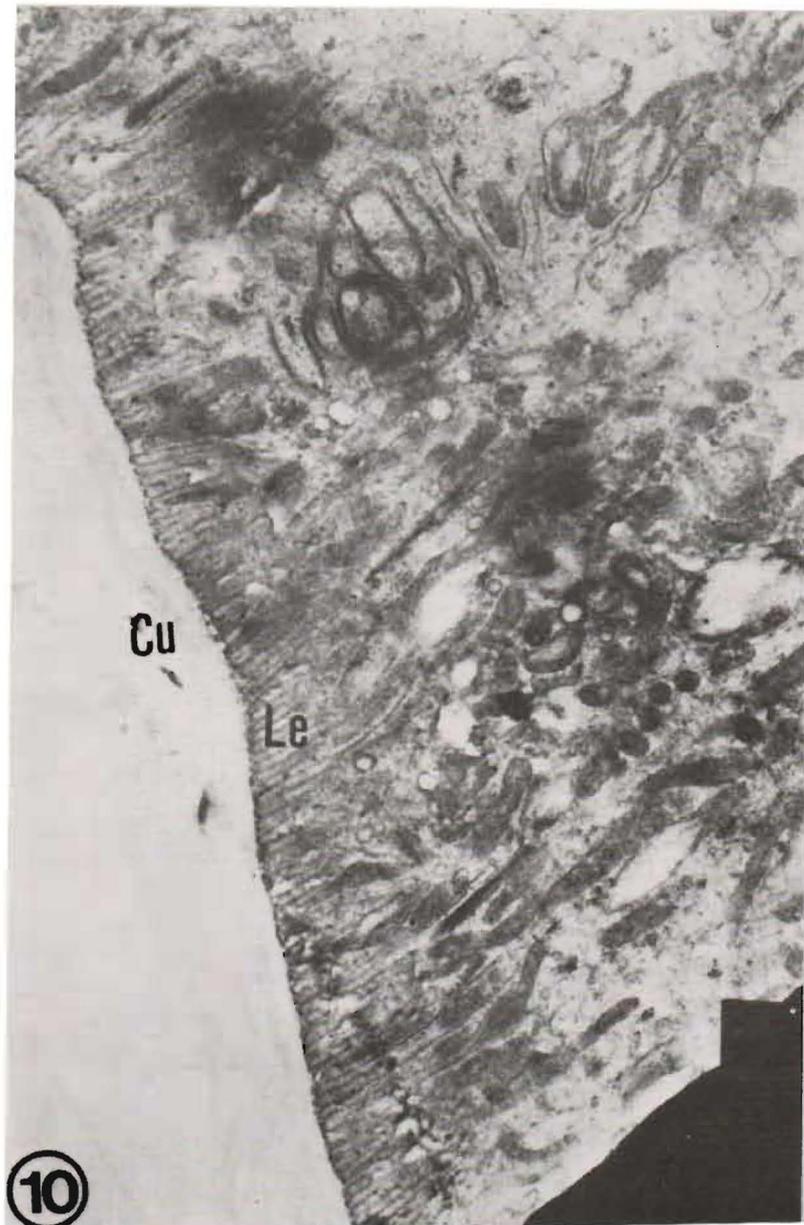


Fig. 10. Luminal portion of rectal cells with infolded plasma membrane (leaflet) structures (Le) covered by cuticle (Cu); X 14,684.

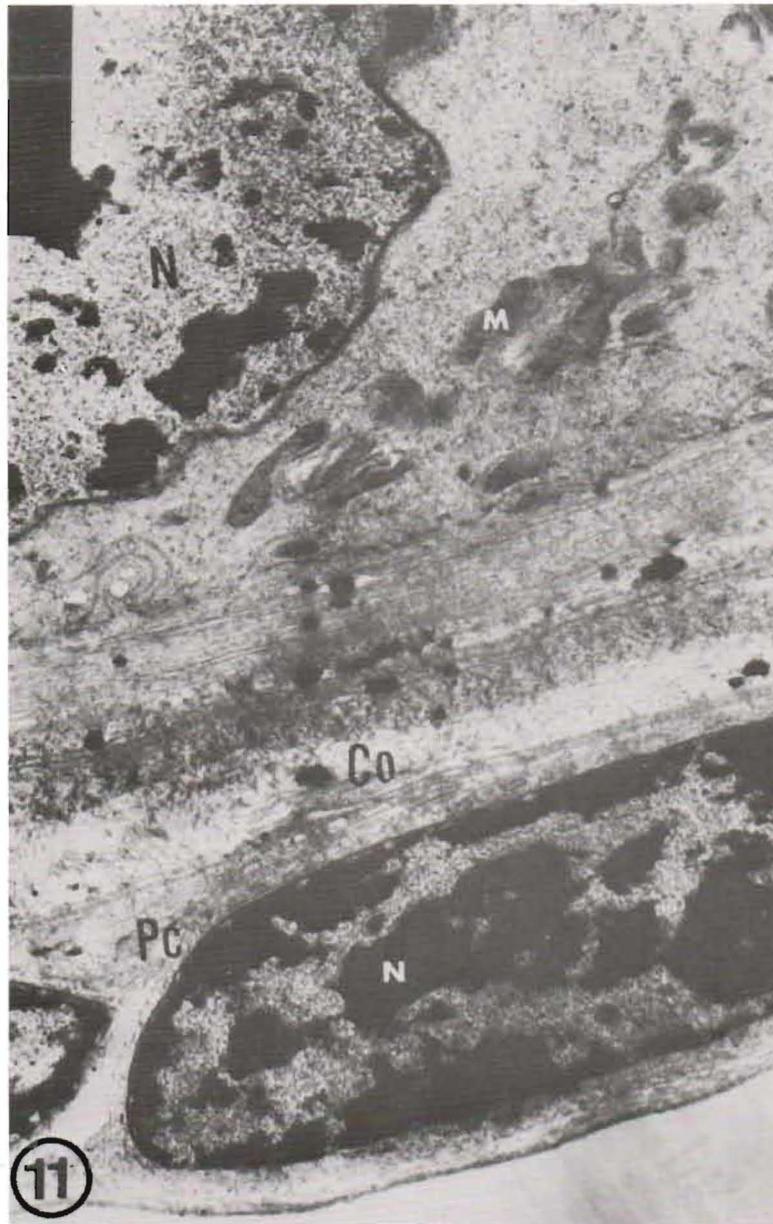


Fig. 11. Basal portion of rectal epithelial cells containing nucleus (N), mitochondria (M), peritubular collagen fibrils (Co) and peritubular cells (Pc) containing elongated nuclei (N); X $\frac{10,000}{14,684}$.

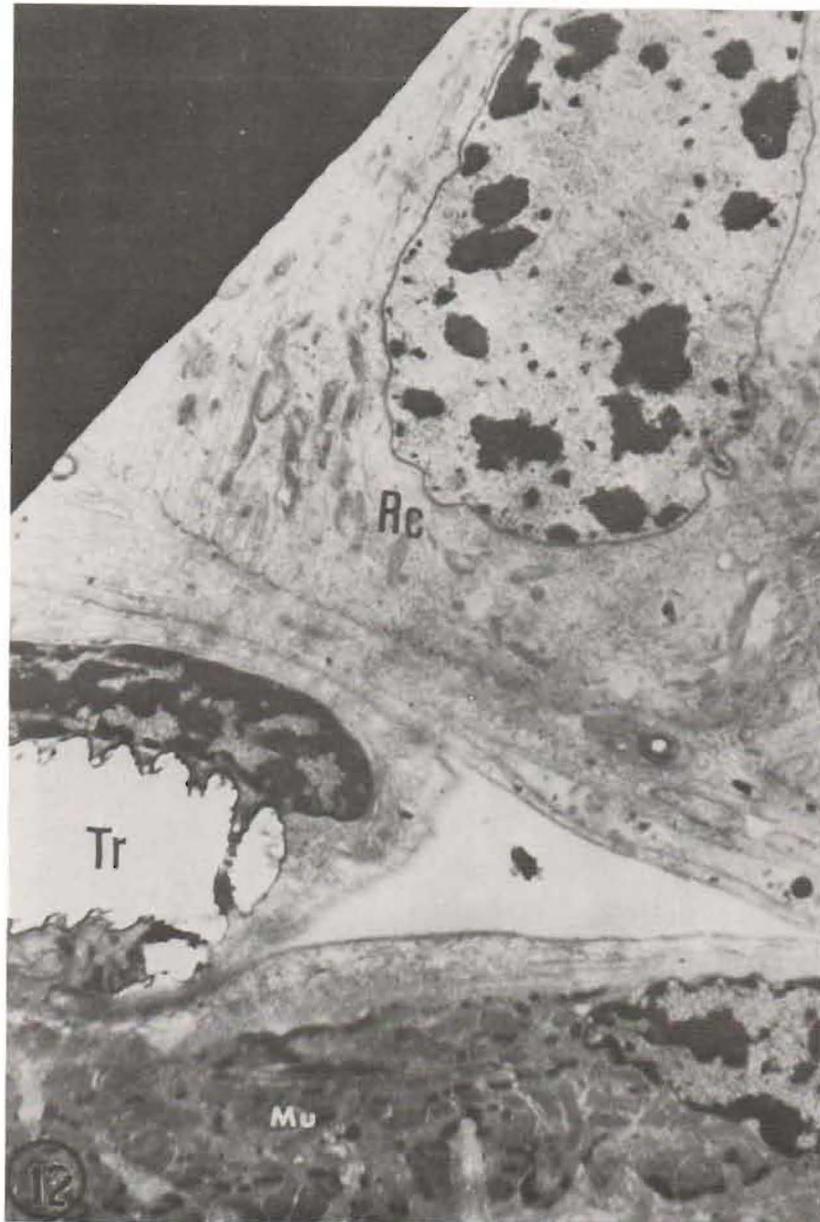


Fig. 12. Muscles (Mu), tracheole (Tr) in the peritubular tissue of rectal epithelial cells (Rc) of *P. bufonius*; X 7,589.



Fig. 13. Electron micrograph of rectal epithelium of *P. bufonius*. The luminal portion of the cells in the form of leaflets (Le) covered with cuticle (Cu). The cytoplasm contains mitochondria (M), smooth vesicles (V) and basal plasma membrane infoldings (arrow); X 24,749.



Fig. 14. Rectal epithelial cells of *P. bufonius* covered with thick laminated layer of cuticle (Cu). Epithelial cells rest on a basement membrane (arrow). The basal plasma membrane and apical plasma membranes are infolded (arrows). The adjoining plasma membranes are closely opposed beneath the border and attached by septate desmosomes (SD). In the cytoplasm, the Nucleus (N), rough endoplasmic reticulum (RER), mitochondria (M), electron dense granules (g), tubular structures (t), tracheoles (Tr) are demonstrated in the peritubular tissue; X 24,749.

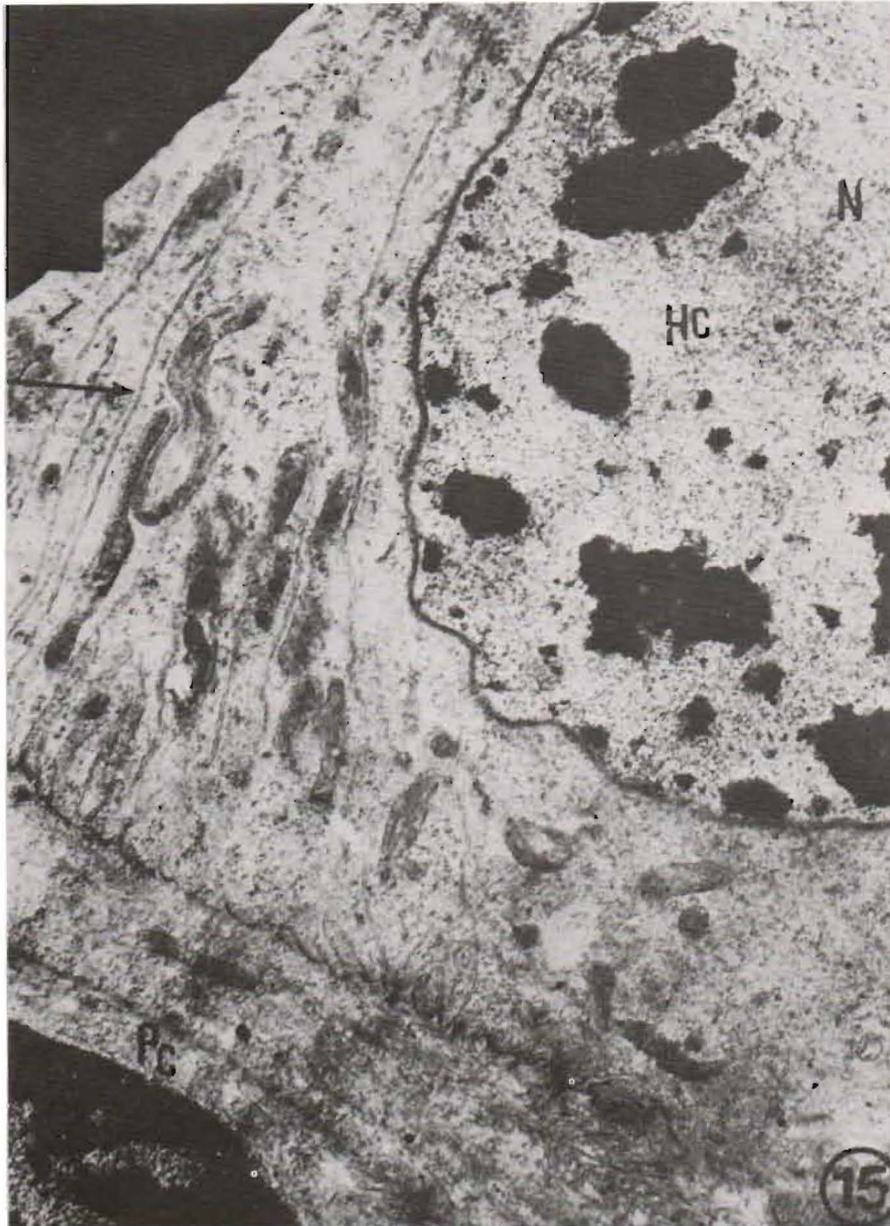


Fig. 15. Basal area of rectal epithelial cells, nucleus with double nuclear membrane (N), heterochromatin and euchromatin, (Hc), mitochondria (M) in association with the basal infolded plasma membrane (arrow), Peritubular cell (Pc); 14,684.



Fig. 16. Electron micrograph of rectal epithelial cells, cuticle (Cu), pinocytotic vesicles (Pv), vacuole (V), nucleus (N), electron dense granule (arrow), mitochondria (M), ribosomes; X 24,749.

التركيب الدقيق لطلائية أنيببات ملبيجى والمستقيم لجرادة نطاط العشر بوكيلوسيرس بوفونيس كلوج (مستقيمة الأجنحة)

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أجريت مشاهدات على أنيببات ملبيجى والمستقيم لجرادة بوكيلوسيرس بوفونيس بعد تجهيزها للرؤيا بالمجهر الإلكتروني النفاذ. وبالرغم من أن التركيب الدقيق للخلايا أنيببية ملبيجى والمستقيم قد درست سابقا في الجراد وبعض الحشرات الأخرى إلا أنه لم تتم دراسة التركيب الدقيق لهذه الأعضاء في جرادة العشر، ولذلك فإن بحثنا هذا يهتم بدراسة التركيب الدقيق لأنيببية ملبيجى والمستقيم لكي نحصل على معلومات يمكن دراستها بطرق الكيمياء الحيوية والنسيجية.

لذلك فإن أنسجة أنيببات ملبيجى والمستقيم قد ثبتت في ٤٪ جلوتارلدهيد في المحلول المنظم كاكوديليت لمدة ثلاث ساعات، ثم غسلت الأنسجة في المحلول المنظم عند درجة حرارة ٤م° ثم أزيل الماء في محاليل تصاعدية في الأسيتون وطمرت في راتنج الالبوكسى، حيث قطعت قطاعات سميكة (١ ميكرون) لصبغها بأزرق التوليدين، أما القطاعات الرقيقة فإنها صبغت بخلات الرصاص وخلات اليورانيل لمدة ثلاثون دقيقة ثم فحصت بالمجهر الإلكتروني النفاذ.

لقد وجد أن طلائية أنيببات ملبيجى لجرادة تيس العشر بسيطة مكعبة، حيث تحتوي على أنوية دائرية أو بيضاوية بها حبيبات كروماتينية في السائل النووي، وترتكز الطلائية للأنيببية الملبيجية على غشاء قاعدى محاط بنسيج ضام يحتوى على ليفات كولاجينية وقصبيات هوائية، ونهايات عصبية وعضلات.

أما الجزء العلوى للأنيببية فإنه مخطط ويتكون من زغب دقيق طويل ومنظم ويختلف في الطول، ويمكن مشاهدة أجسام سبحية (ميتوكوندريا) تمتد داخل بعض من الزغب الدقيق، أما غشاء البلازما القاعدى لخلايا أنيببية ملبيجى فشاهد به

ثنيات بها ميتوكوندريا. وطلائية المستقيم كانت مكعبة كذلك ومغطاة بطبقة سميكة من الكيوتيكل الطبقي وعديم اللون، أما الأنوية فكانت بيضاوية أو مستديرة بها حبيبات كروماتينية وطبقة رقيقة من الكروماتين متصلة بالغشاء النووي الداخلي. ويحتوى سيتوبلازم الخلايا على ميتوكوندريا وصهاريج من الشبكة الإندوبلازمية الحبيبية، ومعظم الميتوكوندريا كانت بالقرب من الأغشية المنشئية. ويتميز غشاء البلازما القمي بانثناءات عديدة داخل الخلية على هيئة وريقات تمتد داخل الجزء العلوى للخلايا الطلائية. وشوهدت الميتوكوندريا بين هذه الانثناءات، وفي الجزء القاعدى للخلايا الطلائية للمستقيم شوهدت الميتوكوندريا بين انثناءات غشاء البلازما القاعدى.

وقد تساعد هذه المعلومات المورفولوجية في تفسير كيفية حدوث الوظائف الامتصاصية والإخراجية لأنبيبات ملبجى والمستقيم في جرادة نطاط العشر التي تتغذى على نبات العشر المنتج لمواد كاردينوليديية (جلوكوسيدات قلبية).