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The surface morphology of the midgut cells of *Rhodnius prolixus* Stål (Hemiptera: Reduviidae) during blood digestion

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Summary

The surface morphology of the midgut cells of *Rhodnius prolixus* is examined using scanning electron microscopy. Before feeding, the microvilli are devoid of any extracellular structures and can be observed in both fracture faces and surface views. By 3 days after feeding, patches of extracellular membrane layers are observed on the surface of the midgut cells and by 7 days the extracellular membrane layers form an incomplete sheet overlying the microvilli, such that the microvilli are no longer visible in surface view. At 15 days after the blood meal the membrane layers are well developed in the intestine forming a continuous sheet, while in the crop the layers are not as completely developed. The results complement previous studies on the midgut ultrastructure of *R. prolixus*. The extracellular membrane layers are thought to function as a peritrophic membrane, allowing the spatial separation of digestive processes.

Key words: *Rhodnius prolixus;* midgut; SEM; extracellular membrane layers.

Introduction

The midgut of *R. prolixus* undergoes several modifications in response to a blood meal. At the gross anatomical level, there is obvious distention of the midgut as the large blood meal is ingested and stored. Differentiation of the midgut into 3 functional regions (Fig. 1) can be made on the basis of the colour of the midgut wall and contents (Wigglesworth, 1943), and on the differential responses to blood feeding of the midgut cells from each region (Billingsley and Downe, 1983; Billingsley, 1985). Physiologically the anterior midgut is concerned with the rapid diuresis of the large amount of water contained in the

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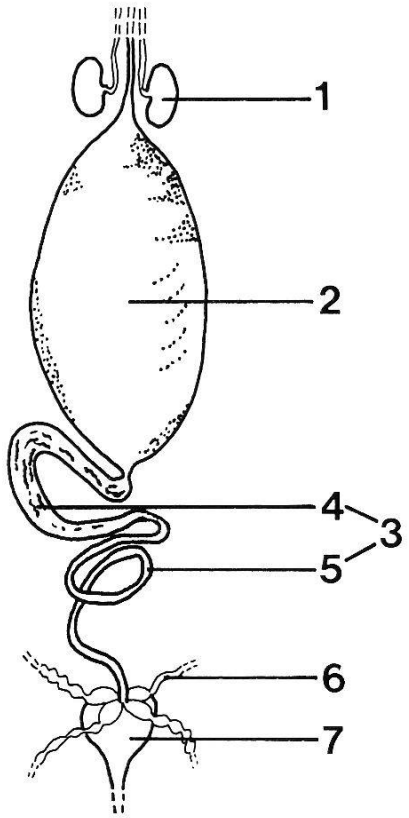


Fig. 1. The alimentary system of *Rhodnius prolixus* (redrawn after Wigglesworth, 1943). 1 = salivary gland; 2 = anterior midgut or crop; 3 = posterior midgut or intestine; 4 = anterior intestine; 5 = posterior intestine; 6 = Malpighian tubule; 7 = rectum. For alternative nomenclature see Bauer (1981).

blood meal (Farmer et al., 1981; Barrett, 1982) and with the digestion of non-proteinacious compounds (Ribeiro and Garcia, 1980; Azambuja et al., 1982; Ribeiro and Pereira, 1984), while the posterior midgut is concerned with the digestion and absorption of the blood meal proteins (Wigglesworth, 1943; Persaud and Davey 1971; Garcia and Garcia, 1977; Houseman and Downe, 1983).

The midgut cells of *R. prolixus* show many significant ultrastructural modifications to blood feeding (Bauer, 1981). In adult females, the lysosomes and rough endoplasmic reticulum (rer) undergo marked structural changes in response to the blood meal (Billingsley and Downe, 1983) which correlate loosely with the localisation of aminopeptidase (Billingsley and Downe, 1985) and cathepsin B (Billingsley, 1985; Billingsley and Downe, in prep.) in these organelles. Similarly, modifications to the organisation of the rer (Bauer, 1981; Billingsley and Downe, 1983) are consistent with a model for the production of digestive proteinases by membrane-bound ribosomes in the midgut of mosquitoes (Hecker, 1977; Gander et al., 1980). The most unusual ultrastructural response to blood feeding by the midgut cells of *R. prolixus* occurs in association with the microvilli. Before feeding, the microvilli possess a typical arrangement of an apical plasma membrane covered on the lumenal surface by a fuzzy layer or glycocalyx (Billingsley and Downe, 1983) but shortly after feeding, a second apical membrane develops on the lumenal surface which is closely associated

with the microvilli and separated from them by a dense staining layer of constant thickness (Lane and Harrison, 1979; Bauer, 1981; Billingsley and Downe, 1983). The outer membrane delaminates during the digestion period to form complex extracellular membrane layers (ECML) which may completely occlude the folds of the midgut epithelium.

The role of the ECML in the spatial organisation of digestion has been discussed (Billingsley and Downe, 1983, 1985). From transmission electron microscopy (TEM) the development of the ECML in association with a few cells at one time can be noted but the overall development to form a complete functional barrier cannot be observed. This study was designed to examine the surface morphology of the midgut cells in *R. prolixus,* particularly to note the timing of ECML development on the lumenal surface of the midgut cells and to compare their development with the timing of previously described physiological (Houseman and Downe, 1983) and ultrastructural (Billingsley and Downe, 1983) events.

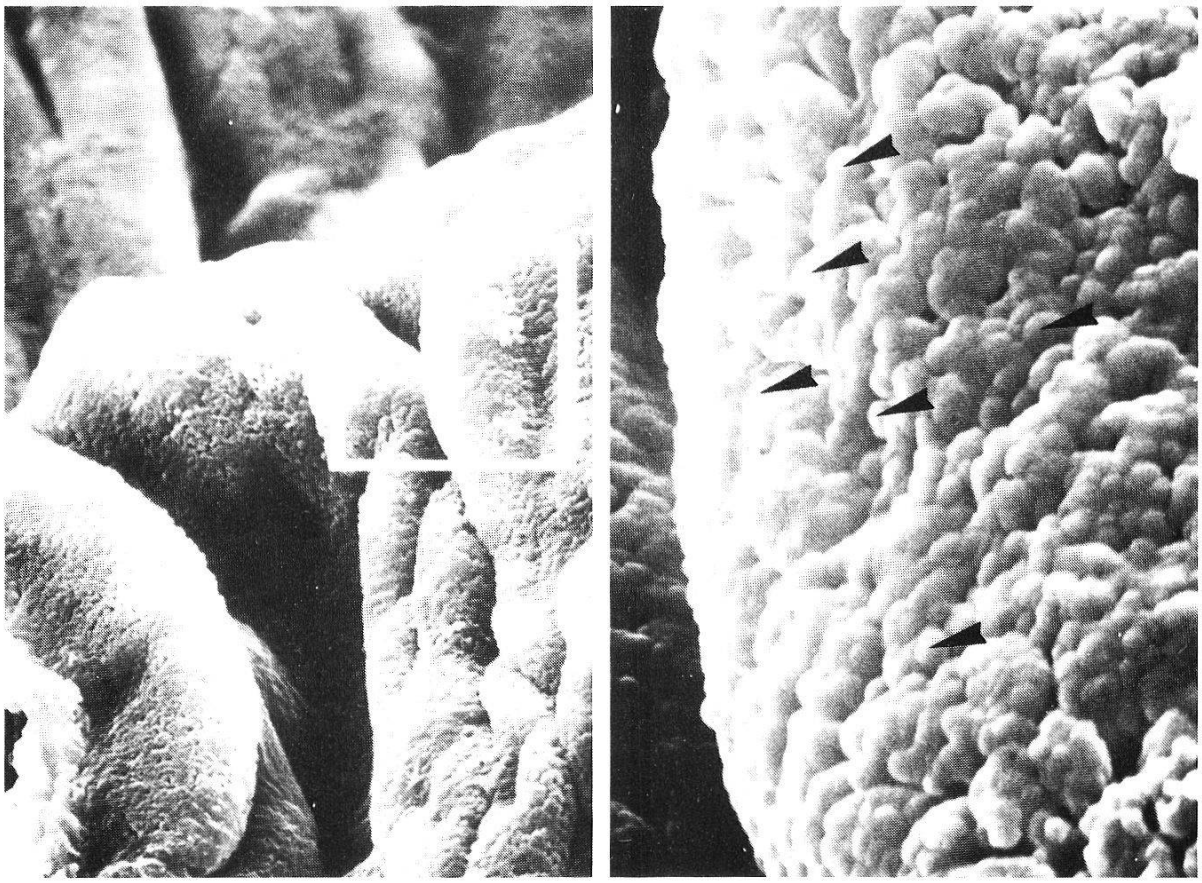
Materials and Methods

Mated female *Rhodnius prolixus,* starved for 6–8 weeks, were obtained from a colony as previously described (Kwan and Downe, 1977; Houseman and Downe, 1983). Midguts were dissected from insects before and at various times after feeding, then prepared for scanning electron microscopy (SEM) as described by Nation (1983). Blocks of midgut tissue from each of the 3 midgut regions (Fig. 1) were fixed for 5 min in 1% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.2, dehydrated through an alcohol series, then immersed in hexamethyldisilazane (HMDS) and air dried before mounting. Alternatively, midguts were fixed by standard procedures in 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide before dehydration through an alcohol series (Billingsley and Downe, 1983). Tissue blocks were then dried in a Polaron (Watford, U.K.) CO_2 critical point dryer.

Blocks of midgut tissue prepared by both methods were mounted on stainless steel stubs using double sided tape. At this point the midguts were fractured longitudinally with a new scalpel blade and unrolled about their long axes, using mounted needles, to reveal the lumenal surface of the midgut cells. Specimens were then coated with gold-paladium in a Polaron E5100 sputter coater for 3 min at 2.2–2.4 kV and 15 mA, and viewed in a Hitachi S450 SEM.

Results

Before feeding, the lumenal surface of the midgut epithelium is clear of debris (Fig. 2). Microvilli form a brush border of irregular height (Fig. 2b) and are devoid of any surface structures such as the ECML. By 3 days after the blood meal, the microvilli of all regions are partially covered by patches of ECML (Fig. 3). The ridges of the epithelial folds run along the midgut (Fig. 3a) and cells may be wholly or partially covered by the ECML (Fig. 3b). At 6 days after feeding, the ECML form an incomplete covering over the intestinal microvilli (Fig. 4). In surface aspects (Fig. 4b), the microvilli are often seen protruding through the ECML and groups of microvilli are held together by patches of ECML. In other parts of the intestine the ECML form a perforated sheet across the lumenal cell surface (Fig. 4a). By 7 days, the ECML are almost continuous;



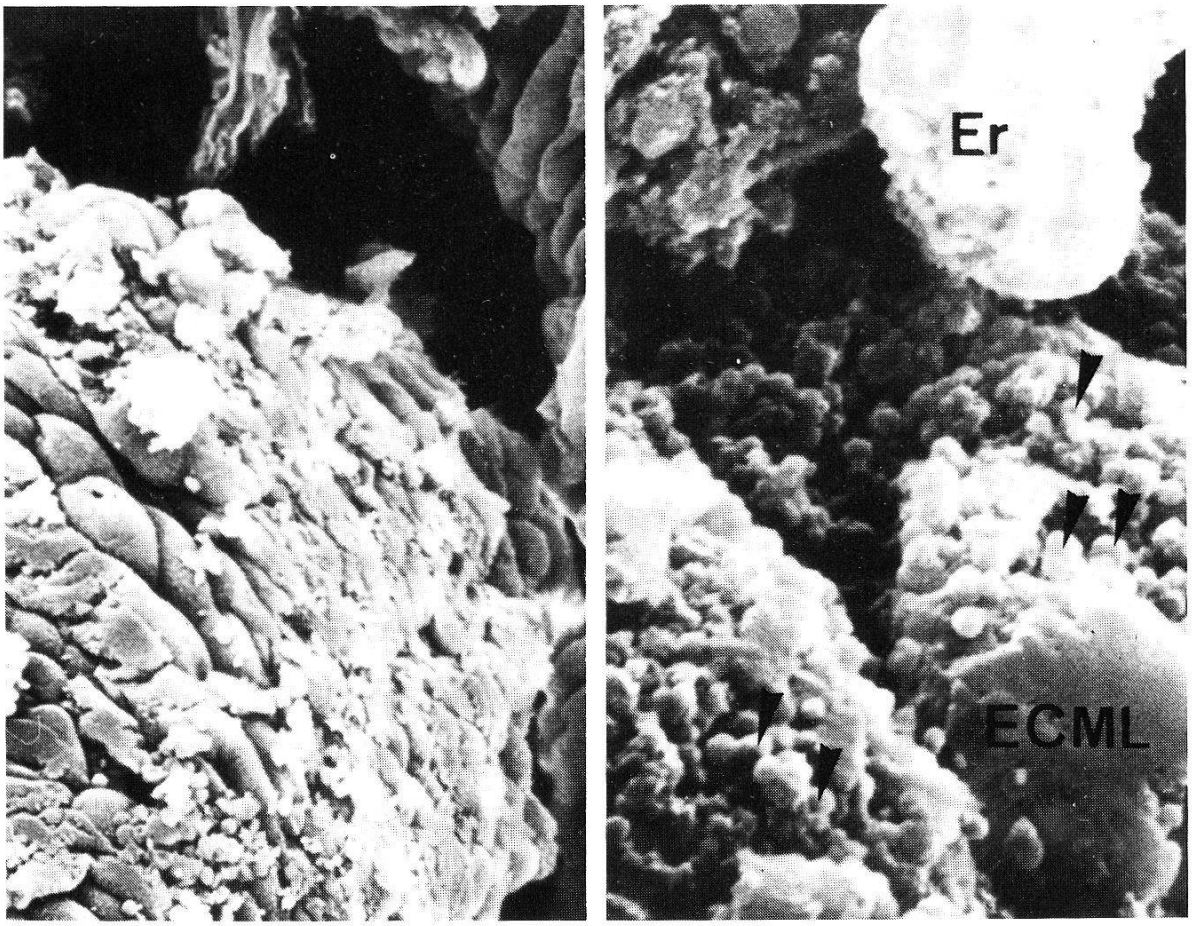
a b

Fig. 2. The surface morphology of the anterior intestinal cells before feeding. a) HMDS-treated, ×1,700. Typical folds of the epithelium, microvilli are exposed to the lumen. b) Detail of square in 2a, ×12,000. The tips of the microvilli are visible (➤).

while microvilli are visible in fracture faces their tips cannot be seen in surface aspects (Fig. 5).

In the crop (Fig. 1), the surface topography of the ECML is very distinctive at 15 days after feeding (Figs. 6a, b). The ECML produced by each individual cell are clearly observed (Fig. 6a) and appear to form a less complete sheet over the microvilli than in the intestinal regions. The ECML from each cell are not always continuous (Fig. 6b), at least on the surface layers, with gaps present in the ECML originating from individual cells (although these may be dehydration or stress artifacts). In the intestine at 15 days after feeding, the ECML may form as a smooth sheet (Fig. 6c) such that the contribution of each individual cell to the ECML cannot be observed. The ECML may also develop to form in ridges along the length of the midgut or the ECML produced by each cell may develop as an individual mound of delaminated membranes (Fig. 6d). In all cases, gaps between the ECML produced by adjacent intestinal cells are not observed.

While the ECML show signs of deterioration at 20 days in the crop and in the posterior intestine, the cells of the anterior intestine continue to produce ECML at this time (Fig. 7). In fractured faces, the delamination of the ECML

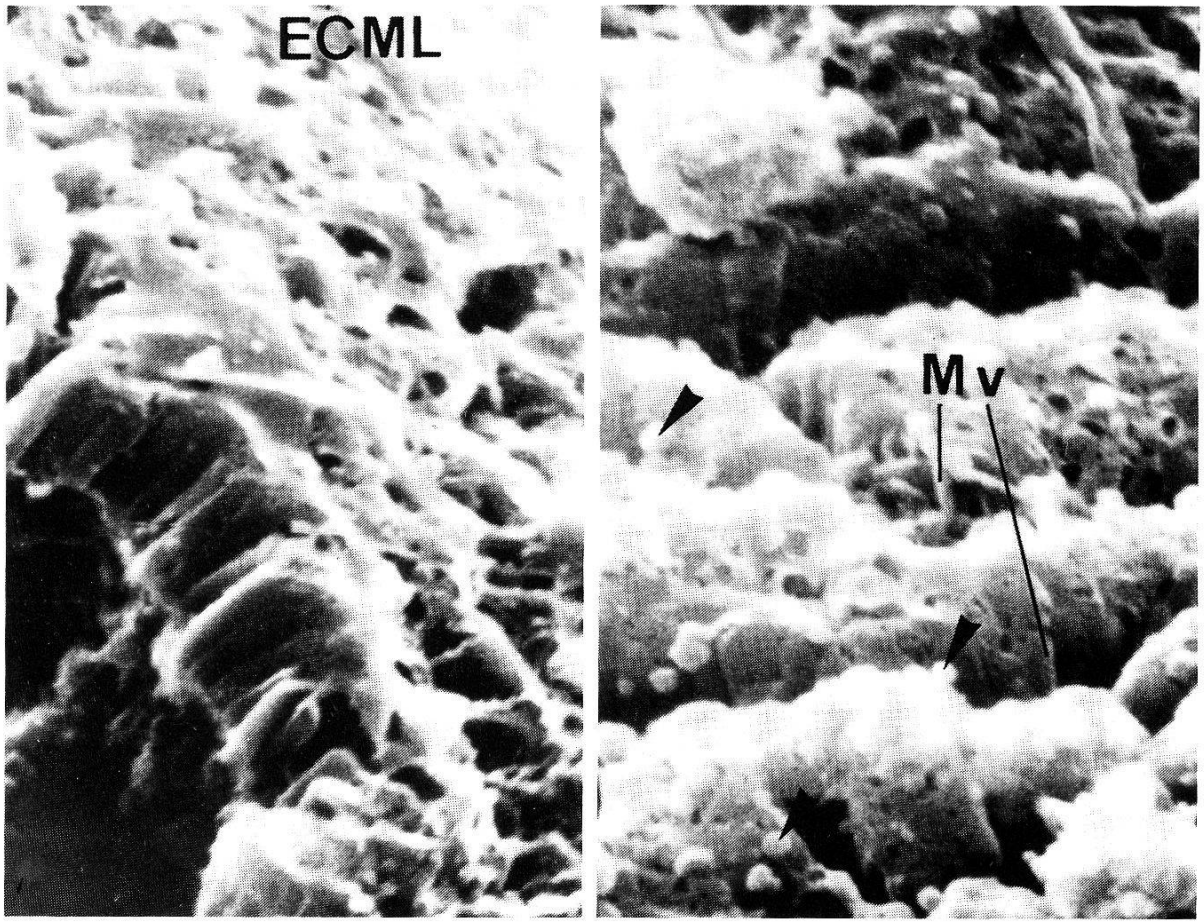


a b

Fig. 3. The development of the ECML in the crop at 3 days post-feeding. a) HMDS-treated, ×420. The topography of each cell is visible and there is considerable debris on the lumenal surface. b) HMDS-treated, ×16,000. Some patches of ECML are evident but the tips of microvilli are still visible (➤). Erythrocyte (Er).

from the microvilli is apparent and the ECML are folded into complex ridges above the microvilli.

Both of the techniques used in this study result in similar, good preservation of the surface morphology of cells from all 3 midgut regions. The only detectable difference is the presence of small "blebs" on the lumenal surface of the ECML, which are present in critical point dried midgut tissue (Fig. 4). Similar surface blebs were also observed on the ECML of the midgut of *Oncopeltus fasciatus* (Baerwald and Delcarpio, 1983), in both SEM- and TEM-prepared tissue. The blebs may thus be an artifact of prolonged glutaraldehyde fixation compared to the 5 minute fixation used in the HMDS treatment (Nation, 1983), or may be real structures that are inadequately preserved in the HMDS treatment. The samples prepared by critical point drying tend to exhibit better intracellular preservation such that organelles are usually detectable in fractured faces (Fig. 7). Nuclei are the only organelles consistently detectable in fracture faces of HMDS-treated midguts, though other organelles are occasionally observed (Fig. 5).



a b

Fig. 4. The development of the ECML in the intestine at 6 days post-feeding. a) Critical point dried, ×17,000. The ECML form a continuous, perforated sheet over the microvilli. b) Critical point dried, ×19,000. The ECML form an incomplete sheet over the lumenal cell surface. Blebs (➢) are present on the ECML surface. Microvilli (Mv).

Discussion

The results concerning the development and distribution of the ECML are consistent with findings from earlier studies using transmission electron microscopy (Billingsley and Downe, 1983; Billingsley, 1985). Between 12 h and 1–2 days after feeding, the second apical membrane develops on the lumenal surface of the microvilli and its subsequent delamination causes the formation of the ECML. The microvilli are partially covered with the ECML at 3 days and are almost completely covered by 6–7 days.

The development of the ECML as a complete "sheet" separating the microvilli from the lumen contents by 7 days has implications for the organisation of digestion in the midgut. Aminopeptidase is associated with the intestinal microvilli of *R. prolixus* (Billingsley and Downe, 1985) and at this site, could be active in a regulated, enclosed ectoperitrophic space (Ferreira and Terra, 1982; Santos et al., 1984). Conversely, cathepsin B is not localised in association with the microvilli (Billingsley, 1985; Billingsley and Downe, in prep.) but is consid-

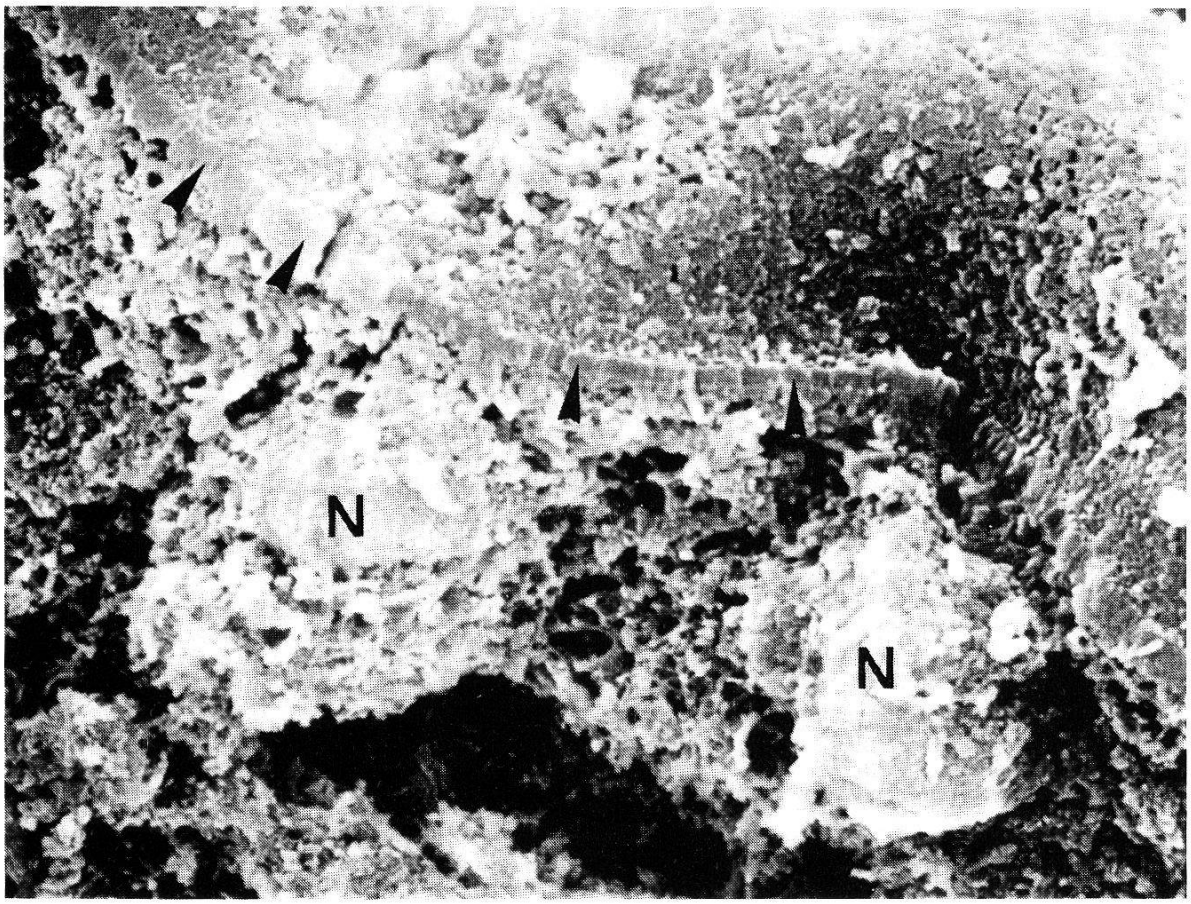


Fig. 5. The ECML in the anterior intestine at 7 days post-feeding. HMDS-treated, ×2,500. Microvilli are visible in fracture faces (➤) but not in surface aspect. Nuclei of epithelial cells (N).

ered to be active in the endoperitrophic space (Houseman and Downe, 1983). The development of the ECML over the microvilli supports a model for the spatial separation of these two enzymes so that they may both function in more optimal conditions (Billingsley, 1985).

The cytology and ultrastructure of the surface coat of the midgut cells of *Triatoma infestans* starved for 14 days has been described (Burgos and Gutierrez, 1976; Gutierrez and Burgos, 1978) and it was found that the ECML formed a well organised "plexiform" coat, comprised of phospholipid membranes which contained polysaccharides. Acid phosphatase was also detectable within the surface layers and the surface ultrastructure of the ECML in *T. infestans* appears to be very similar to that found in *R. prolixus* at 15–25 days after blood feeding (Billingsley and Downe, 1983; present study).

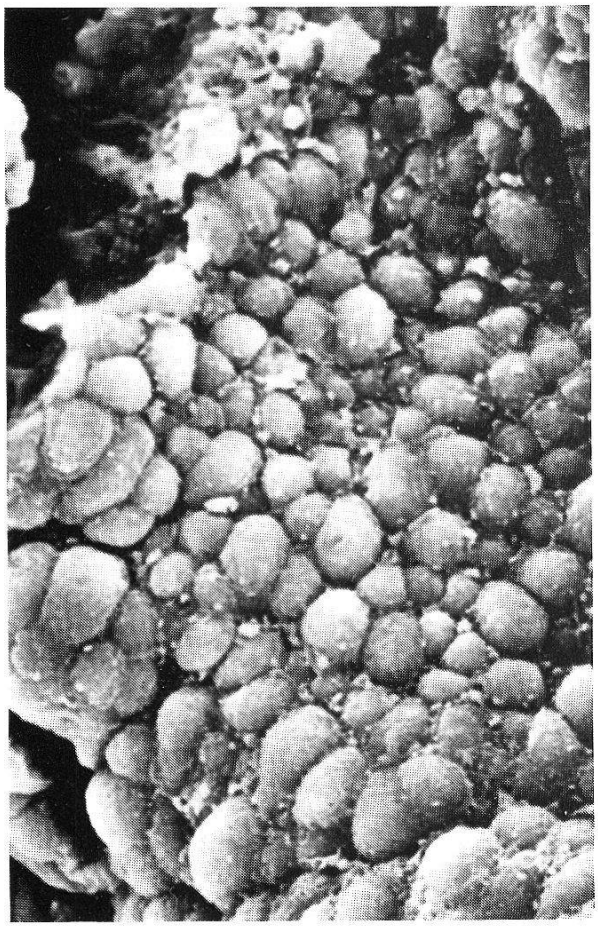
A double membrane system, similar to that found on the midgut microvilli of *R. prolixus,* has been described in the midgut of non-haematophagous Hemiptera (Andries and Torpier, 1982; Baerwald and Delcarpio, 1983; Tieszen et al., 1985, 1986). In the water scorpion, *Nepa cinerea* (Andries and Torpier, 1982), the ECML develop from Golgi vesicles containing internal membrane layers, which form the ECML by exocytosis. In *R. prolixus* similar vesicles were apparent in the midgut cells 2 h after feeding (Billingsley and Downe, 1983) but

no fusion of any such vesicles with the apical membrane was observed. In the seed-feeding hemipteran, *Oncopeltus fasciatus* (Baerwald and Delcarpio, 1983), cross sections of the ECML are similar to those of *R. prolixus,* but, as in the studies on *T. infestans* (Burgos and Gutierrez, 1976; Gutierrez and Burgos, 1978), the development of the ECML in *O. fasciatus* has not been studied, although the ECML may be present all the time as this insect is more of a continuous feeder.

Several authors have noted the presence of a second apical membrane associated with the microvilli in the midgut of *R. prolixus.* Pacheco and Ogura (1966) and Pacheco (1970) examined the ultrastructure of the anterior and posterior midgut respectively but did not examine the ECML in detail. Bauer (1981) studied the changes in the midgut ultrastructure of 1st- and 2nd-instar larvae of *R. prolixus* and noted the presence of additional apical membranes. The most detailed study of the apical membranes of the midgut cells of *R. prolixus* was provided by Lane and Harrison (1977) who used a combination of TEM, freeze fracturing and lanthanum staining to determine the relationship between the apical plasma membrane and the additional extracellular membranes. While the insertion of particles into the membranes demonstrated that they were more than just a phospholipid bilayer, the lumenal apical membrane was thought to have a strengthening function, allowing rapid distention of the midgut during blood feeding without damage to the cells. While this may be the case, the absence of the second apical membrane before feeding in *R. prolixus* (Billingsley and Downe, 1983) and the absence of any such structure in non-hemipteran blood feeding insects (Richards, 1975), suggests that the lumenal apical membrane and the ECML must have some other function. Although the ECML have not been demonstrated to be a "true" peritrophic membrane (Richards, 1975), the present study adds to the evidence that the ECML may function in this capacity (Bauer, 1981) where they may separate digestive processes from one another.

Previous studies have been concerned with the ultrastructure and life-cycle stages of the parasite *Trypanosoma cruzi* in the midgut and hindgut of *R. prolixus* (Brack, 1968; Sanabria, 1966) and in the rectum of *Triatoma dimidiata* (Zeledon et al., 1984) and *T. infestans* (Böker and Schaub, 1985). The midgut of the reduviid vector has been shown to be an important site for the development and multiplication of *T. cruzi,* but EM studies on the interaction of the trypanosomes with the midgut cells are seriously lacking. The development of the ECML after blood feeding in *R. prolixus* may affect the concurrent development

Fig. 6. The surface morphology of the midgut cells 15 days after feeding. a) Crop, HMDS-treated, ×450. The surface topography of the ECML from each crop cell is clearly visible. b) Crop, HMDS-treated, ×6,800. Although the ECML cover the microvilli, there are gaps (➤) between the ECML of adjacent cells. c) Posterior intestine, HMDS, ×7,500. The ECML from each cell is sometimes discernable (✻), but gaps between them are not present in the intestine. d) Anterior intestine, HMDS-treated, ×1,500. The ECML may form a smooth, perforated sheet over the midgut cells. Microvilli (Mv).



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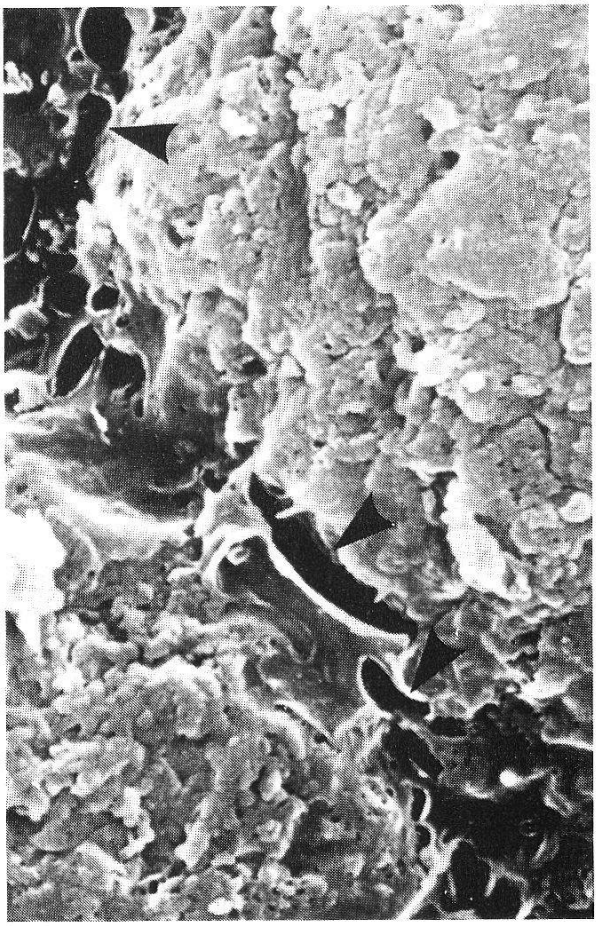

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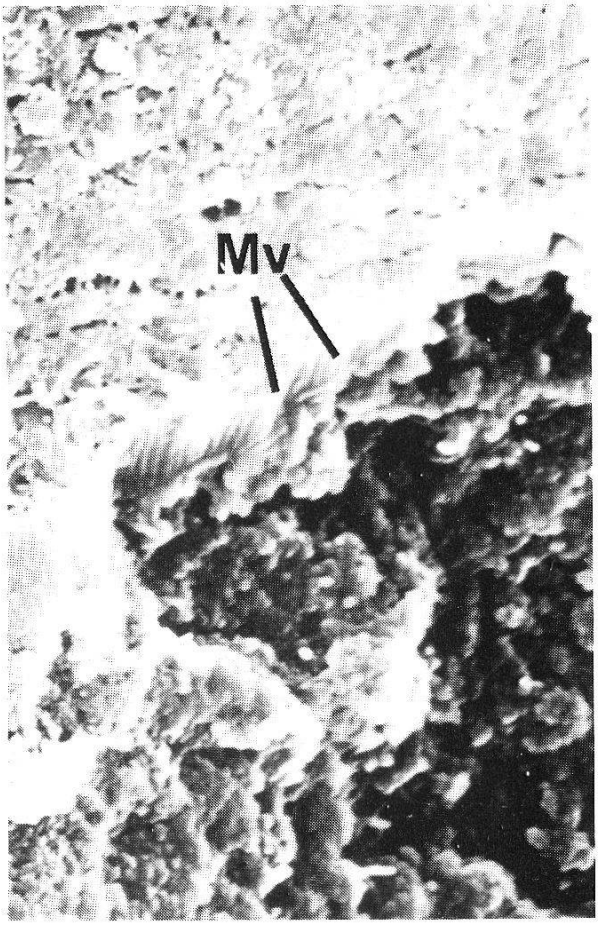

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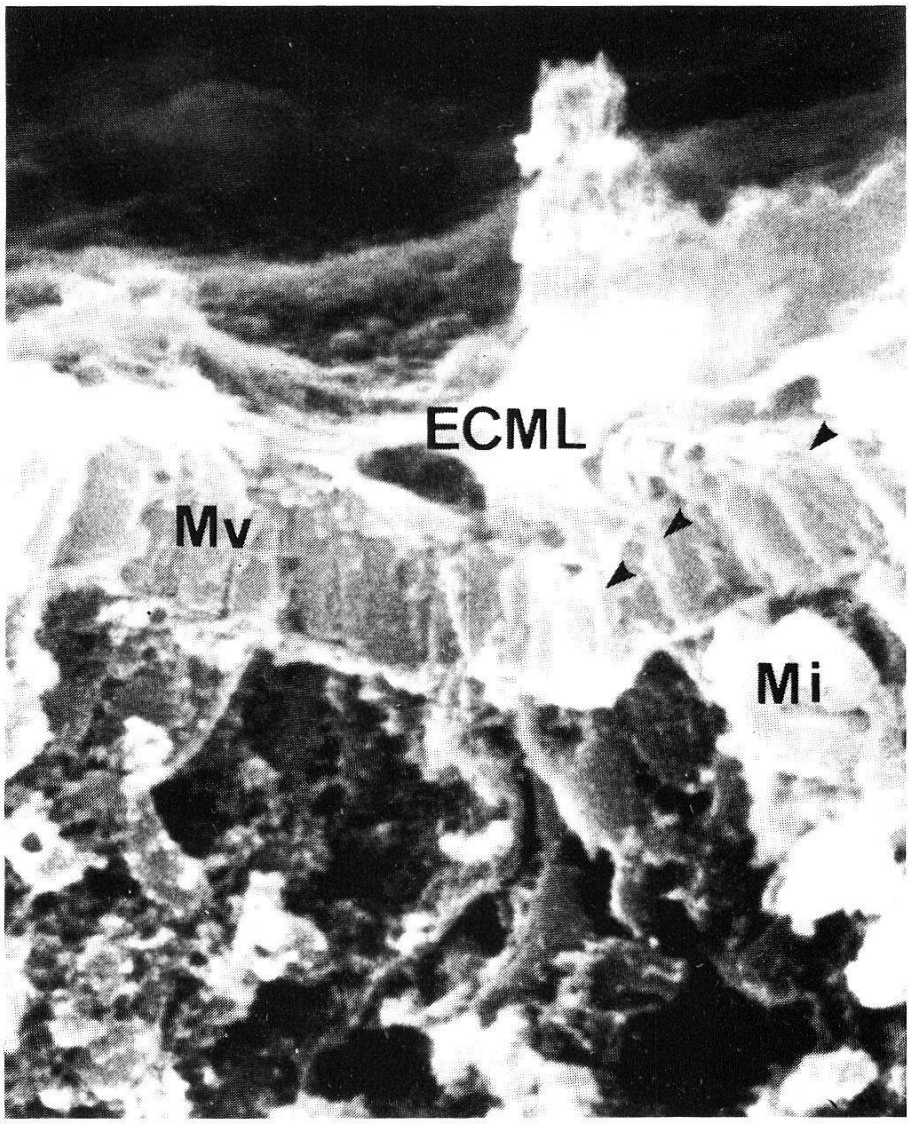

Fig. 7. The ECML in the anterior intestine at 20 days. Critical point dried, ×26,000. The ECML are still well developed and large sheets protrude into the lumen. Delamination of membranes from the microvilli can be seen (➤). Microvilli (Mv), mitochondria (Mi).

of the trypanosomes in the midgut and may play a role, along with other digestive processes, in causing the stage differentiation of the parasites. Studies on the interaction of blastocrithidial protozoans in gerrid and lygaeid species (Tieszen et al., 1983, 1985) have concentrated mainly upon the development of the parasites in the insect gut. More recently the number of blastocrithidial parasites attached to a cell in the midgut of *Lygaeus pandurus,* was found to be related to the extent of ECML development and the presence of the second, lumenal apical membrane (Tieszen et al., 1986). While quantitative analysis has not been carried out on this host-parasite association (Molyneux, personal communication), it is becoming evident that the ECML plays a significant role in the ability of the parasite to remain and develop in the insect gut.

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