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Trypanosoma (Megatrypanum) melophagium: Modes of attachment of parasites to mid-gut, hindgut and rectum of the sheep ked, *Melophagus ovinus*

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Introduction

Since HOARE (1923) described in great detail the life-cycle of the sheep trypanosome, *Trypanosoma (Megatrypanum) melophagium* in the sheep ked *Melophagus ovinus,* only NELSON (1956, 1958, 1961) and HERBERT (1965) have investigated this parasite. NELSON (1956) reported that infected *M. ovinus* died as a result of the infection with *T. (M.) melophagium* but HOARE (1972) disputes that the trypanosomes were the cause of death. HERBERT (1965) studied the cytochemistry of the culture forms of *T. (M.) melophagium.* This paper describes the host-parasite relationship of *T. (M.) melophagium* in *M. ovinus* with particular reference to the mode of attachment of the parasites to the different regions of the gut wall.

Materials and Methods

Melophagus ovinus were obtained from sheep kept at the Wellcome Research Laboratories, Berkhampstead and from sheep kept at the School of Tropical Medicine, Liverpool. *M. ovinus* from Berkhampstead were infected with *T. (M.) melophagium* whilst those from Liverpool were not infected and provided a control. *M. ovinus* were dissected in physiological saline or in ice-cold 3% glutaraldehyde in 0.2 M cacodylate buffer or in ice-cold 2.5% glutaraldehyde in 0.05 M cacodylate buffer containing 0.15 M sucrose. The various parts of the gut were removed and separated into posterior mid-gut, hind-gut triangle, hind-gut and rectum. After fixation, the material was washed in the appropriate ice-cold buffer for one hour and post-fixed in cold 1% osmium tetroxide in the appropriate buffer for one hour. Dehydration took place in an ethanol series, after which the pieces of gut were treated with propylene oxide before infiltration with Taab embedding medium. After overnight treatment in this medium at room temperature, the material was embedded in fresh medium. Embedded material was polymerised for 48 hours at 60 °C. Sections were cut on an LKB ultramicrotome, stained with uranyl acetate and lead citrate and examined in an AEI EM 6B electron microscope.

Results

(a) Mid-gut

The flagella of the parasites in the mid-gut of *M. ovinus* interdigitate between the microvilli forming the lining brush border of the epithelial cells. Between the microvilli, large masses of mycoplasma-like organisms (MLOs) are also present and the flagella and anterior end of the epimastigotes are found packed closely amongst microvilli and MLOs (Fig. 1 and 2). The MLOs have a trilaminar outer unit membrane, are spherical and ovoid in section and their size range is within that of other mycoplasmas (circa 400 nm) (HORNE 1972, MANILOFF 1972). These

organisms are extracellular and are therefore not *Rickettsia* or *Chlamydia* which are intracellular as are the bacteroids of the *Glossina* mid-gut mycetome (REINHARDT et al. 1972). Present in the mid-gut epithelial cells of all the specimens of *M. ovinus* examined, are virus-like particles (VLPs) approximately 50 nm in diameter (Fig. 6). These particles are found in the microvilli and also free in the mid-gut lumen (Fig. 5).

The flagella of *T. (M.) melophagium* come into close contact with the plasma membrane of the mid-gut epithelial cells themselves, thus extending the whole length of the microvilli and running both parallel (Fig. 4) and at right angles to the direction of the microvilli (Fig. 2). Occasionally, the anterior ends of the epimastigotes are found close to the base of microvilli (Fig. 3). No modifications of the flagella such as formation of myelin figures within the flagellar sheath have been observed in the mid-gut, nor have multiple axonemes been found (MOLYNEUX 1969, KILLICK-KENDRICK et al. 1974). No hemidesmosomal junctional complexes are observed between flagella and host material or MLOs. STEIGER (1973) found that the flagella of *T. brucei* epimastigotes formed hemidesmosomes with the microvilli of the salivary gland epithelium of *Glossina* but KILLICK-KENDRICK et al. (1974) found no such junctional complexes between flagella of *Leishmania mexicana amazonensis* and sandfly mid-gut microvilli.

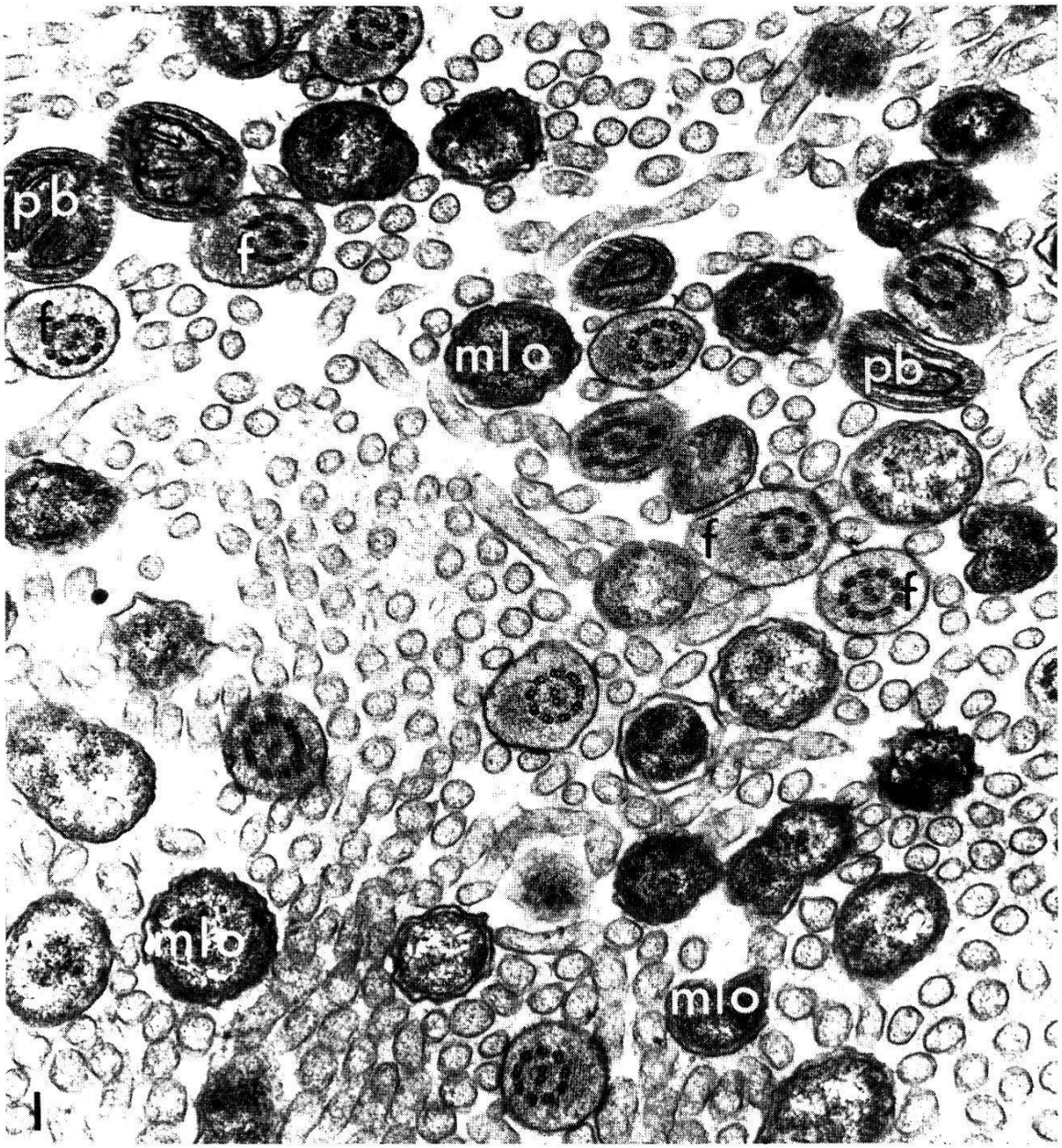
(b) Hind-gut triangle and hind-gut

These parts of the gut are lined with a smooth lining of cuticle. These were termed respectively by HOARE (1923) the iliac bulb and the colon. Infected *M. ovinus* have a "pile carpet" of epimastigotes and metacyclic trypomastigotes lining the cuticular epithelial cells (Fig. 7). The whole of the cuticular surface is found to be lined by expanded flagella with hemidesmosomal plaques closely applied to the flagellar membrane close to the adjacent cuticle (Fig. 7 and 8), and running parallel to it. The term hemidesmosome has been used following earlier publications on this subject by BROOKER (1970, 1971), VICKERMAN (1973), STEIGER (1973) KILLICK-KENDRICK et al. (1974).

The hemidesmosomes of *T. (M.) melophagium* applied to the hind-gut cuticle are electron dense plaques which run parallel to the flagellar membrane and the cuticle lining the hind-gut. The intensely electron dense area of the hemidesmosome is a band approximately 30–40 nm wide. The trilaminate flagellar membrane is 7–9 nm and the distance between the outer layer of this plasmalemma and the hind-gut cuticle is 12–15 nm where a hemidesmosome is present. The hemidesmosomal material is laid down within 2–3 nm of the inner lamina of the flagellar membrane and is so clearly defined at the distal end, that it sometimes gives the impression of being an extra component to the flagellar membrane (Fig. 8). At the proximal surface of the hemidesmosome material is seen flowing towards the dense plaque. The material, however, is not organised into any configuration.

Fig. 1. Transverse section through the microvilli lining the epithelial cells of *Melophagus ovinus* mid-gut infected with *Trypanosoma (M). melophagium*. Section of both flagella (f) and anterior ends of epimastigote parasites (pb) showing insertion into microvillar borders. Mycoplasma-like organisms (mlo) are found in the same region. × 30,000.

Fig. 2. Cross sections of flagella close to base of microvilli (mv) and mid-gut epithelium (me) from which the microvilli arise. No hemidesmosomes are present in the flagella despite their close relationship with the microvilli. Mycoplasma-like organisms (mlo). × 50,000.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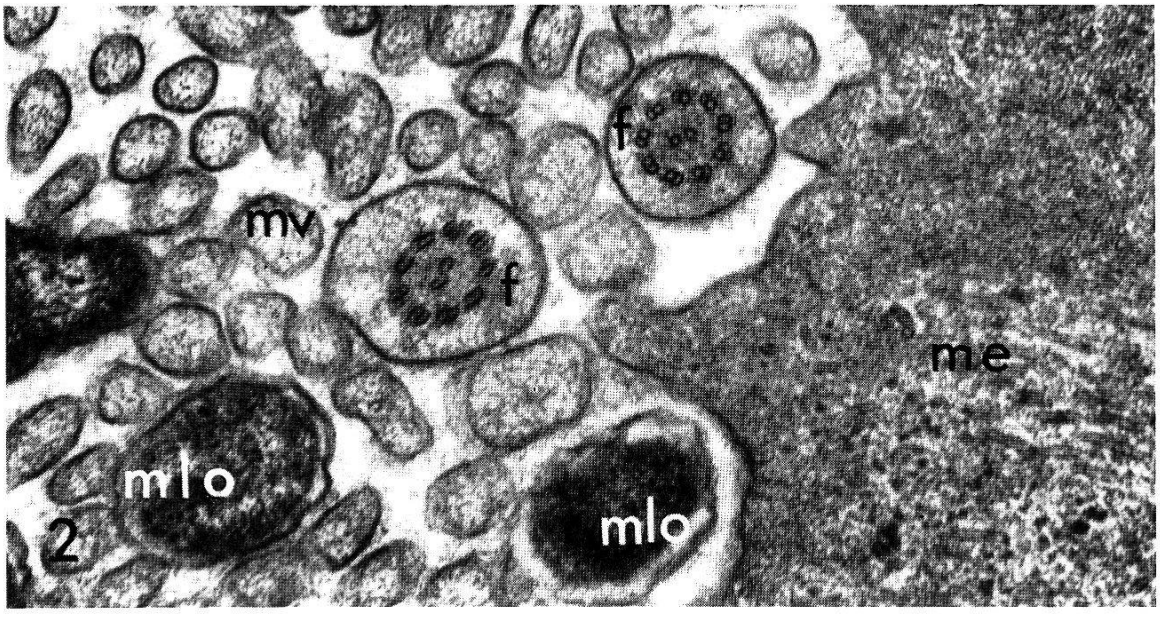
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The flagella of individual parasites in the hind-gut form desmosomes between adjacent flagellar membranes (Fig. 10) and there is a great expansion of the intraflagellar region of all attached parasites. The desmosomes formed between flagella have electron dense deposits on both sides of the opposed membranes as well as a deposit between these membranes. The flagellar membranes are separated by 10–12 nm but deposits occur on both sides of the membranes and in the space between. The deposit on the flagellar side of the membrane only extends 10–15 nm into the intraflagellar region. The outer component of the trilaminar flagellar membranes forming the flagellar desmosomes is more electron dense than the inner ones (Fig. 10). Rosette formation of epimastigotes in the lumen of the hind-gut is also mediated through desmosomal connections between flagella with expanded intraflagellar regions (Fig. 9).

(c) Rectum

The rectum of *M. ovinus* is not colonised heavily with parasites but a few groups of small numbers of epimastigotes and metacyclic trypomastigotes are found attached to the folded cuticular epithelium of the rectum. The mechanisms by which they are attached seem to be similar to that observed in the hind-gut. Hemidesmosomal plaques are found (Fig. 11) of the same structure and in the same relationship to the flagella and to the host cuticle. The flagella themselves are not as closely packed in the rectum as in the hind-gut because of the smaller numbers present. The enlargement of the intraflagellar space is not as prominent as in the hind-gut nor are desmosomal connections between the flagella of different parasites so common in the rectum. No multiple axonemes were seen as described by MOLYNEUX (1969) in *T. lewisi*.

Discussion

The methods of attachment of trypanosomatids to the gut wall of their insect hosts has been studied over the past years by several workers. The results obtained so far show that attachment of flagellates to the surface with which they come into contact are mediated through what are now termed hemidesmosomes. Although the molecular basis of this type of attachment mechanism is unknown, it has been demonstrated in *Trypanosoma* subgenera *Herpetosoma* (MOLYNEUX 1969), *Trypanozoon* (STEIGER 1973), *Duttonella* (VICKERMAN 1973); in the insect flagellate genera *Crithidia* (BROOKER 1971) and *Herpetomonas* (BRUN 1974), and in the genus *Leishmania* (KILLICK-KENDRICK et al. 1974). This communication reports the presence of a hemidesmosomal attachment mechanism in a species of trypano-

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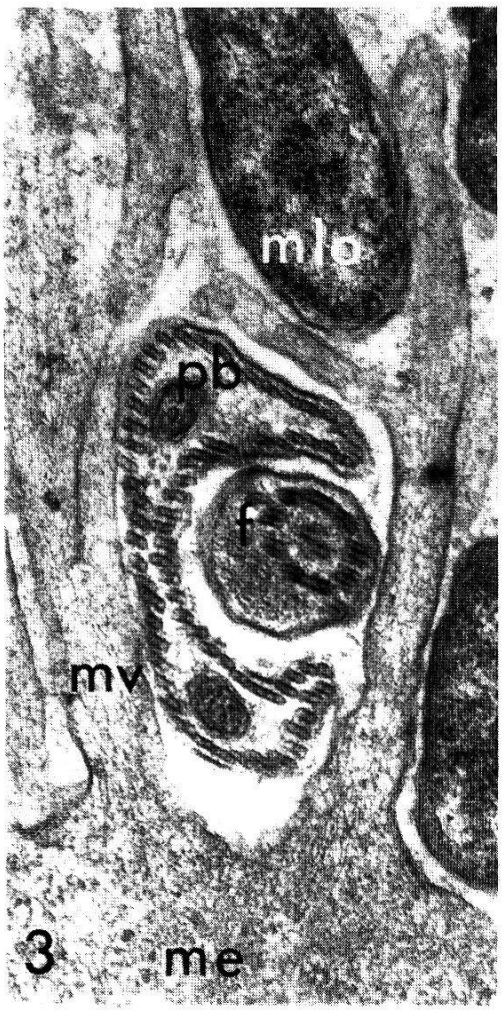
Fig. 3. Mid-gut of *M. ovinus*. Transverse section of an epimastigote (pb) coming into close contact with a mid-gut cell (mc) and the base of the microvilli (mv). Mycoplasma-like organisms (mlo) present between microvilli. × 50,000.

Fig. 4. Longitudinal section of flagellum with its axoneme (ax) reaching base of microvilli. × 40,000.

Fig. 5. Mid-gut epithelial cell of *M. ovinus* containing virus-like particles in the apical part (v) and in the microvilli (vv). Flagella (f) in contact with base of microvilli. × 22,500.

Fig. 6. Virus-like particles (v) in mid-gut cells of *M. ovinus*. Base of microvilli (mv) and gut lumen to the left of the figure. × 80,000.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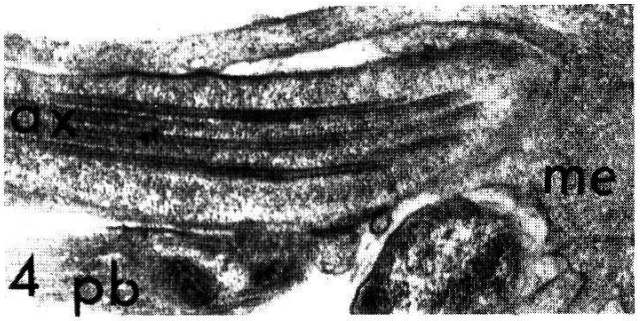
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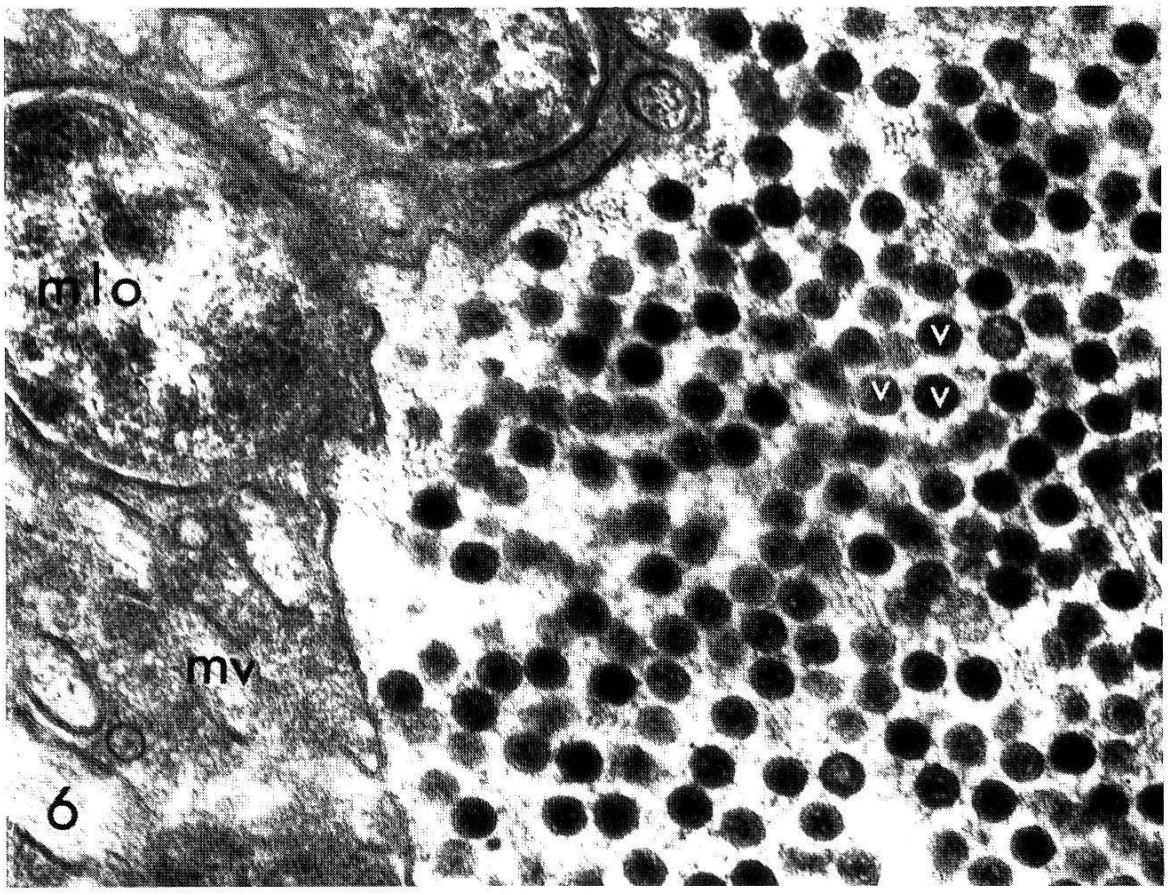
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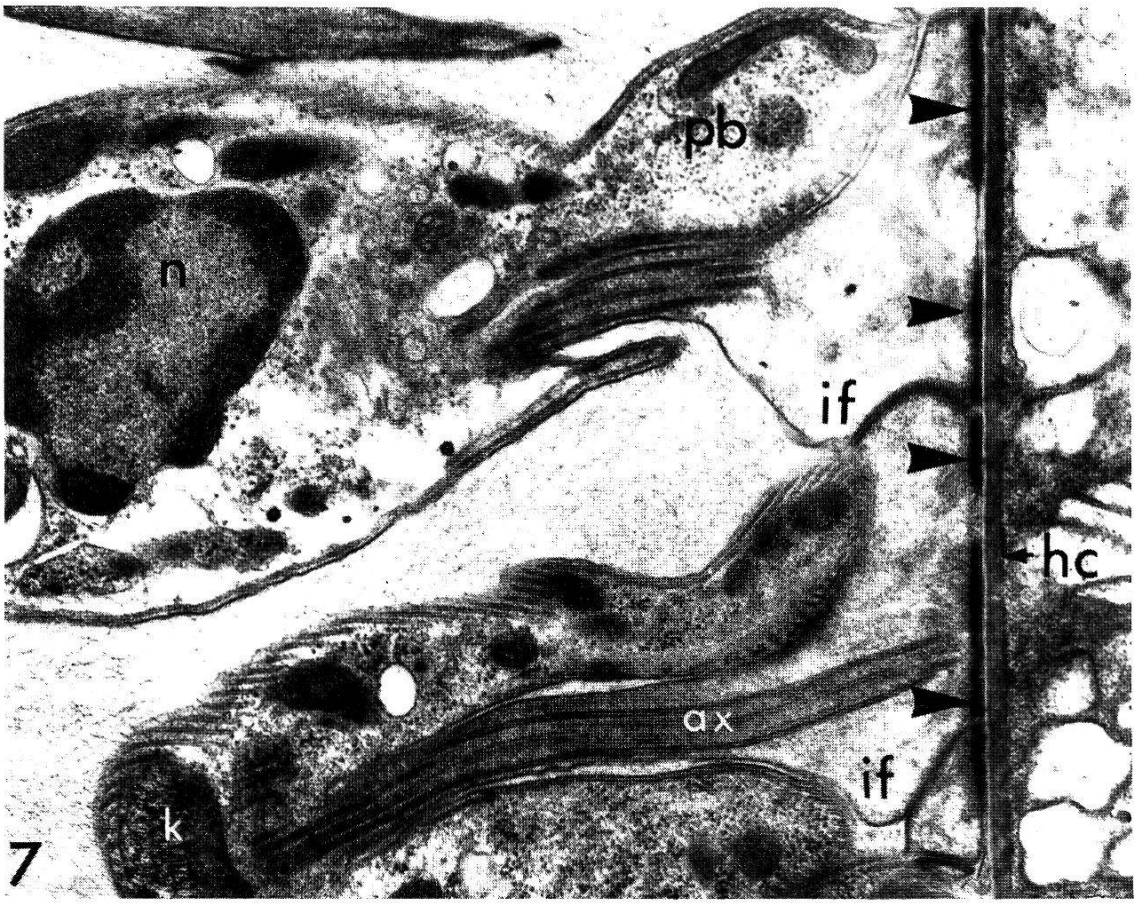
some of the subgenus *Megatrypanum, T. (M.) melophagium* in the hind-gut and rectum of *M. ovinus*. Hemidesmosomes were not seen to mediate attachment to the microvilli of the mid-gut epithelial cells, attachment of the epimastigotes here being by interdigitation of the anterior end of the epimastigotes and the flagella amongst the microvilli and mycoplasma-like organisms lining mid-gut cells. KILLICK-KENDRICK et al. (1974) found a similar situation in *Leishmania* infected sandflies where the nectomonad promastigotes penetrated between the microvilli but no hemidesmosomes were seen. STEIGER (1973), however, found that in *T. brucei* infections in the salivary glands of *Glossina,* the flagella of the epimastigotes were inserted between the microvilli of the salivary gland epithelial cells and hemidesmosomes were formed. The formation of flagella-microvillar hemidesmosomes in salivary glands and not in the mid-gut, suggests that there may be a difference in surface structure between the two types of microvilli, possibly associated with the ionic composition and relative charges on the glycocalyx surface coat of the microvilli. No evidence of a peritrophic membrane has been found in *M. ovinus* mid-gut, and no penetration of flagella into the mid-gut epithelial cells has been observed (KILLOCK-KENDRICK et al. 1974), nor have any parasites been observed in the mid-gut cells (BAILEY & BROOKS 1972).

The close association between the flagella of *T. (M.) melophagium,* microvilli and the MLOs in the mid-gut of *M. ovinus* and the presence of the MLOs may help establishment and anchorage of the flagella amongst the microvilli. The attachment of *Eperythrozoon coccoides* to trypanosome flagella was observed by MOLYNEUX (1970) and as mycoplasmas are very similar in structure to *Erythrozoon* and *Haemobartonella* (TANAKA et al. 1965, PETERS et al. 1974), it is possible that flagella-mycoplasma attachment in this site is an important factor in establishment of the parasites in the mid-gut of *M. ovinus* as all keds examined have been found infected with these MLOs.

The axoneme of *T. (D). vivax* epimastigotes attached to the labrum of *G. fuscipes* is oriented parallel to the cuticle lining of the proboscis and thus runs alongside the hemidesmosomes (VICKERMAN 1973). In *T. (M.) melophagium* in the hind-gut of *M. ovinus,* however, the axoneme usually approaches the hemidesmosome at right angles to the cuticular lining (see Fig. 7 and 8), and does not run parallel to the hemidesmosome. There is also no evidence in *T. (M.) melophagium* for any deposition of material between the flagellar membrane and cuticle where such material has been found in earlier work on cuticle-trypanosomatid attachment (MOLYNEUX 1969, BROOKER 1971, VICKERMAN 1973, KILLICK-KENDRICK et al. 1974). The change in flagellar structure from mid-gut to hind-gut in *T. (M.) melophagium* shows the adaptability of the trypanosome to its environment and emphasises the function of the flagellum as an organelle of attachment in addition to one of locomotion.

Fig. 7. Anterior part of hind-gut triangle region showing two epimastigotes of *T. (M.) melophagium* attached to cuticular lining of the hind-gut (hc →). Massive expansion of the intraflagellar space (if). The axoneme (ax) extends within a short distance of the hemidesmosome. Dark electron dense bands (= hemidesmosomes) parallel to flagellar membrane and cuticle (→). Nucleus (n), kinetoplast (k), parasite body (pb). × 22,500.

Fig. 8. Details of flagellum of epimagtigote of *T. (M.) melophagium* attached to hind-gut of *M. ovinus*. The flagellar membrane (fm) runs parallel to the cuticle lining the hind-gut. Space between parasite membrane and host cell (→) devoid of electron dense material. The hemidesmosome (hd) has a thick black edge being more diffuse proximally. The axoneme (ax) courses towards the hemidesmosome. × 100,000.



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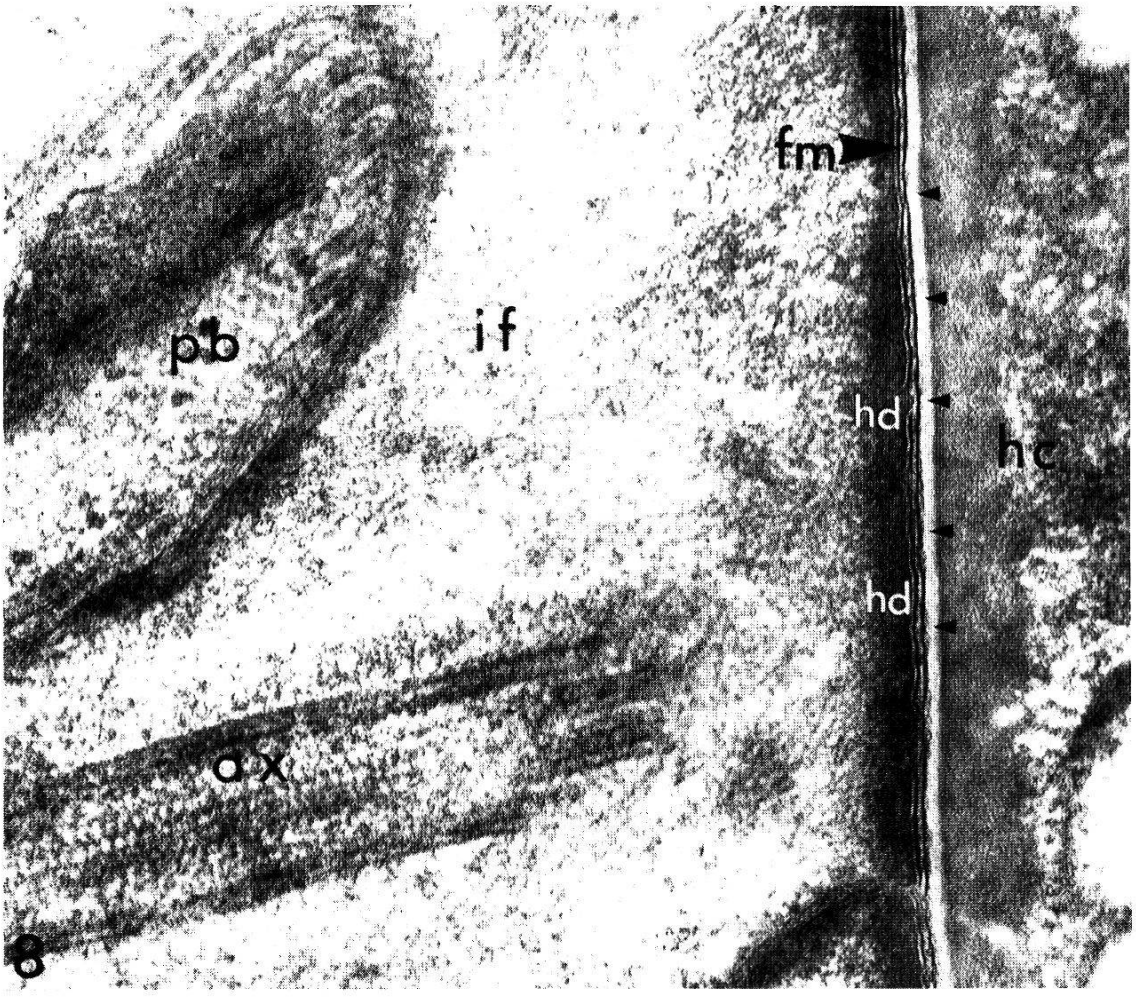

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T. (M.) melophagium in *M. ovinus* will provide an excellent model for a detailed biochemical study of the mechanism of attachment by hemidesmosomes to cuticle. The significance of cuticle to the establishment of trypanosomatids in insect vectors has been discussed by KILLICK-KENDRICK et al. (1974), who suggested that an inability to attach to cuticular surfaces may explain the relatively low infection rates in *Glossina* infected with trypanosomes of the subgenus *Trypanozoon* whilst the subgenera *Nannomonas* and *Duttonella* are able to assume such junctional complexes by flagellar modification with the cuticle lined mouthparts. HOARE (1923) (Plate 14, Fig. 94–96), figures the relationship of *T. (M.) melophagium* in *M. ovinus* as observed in light microscope sections of the hind-gut. If this diagram is compared with a later one of HOARE (1931) (Plate 18, Fig. 14), of the crocodile trypanosome *T. grayi* in the hind-gut of *Glossina,* the association between parasites and hind-gut is very similar. This suggests that in *T. grayi* attachment is mediated in the same way as that of *T. (M.) melophagium.* If *T. grayi* can form hemidesmosomes with the hind-gut of *Glossina* and *T. (D.) vivax* with the proboscis cuticle (VICKERMAN 1973), the absence of any attachment of *T. brucei* sub-group to cuticular lined organs of *Glossina* could be based on the parasites inability to undergo the required morphogenesis rather than the fact that the fly may not provide conditions suitable for establishment.

From this work, it seems that hemidesmosomes are a widespread attachment mechanism in trypanosomatids. They have not yet been shown to mediate attachment in the subgenera *Schizotrypanum* and *Nannomonas* but it seems predictable that the flagella-cuticle relationship will be found to be similar in these two groups.

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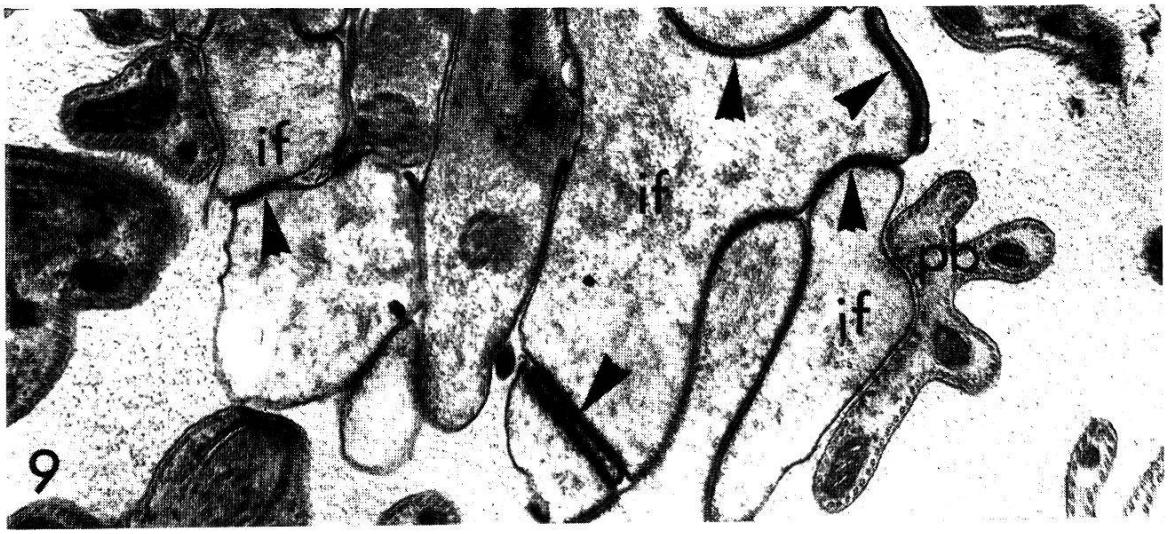
Fig. 9. Enlarged intraflagellar spaces (if) of separate parasites anchored together to form a rosette in the lumen of the anterior hind-gut. Flagellum-flagellum desmosomes (→). Some flagella have flagellar desmosomes between several different flagella. × 22,500.

Fig. 10. Detail of hemidesmosome (hd) attachments to hind-gut cuticle and flagellar desmosomes (fd) between two flagella. Material (m) within flagellar sheath is seen which is streaming towards hemidesmosome. Thickenings on flagellar desmosomes (fd) are not as large as those on hemidesmosomes. Material is present between apposed membrane of a flagellar desmosome (compare with Fig. 8). × 80,000.

Fig. 11. T. (M.) melophagium in rectum of the sheep ked with three flagella attached by hemidesmosomes (hd) to cuticle lining rectal wall (rc). There is less enlargement of intraflagellar space in this site compared with hind-gut. × 60,000.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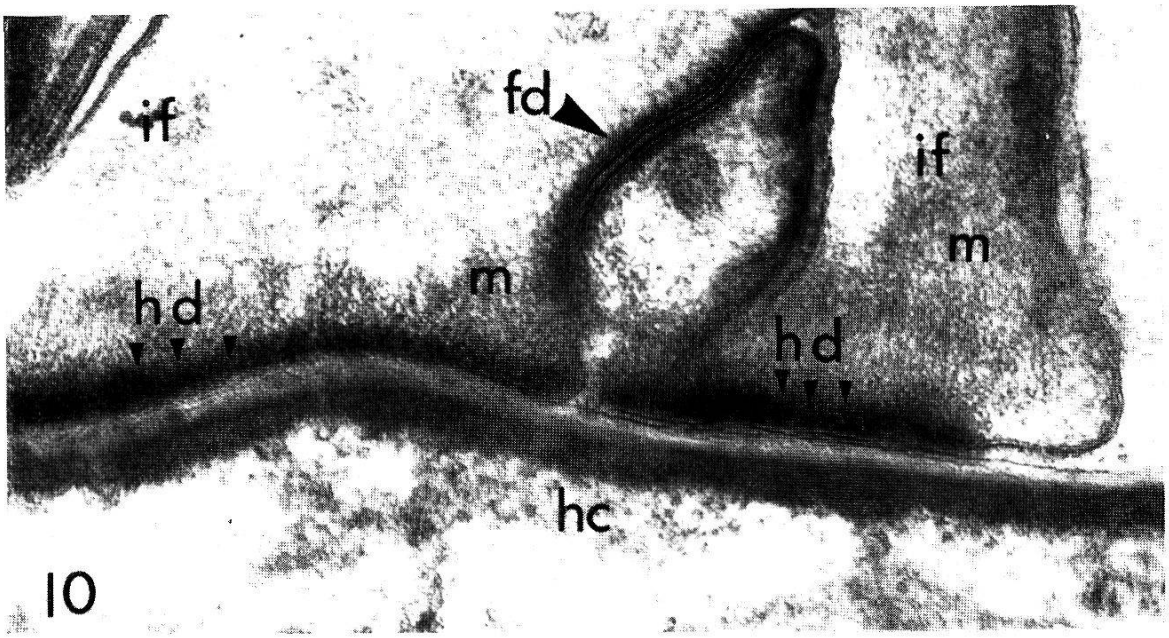
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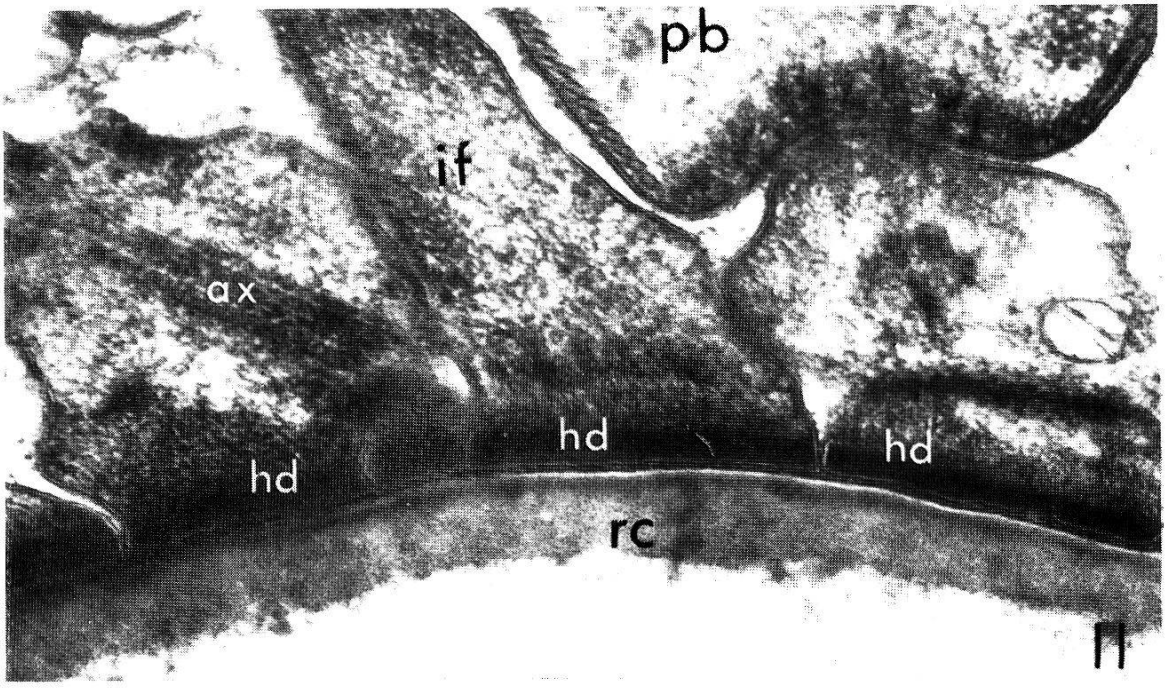
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