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Trypanosoma (Megatrypanum) melophagium in the sheep ked, *Melophagus ovinus*

A scanning electron microscope (SEM) study of the parasites and the insect gut wall surfaces

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Summary

A description of the different stages of *Trypanosoma (M.) melophagium* in different regions of the gut of the sheep ked (*Melophagus ovinus*) as observed by the SEM is presented. The extensive pile carpet or palisade colonization of the midgut and pylorus is described. The method of attachment and the relationship of the parasites to the microvilli in the midgut and the cuticle of the pylorus and ileum observed by other methods are confirmed. The micro-structure of the surfaces themselves in the regions of the gut to which parasites attach are described. The use of the technique for the study of other similar systems is discussed.

Key words: Trypanosoma (Megatrypanum) melophagium; sheep ked; *Melophagus ovinus;* scanning electron microscopy.

Introduction

Recent studies by Sinden (1974, 1975a), Strome and Beaudoin (1974) and Cochrane et al. (1976) on scanning electron microscopy (SEM) of *Plasmodium* in *Anopheles* have considerably furthered our understanding of the structure and behaviour of the sporogonic stages of malaria parasites in the mosquito. SEM studies have also been made on malaria parasites in their mammalian hosts (Sinden, 1975b; Aikawa, 1977) and *Entamoeba histolytica* in tissue culture (McCaul and Bird, 1977).

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Studies on trypanosomes and *Leishmania* parasites using the SEM have been carried out by Pennisi and Faraone (1973), Pal et al. (1974), Killick-Kendrick et al. (1975) and Seed et al. (1976) but no attempt has been made to relate the parasite to its host environment as far as we are aware. This paper presents a description of the association and configuration of trypanosomes in a vector in situ by SEM. The system studied was *Trypanosoma (Megatrypanum) melophagium*, the sheep trypanosome, in its vector *Melophagus ovinus* (Diptera: Hippoboscidae), the sheep ked. It was selected because of the density of the infection in the vector, the relative size of the gut of this insect and consequently its ease of dissection and manipulation, and other existing knowledge of the life cycle and morphology of parasite and host (Hoare, 1923; Waterhouse, 1953; Nelson, 1956; Hoare, 1972; Molyneux, 1975, 1977). The surface structure of the different regions of the gut with which parasites are associated was examined to provide information on the host-parasite relationship and the nature of the surfaces to which trypanosomatid parasites attach within their insect hosts.

Materials and methods

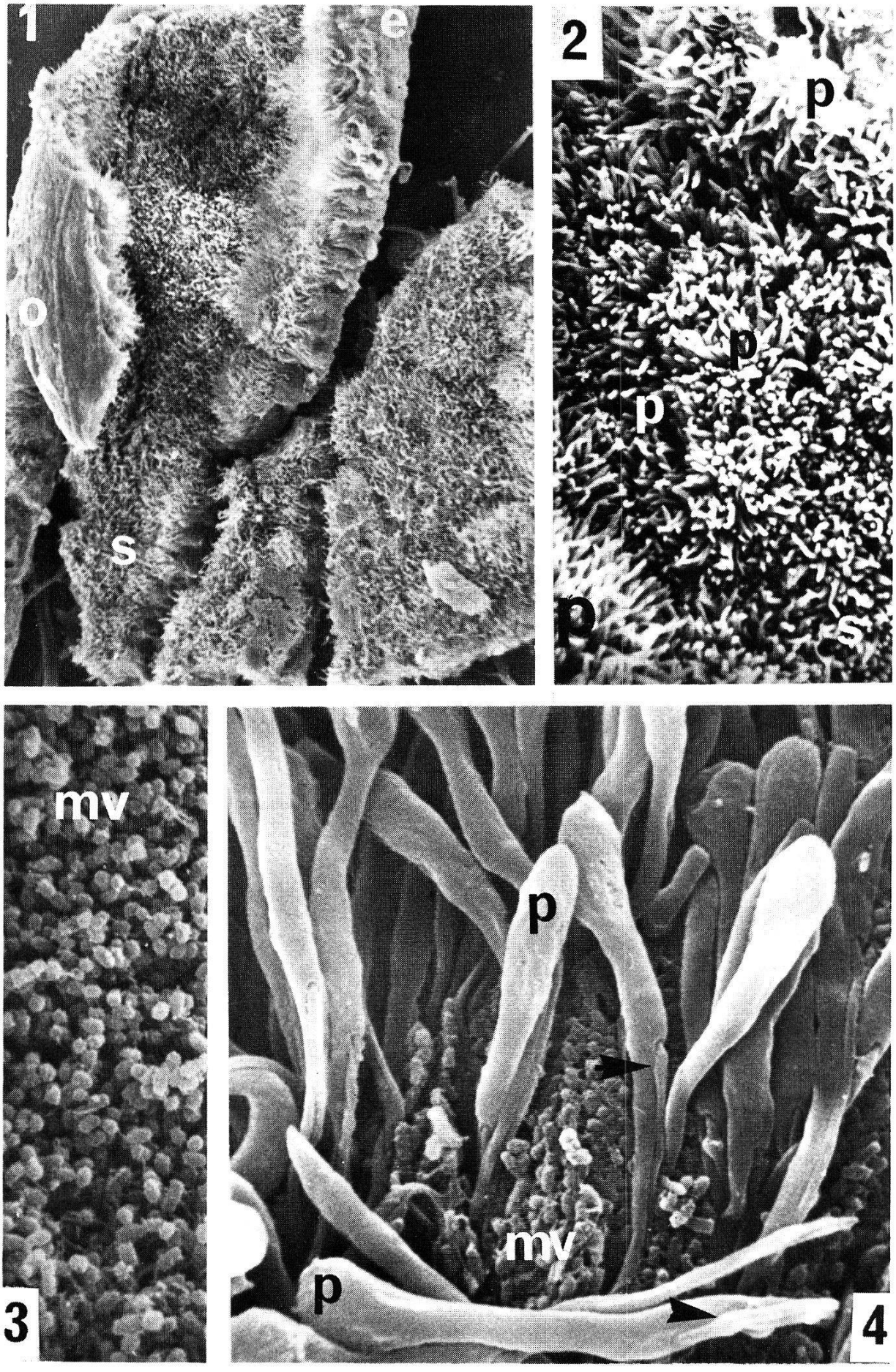
M. ovinus were dissected and whole guts removed into physiological insect saline (NaCl 14.63 g; KCl 0.45 g; $CaCl_2$ 0.5 g; $NaHCO_3$ 0.19 g made up to 1 litre) and examined for the presence of trypanosomes without damaging the gut. Immediately after examination the guts, either infected or uninfected, were fixed in ice cold 3% glutaraldehyde in 0.2 M cacodylate buffer for 1–2 h, washed in the buffer for varying periods from 1½–24 h prior to dehydration in acetone and drying by the critical point method. The dissected guts were either maintained intact until after critical point drying at which time they were fractured by fine dissecting needles at the required points or dissection and opening of the gut in the required regions was carried out whilst the specimens were in the cacodylate buffer solution prior to dehydration. The critically point dried specimens were then mounted on double sided cellotape on aluminium stubs, gold coated in a Polaron splutter coaster which gave a 400 Å coat on the specimens. They were then examined in a Cambridge Stereoscan IIA at accelerating voltages of 20–30 kV.

Fig. 1. General view of opened infected midgut. The whole of the exposed surface is colonised by parasites. Epithelium (e); parasitised surface (s); outerwall of gut exposed to haemocoele (o). ×170.

Fig. 2. Higher magnification SEM of infected gut showing parasites occupying all available surface. No microvilli can be seen below the palisade of parasites (p). ×550.

Fig. 3. SE micrograph of uninfected midgut surface of *M. ovinus* showing microvilli of epithelial cells. Note closely packed nature of the microvilli (mv) and absence of any surface structure or material suggestive of peritrophic membrane. ×4800.

Fig. 4. SEM of infected midgut and parasites (p); microvilli of epithelial cells (mv). Note expansion of flagella near exit from reservoir (arrowed). ×5200.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Results

The surfaces of uninfected midgut, pylorus (hindgut triangle or iliac bulb of Hoare, 1923), ileum (= hindgut and colon of Hoare, 1923) and rectum were examined using the SEM. The terminology of the regions of the gut follows Killick-Kendrick et al. (1977). The different gut surfaces to which the parasites were attached are illustrated in Figs. 3, 4, 8, 14 and 15. The surface structure of the midgut was typified by densely packed microvilli rising from the epithelium (Figs. 3–5). No peritrophic membrane was present (Waterhouse, 1953). There was a sudden transition between the microvillar surface of the midgut and the cuticular surface of the pylorus which was thrown into small regular corrugations at the pyloric valve (Figs. 5–8). The ileum itself was lined with cuticle, with folds which run longitudinally and are associated with longitudinal muscle layers (Figs. 14, 15). Spinous excresences (Figs. 14, 15) were found in the ileum but not in the pylorus, and these spines were not associated with any break in the cuticular surface.

In *M. ovinus* infected with a mature *T. (M.) melophagium* infection the microvilli lining the midgut were obliterated from view by an attached 'pile carpet' or palisade of epimastigotes (Figs. 1, 2). The parasites were orientated with their flagella towards the epithelium and only the body of the parasite appeared above the microvilli lining the gut wall (Figs. 4, 5), the anterior end of the body of the epimastigote itself being partially inserted between the microvilli (Figs. 4 and 5). The posterior ends of the attached forms in the midgut were orientated in many directions (Figs. 3, 4) probably related to the peristaltic movement of the gut and their position at the moment of fixation as attached forms in the midgut when observed before fixation move their posterior ends rapidly in the lumen. Lateral SEM views (Figs. 4, 5) of the epimastigotes in the midgut confirmed the earlier descriptions (Hoare, 1923; Molyneux, 1975) that insertion of the flagella of the parasites is between the microvilli of the epithelial cells. The parasites themselves show the typical epimastigote configuration with undulating membrane and exit of the flagellum from the flagellar pocket in a lateral position. A swelling of the flagellum is observed in some specimens close to the exit of the flagellum from the pocket (Fig. 4). The loss of parasites from the surface of the midgut leaves gaps between the microvilli lining the epithelium and microvilli are pushed apart where parasites insert. The valvular junc-

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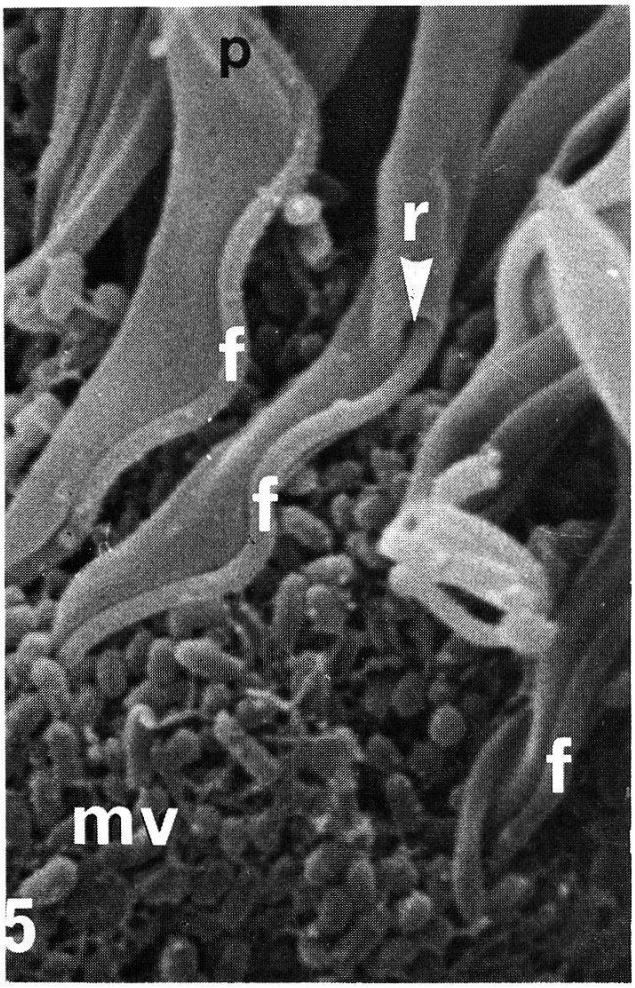
Fig. 5. High power of epimastigote parasites (p) inserting flagella between microvilli (mv) of epithelial cell. Note flagella (f) and reservoir opening (r, arrowed) in lateral position of a parasite. Anterior end of parasite is also embedded in microvilli. ×8300.

Fig. 6. SEM of valvular junction (v) between midgut (m) and pylorus (py). ×240.

Fig. 7. Infected pylorus showing unparasitised cuticular lining (cu) and groups of parasites (p) attached or as rosettes in the lumen. ×1050.

Fig. 8. SE micrograph of the surface cuticle of pylorus of *M. ovinus* showing extensive folding of cuticular surface. Note absence of spicules. ×5800.



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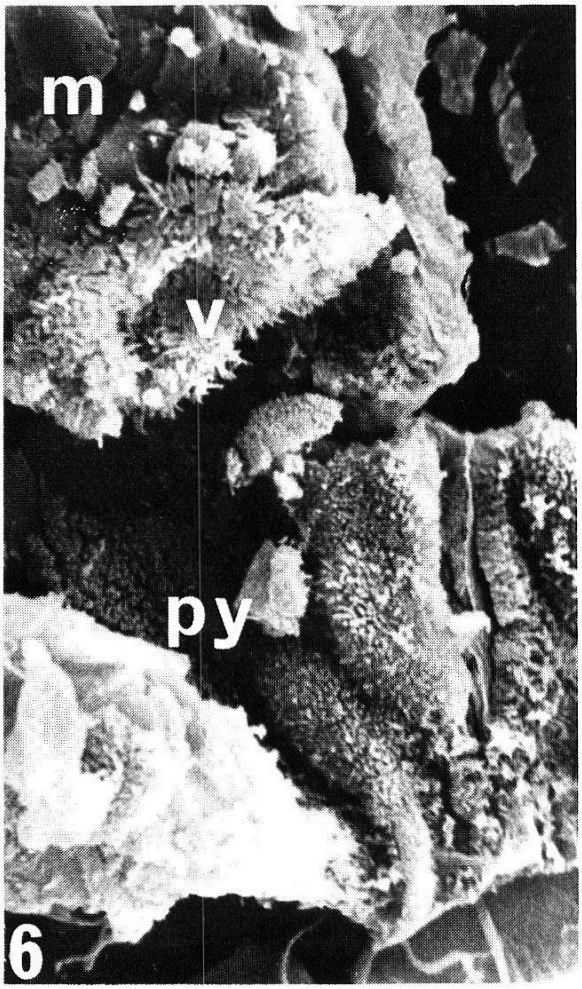

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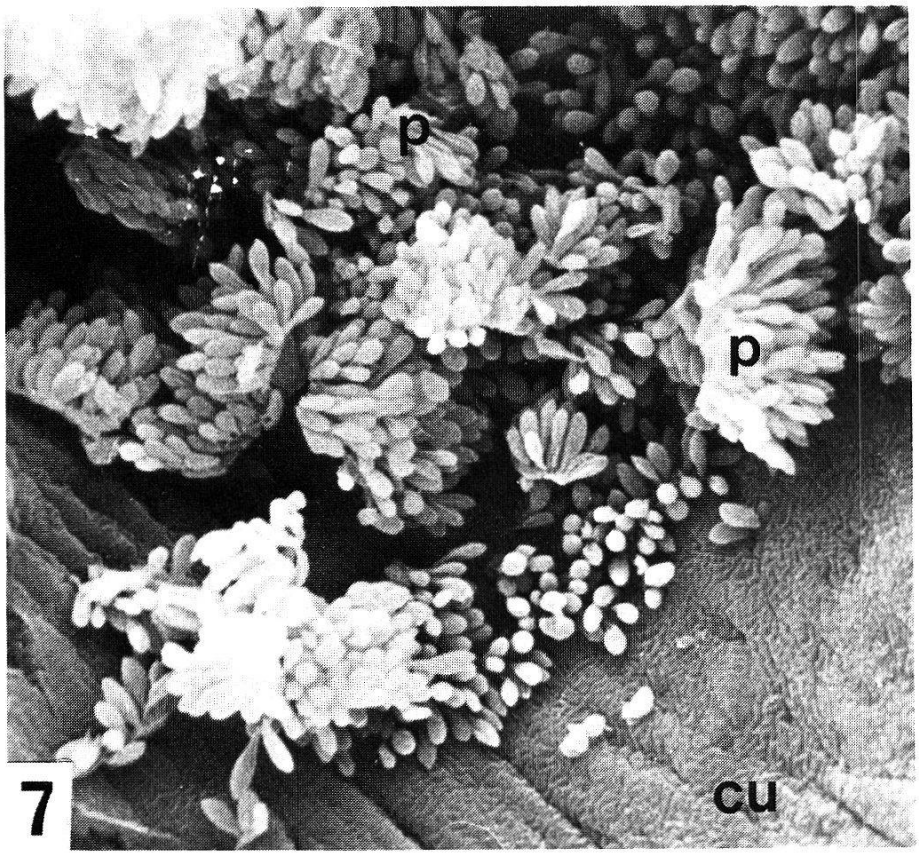

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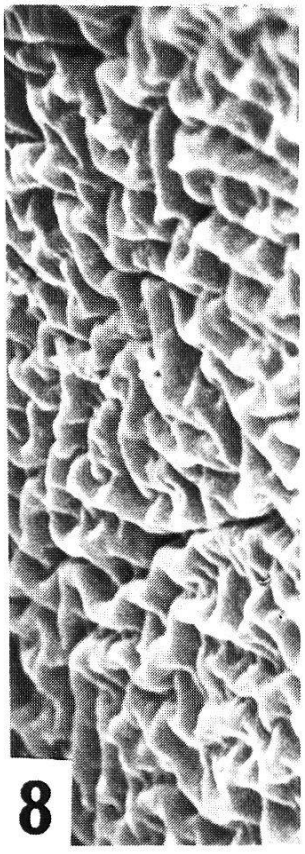

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tion between the midgut and pylorus is seen in infected keds by the immediate transition from the types of parasite colonizing the two surfaces as observed by Hoare (1923) (Figs. 5–7). The elongate epimastigotes of the midgut give way to smaller pyriform epimastigotes associated with the cuticular surface of the pylorus, the parasites being attached to the corrugated cuticular lined epithelium (Figs. 7, 9, 10, 12, 13). In infected keds the surface of the pylorus is heavily if not completely colonized by the parasites. The 'pile carpet' of layered parasites lining this region is more uniform in height and the parasites more closely packed than that formed by the parasites lining the midgut. In addition, the similar size of the parasites and the shorter distance between the attachment zone and the distal surface of this parasite prevents the distal end of the parasites moving in the rapidly contracting gut and the fluid contained therein. This observation is confirmed by studies on living material which reveals only occasional twitches of the attached parasites, a fact related to the absence of a free flagellum and the shortness of the parasite.

Colonization of the pylorus by epimastigotes from the midgut would appear to take place by association of these free swimming forms with the edge of the attached haptomonads forming this palisade. The structure and appearance of the attachment zones of haptomonads to the cuticle when observed by SEM is illustrated in Figs. 16–18, where it is seen that the anterior part of the parasite is expanded. The flagellum is not as evident as it is in the parasites of the midgut nor is the reservoir clearly visible because of the expansion of the intraflagellar space to form the attachment plaque (Molyneux, 1975). The rosettes in the pylorus are sometimes detached from the surface whereas the bulk of parasites are associated with the surface itself. True rosettes of parasites seem attached to each other by their flagellar extremities, a view supported by TEM studies (Fig. 11) (Molyneux, 1975). Attachment occurred despite the corrugations (Figs. 7, 8) of the surface cuticle of the pylorus. These corrugations were more extensive than those observed in the ileum which although it was longitudinally folded did not have the regular corrugations found in the pylorus. Individual parasites were more frequently seen in the ileum and this allowed closer observation of the expanded flagella in contact with the surface (Figs. 16–18). Occasional

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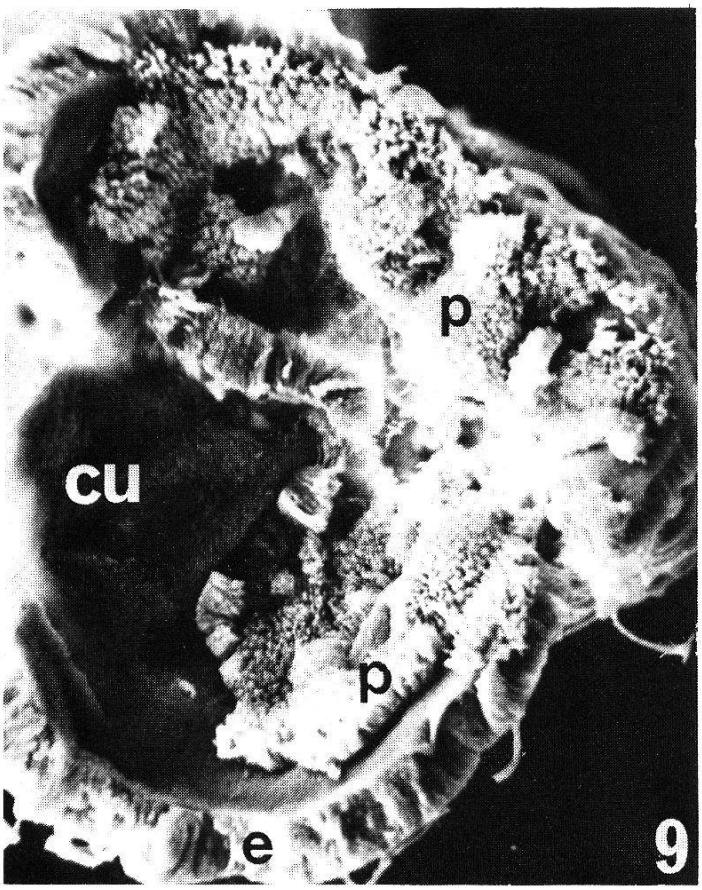
Fig. 9. SEM of pylorus of infected *M. ovinus* fractured immediately posterior to the midgut junction. Pile carpet of parasite (p); unparasitised cuticular lining (cu). Epithelial wall of hindgut triangle (e). ×230.

Fig. 10. SEM of pylorus showing lateral view of pile carpet or palisade of parasites (p); ranks of attached parasites are observed. Cuticle (cu); epithelial cell (e). × 1150.

Fig. 11. Higher magnification SEM of a rosette in pylorus. Note attachment by flagellar extremities and absence of reservoir opening as seen in Fig. 5. ×5100.

Fig. 12. Similar subject as Fig. 10. ×600.

Fig. 13. Attached parasites with flagellar membrane expanded attached (arrowed) and orientated with anterior end of flagella of parasite on cuticle (cu) lining. Note absence of apparent reservoir opening. × 10,500.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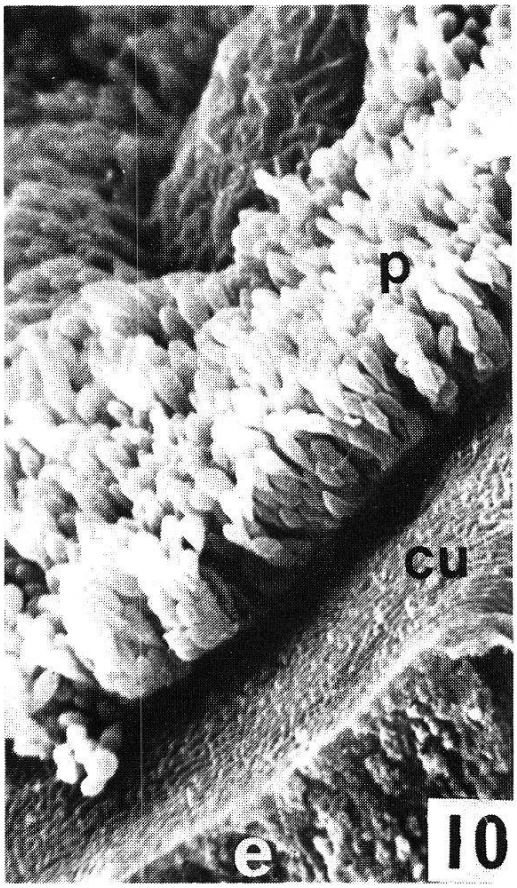
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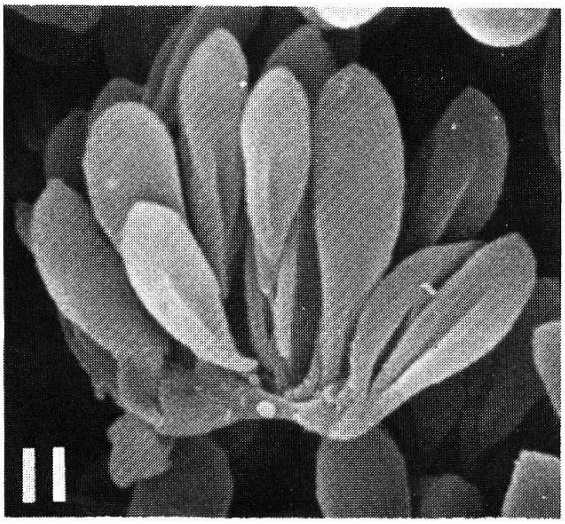
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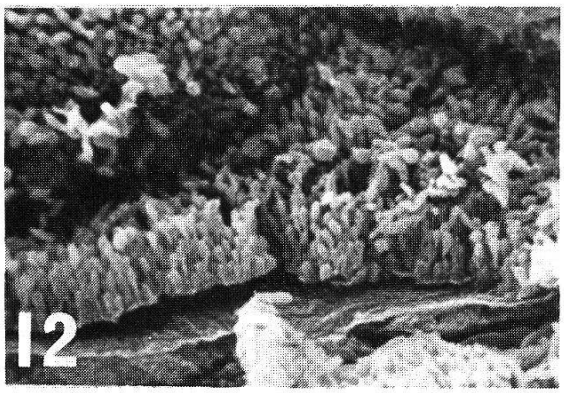
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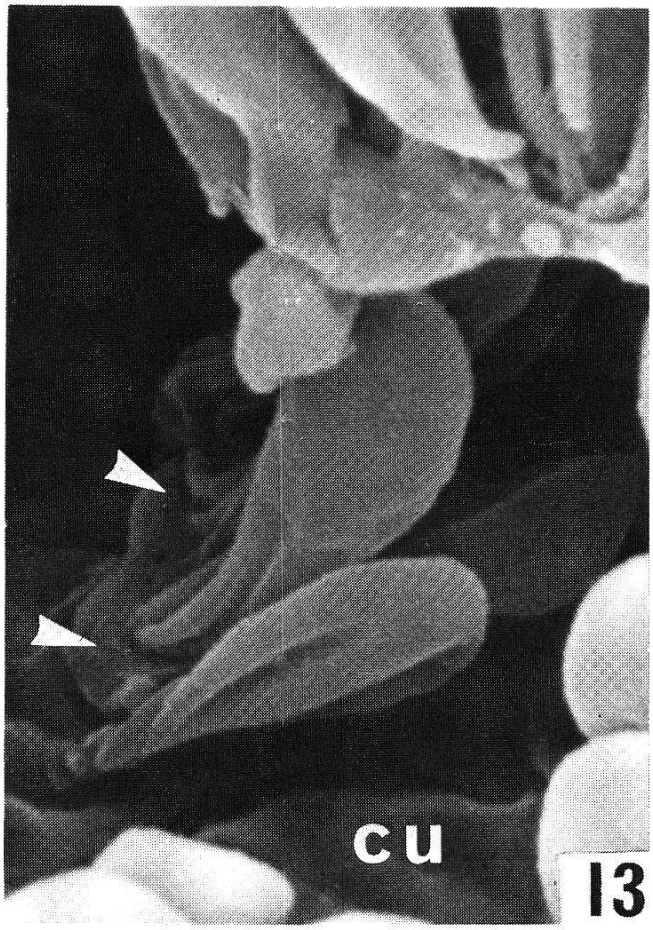
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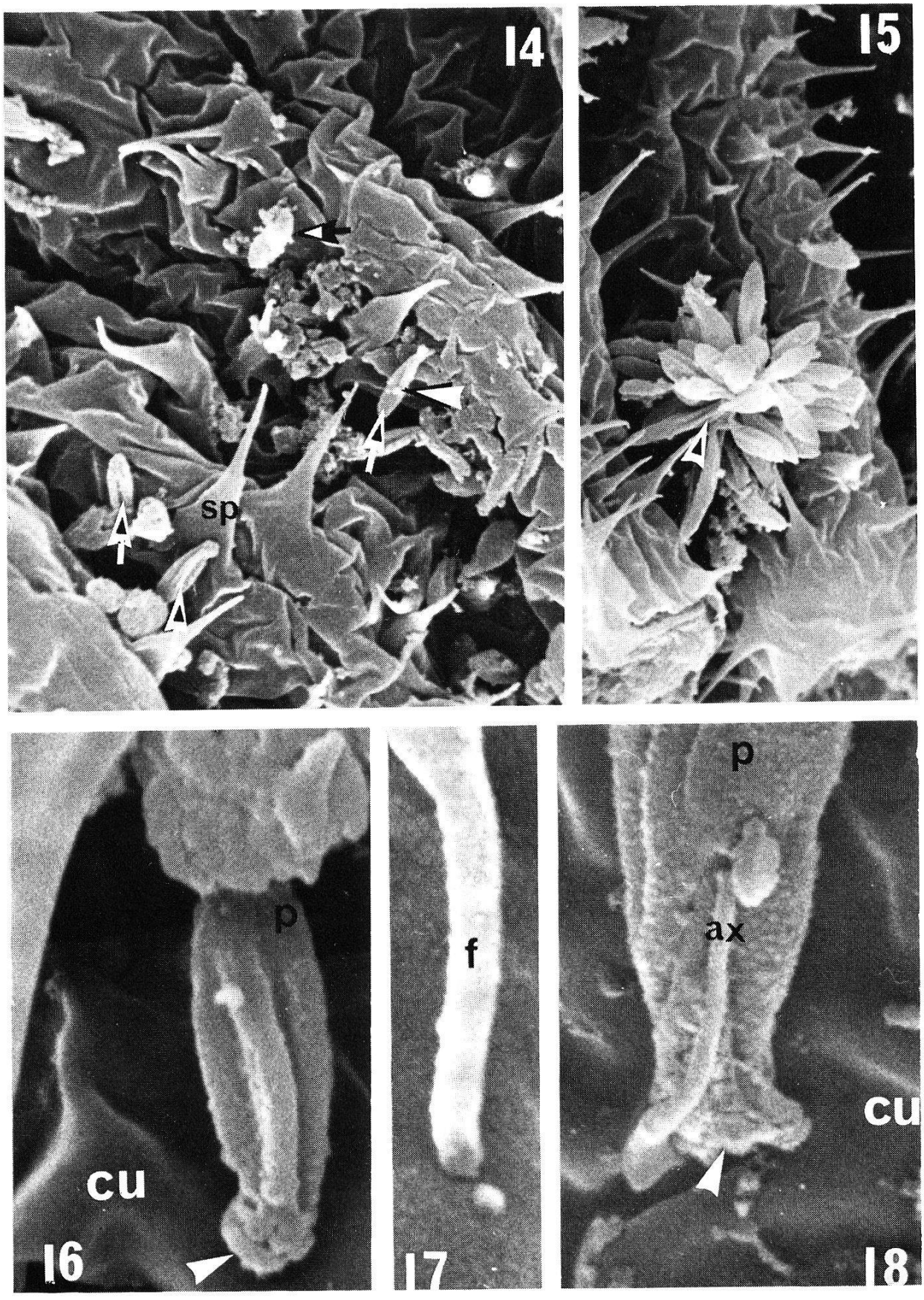
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rosettes of parasites associated with the surface in this part of the gut were observed but intense colonization was not evident (Figs. 14, 15).

Discussion

The spacial relationships of a trypanosome in the insect host have been revealed in the midgut, pylorus and ileum and the differences in parasite configuration and in the surface to which they attach have been demonstrated. The adherence of the parasites during fixation and processing to their respective surfaces indicates that attachment is strong and the structure of the parasites as observed by the other microscopical techniques is confirmed. This technique, when applied to other insect trypanosomatid systems, may enable the differentiation of the factors associated with the host surface which permit establishment, multiplication and hence transmission of infections in vectors to take place. The surface structure for example of the probosces of different species of *Glossina* in relation to susceptibility to *Nannomonas* and *Duttonella* infections or the oesophageal valves of sandflies in relation to susceptibility to *Leishmania* may be relevant in this context. The technique will also allow the definition of the numbers of parasites capable of occupying a unit area of gut, thus allowing quantification of levels of infection in insects. The extraordinary numbers of parasites colonizing the gut suggest that a symbiotic association may exist between parasite and host in the absence of observed pathogenicity despite the views of Nelson (1956). This possibility requires investigation.

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Fig. 14. SEM of cuticle lining ileum itself of *M. ovinus*. Note presence of spicules (sp), less extensive corrugation on surface and longitudinal striations of gut wall associated with muscle layers and folding in parts. Parasites (arrowed). ×2400.

Fig. 15. Rosette of parasites (arrowed) associated with ileum between two striations. ×2050.

Fig. 16. Pyriform parasite (possibly metacyclic) attached to ileum cuticle (cu). Note absence of detectable reservoir as observed in midgut epimastigotes (Figs. 4 and 5) and expanded flagellar extremities (arrowed) in contact with surface cuticle. ×11,900.

Fig. 17. Flagellum (f) of parasite in ileum associated with surface but without expanded flagellar extremity. ×20,000.

Fig. 18. Attached pyriform parasite (p) in ileum with expanded flagellar extremity (arrowed): axoneme (ax) of flagellum forms ridge associated with expanded region. ×20,000.

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