Distribution and attachment of "Trypanosoma (Nannomonas) congolense" in the proximal part of the proboscis of "Glossina morsitans morsitans"

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Distribution and attachment of *Trypanosoma (Nannomonas) congolense* in the proximal part of the proboscis of *Glossina morsitans morsitans*[1]

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Summary

The distribution and attachment of *Trypanosoma congolense* were investigated in the proximal part of the proboscis of *Glossina m. morsitans*. In the food canal, epimastigotes and trypomastigotes formed tufts or compact layers. Trypanosomes were attached to the cuticle by their flagella, which formed zonar hemidesmosomes. The flagella were mostly attached parallel to the axis of the labrum and often pointed to its tip. Foot-like processes of the flagella came into contact with adjacent flagella leading to dense grouping of the trypanosomes. Despite narrow contacts between adjacent flagella, no desmosomes were differentiated. The trypanosomes were attached to all parts of the LCl sensilla and might thus impair their function.

Uncoated and coated forms were present in the labrum, which indicated that transformation to metacyclic forms is not strictly limited to the hypopharynx.

Uncoated forms were often attached to the cuticle of the common salivary duct and hypopharynx by hemidesmosomes. Coated forms could also be attached. Neither compact layers nor tufts of trypanosomes were found. This attachment may partly explain the low number of metacyclics deposited when flies probed on a warm glass slide.

Key words: Trypanosoma congolense; Glossina m. morsitans; fly proboscis; parasite development; cell attachment; ultrastructure.

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Introduction

The morphology and behaviour of *T. congolense* in the bloodstream of vertebrate hosts has been investigated by several authors (e.g. Vickerman, 1969; Nantulya et al., 1978; Banks, 1978, 1979). Further work has been done on the various forms in the organs of the tsetse vector (Robertson, 1913; Bruce et al., 1914; Lloyd and Johnson, 1924; Hoare, 1972). Only a few papers exist on ultrastructural aspects of *T. congolense* in the fly. Evans et al. (1979) and Kaddu and Mutinga (1980) studied forms in the midgut. Evans et al. (1979) described preliminary aspects of the attachment of epimastigote forms in the labrum.

The present paper describes the distribution and attachment of various forms of *T. congolense* in the proximal part of the proboscis of *G. m. morsitans*. The interactions within the trypanosome population and between the parasite and the vector are shown. These relationships may influence the feeding behaviour of the tsetse fly and are of epizootiologic importance (Molyneux et al., 1979; Jenni et al., 1980). This study is part of an ultrastructural investigation of the whole life cycle of *T. congolense*.

Materials and methods

Trypanosoma congolense: clone STIB 68-F-A which derived from stock STIB 228 was used (Schläppi and Jenni, 1977). STIB 228 was isolated from a lion *(Panthera leo)* in the Serengeti in 1971.

Glossina m. morsitans were forwarded to us as pupae by Drs. A. M. Jordan, Langford, Bristol and J. Itard, Maisons-Alfort, Paris. Within 24 h after hatching, flies were infected on white ICR female mice which were at the first peak of parasitemia. Afterwards the flies were fed on uninfected mice 3 times a week. The saliva of the flies was checked for trypanosomes 12 and 18 d after the infectious bloodmeal. *G. morsitans* containing metacyclic forms in their saliva were isolated. Proboscis and various other parts of the digestive tract were dissected between 17 and 40 d after the infectious bloodmeal and 2 d after the last feeding.

Twenty flies (14 males and 6 females) were prepared for electron microscopy following a standardized procedure. In order to avoid evacuation of trypanosomes from the proboscis, flies were immobilized over dry ice for 5–10 sec. The head was then cut off, the proboscis dissected at the level of the bulbous membrane (terminology according to Jobling, 1933) and placed in 3% glutaraldehyde (v/v) in 0.2 M phosphate buffer containing saccharose (buffer and saccharose = 430 mOsm, complete fixation solution = 735 mOsm). Prefixation took place at room temperature for 90 min. Samples were washed in 3 changes of buffer containing saccharose (430 mOsm) overnight at 4° C. Postfixation was carried out in 2% OsO$_4$ (w/v) in 0.2 M cacodylate buffer at 4° C for 2 h. Block staining occurred in 2% uranylacetate (w/v) in acetone 70% for 1 h; dehydration in several steps of graded acetone and anhydrous propylene oxide. Specimens were embedded in epon. Semithin and ultrathin sections were cut with glass and diamond knives on a Reichert OmU2.

Three zones of the proboscis were cut transversally and the position of the trypanosomes investigated (from proximal to distal with respect to the proboscis):

Zone 1 *Hyoid* (= zone 11, Jobling, 1933: hyoid and common salivary duct completely separated).

Zone 2 *Subhyoid* (= zone 12, Jobling, 1933: food canal and common salivary duct separated, but surrounded by a single cuticle, proximal with respect to LCl sensilla, Rice et al., 1973).

Zone 3 *LCl sensilla* in the labrum (= zones 15 and 16, Jobling, 1933: food canal laterally extended, containing LCl sensilla, hypopharynx ventral to food canal, cavities containing the labral nerves located laterally to food canal).

Thin sections were stained with lead citrate and photographs taken in a Philips EM 300 on 70 mm roll film. Semithin sections were stained with azur-II-methylenblue and used for light microscopic observations.

Results

### 1. T. congolense in the hyoid and labrum of G. m. morsitans

Density of the infection

Five of the investigated flies showed a high infection with trypanosomes. The parasites often covered more than ¾ of the cuticular lining of the subhyoid (zone 2) and of the labrum (zone 3), forming a compact layer. In 9 flies the infection was less dense, the trypanosomes forming tufts covering about 50% of the inner surface of the canal. Six glossines were only slightly infected. The trypanosomes then formed small and dispersed groups attached to the cuticular lining of the lumen. No correlation was observed between the density of the infection, the sex and the age of the flies. As the present investigation was limited to the proximal part of the labrum, no information is given about the density of infection in more distal parts.

Distribution and position of the trypanosomes

The examination of semithin sections revealed that small groups of trypanosomes may be fixed to the cuticular lining of the labral lumen in isolated tufts. On the other hand, they may form a continuous layer, or show an intermediate distribution between the 2 possibilities described. In zone 1 (hyoid) the trypanosomes were not attached but freely distributed in the lumen. Only in one of the flies examined 2 small tufts were seen, attached lateroventrally. In highly infected zones 2 (subhyoid) the trypanosomes were attached to the dorsal and ventral regions of the canal. They formed layers which were interrupted laterally. An uninterrupted layer was found only exceptionally (Fig. 1). In zone 3 (LCl sensilla) the attachment sites of the trypanosomes were found to be on the lateral walls of the tube. In high infections an extension of the attachment areas towards the ventral side of the tube could also be observed.

The preferred attachment sites in zones 2 and 3 were constant and not dependent on the density of the parasites. The attached trypanosomes were fixed to the cuticle by their flagella, the cell bodies tended to point to the center of the lumen (Figs. 1, 3). In 2 heavily infected flies most of the trypanosomes were longitudinally orientated with respect to the canal and grouped closely together, forming compact layers (Figs. 2, 10). The sectioned posterior parts of the cells were always found near the free lumen (Fig. 2). The maximal thickness of these trypanosome layers ranged from 8–12 μm. The boundary between the layers of attached trypanosomes and the free lumen was clearly visible (Figs. 2, 10). In zones 2 and 3 the layer of trypanosomes converted the originally free oval lumen to a circle. Only a few free trypanosomes were present in the lumen of the food canal (Fig. 1).

cu

cu

1

lu

cu

nu

fl

2

cu

nu

tf

3

tf

nu

4

166
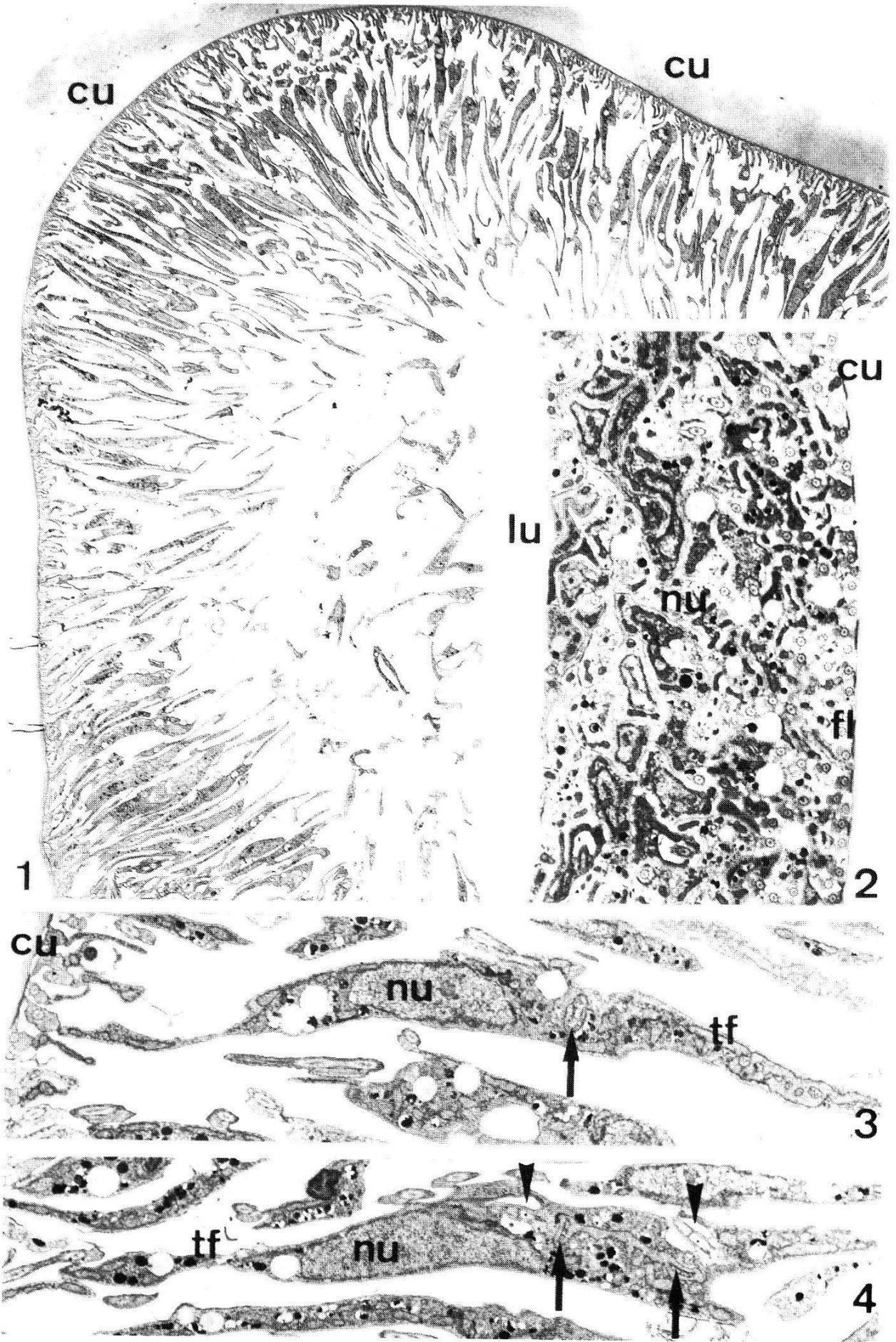
Forms of T. congolense attached to the labrum

Most of the long forms attached to the cuticle of the labrum were epimastigote forms. Trypomastigotes and intermediate forms between trypomastigotes and epimastigotes were less frequent (Fig. 3). The surface of the parasites was often covered by electron dense and irregular deposits (Figs. 7, 8). In addition, trypanosomes covered by a regular surface coat were also rarely found (Fig. 14). Dividing trypo- and epimastigote forms could be observed (Fig. 4).

Mode of attachment and contacts between trypanosomes

Vickerman (1973) described the structures of attachment of *T. vivax* in the labrum of *G. fuscipes*. The structures found in the present investigation for *T. congolense* were comparable to these findings.

Twelve or more rows of small desmosomes (maculae adherentes) were observed between the cell body of the trypanosome and its flagellum (Fig. 6). These rows run parallel to the axoneme. The halves of the junctions located in the cell body were denser and better visible than their counterparts in the flagellum (Figs. 6–8). Microfilaments originating from the desmosomes of the flagellum reached the flagellar matrix and some were connected with microfilaments originating from hemidesmosomes of the flagellar – cuticular junction (Fig. 7).

The trypanosomes were attached to the cuticle of the proboscis by their flagella and never by the cell body (Figs. 3, 5–10). The membrane and the matrix of an attached flagellum may be enlarged considerably, and zonar hemidesmosomes were formed (Figs. 5–8). The latter were more frequently found in the region of the flagellum, where this is in contact with the cell body. They were never seen at the flagellar tip, where the paraxial rod is absent. With respect to the axoneme, hemidesmosomes were located near the microtubular doublets 8 to 2 (Afzelius, 1959; Fig. 8). The structure of the hemidesmosomes of *T. congolense* was similar to that described for *T. vivax* (Vickerman, 1973).

In the present investigation, it was not possible to confirm the observation of Evans et al. (1979) describing desmosomes between flagella of adjacent trypanosomes. We found that the flagella of neighbouring parasites tended to gather together (Fig. 5). Single flagella could be observed in close contact with
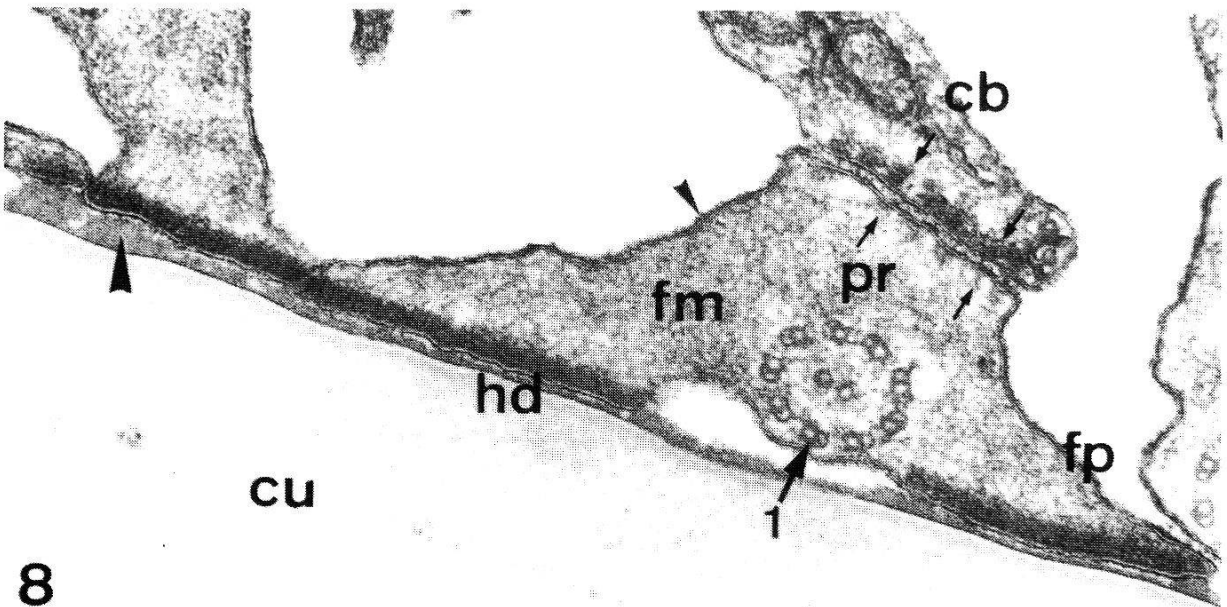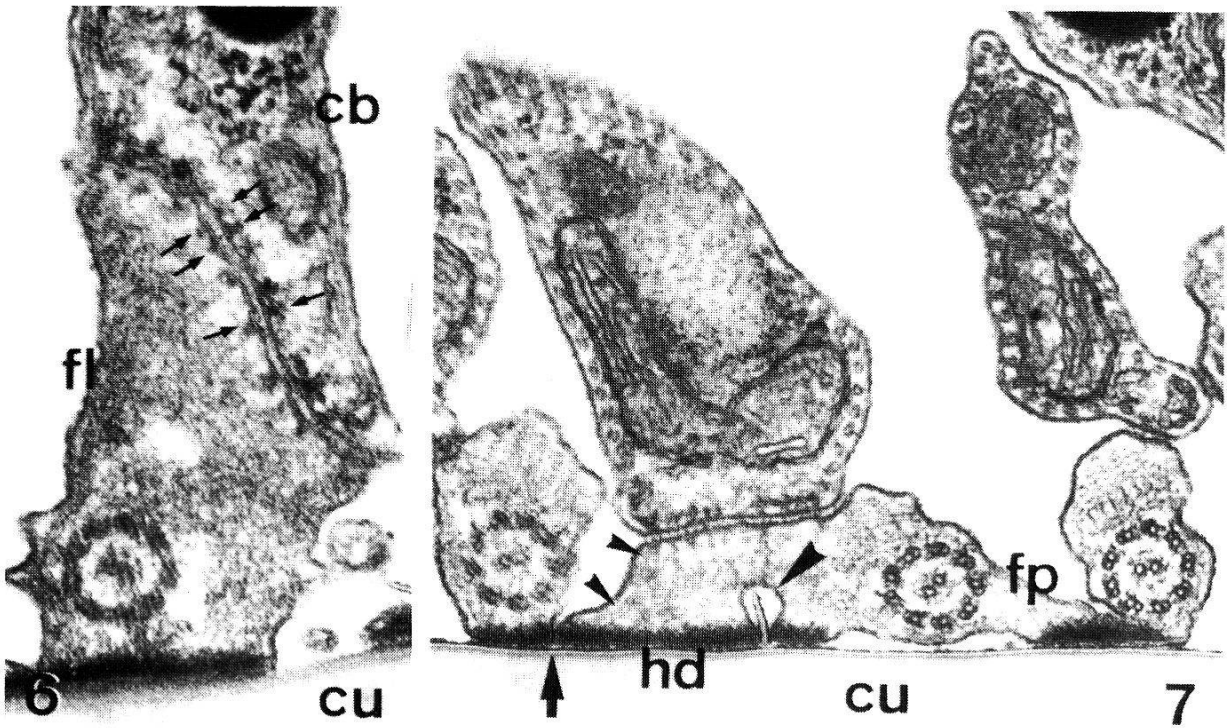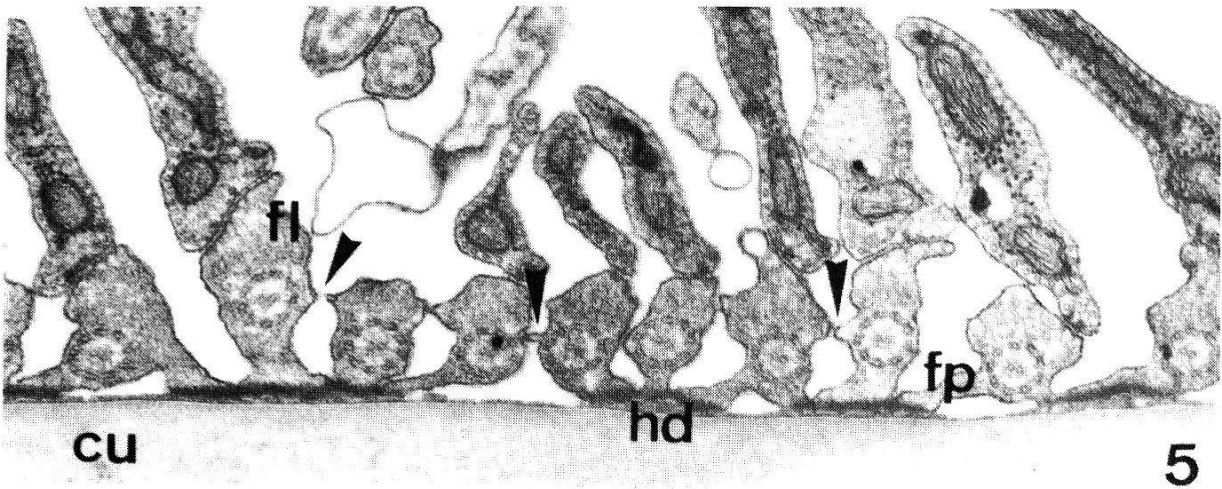

All figures are of *T. congolense* in the proboscis of *G. m. morsitans*.

Fig. 1. Subhyoid level (zone 2). Distribution of long forms attached to the cuticle (cu) of the labrum by the flagella, forming a continuous layer. Posterior end of the trypanosomes pointing to the center of the food canal; × 1300.

Fig. 2. Subhyoid level (zone 2). Trypanosomes attached to the cuticle (cu) of the labrum, orientated longitudinally with respect to the latter and forming a compact layer. Stratified appearance of the layer: attached flagella (fl), zone of nuclei (nu), posterior parts near the free lumen (lu), × 5200.

Fig. 3. Subhyoid level (zone 2). Trypomastigote form (tf) attached to the cuticle (cu) of the labrum. Nucleus (nu), kinetoplast (↗), × 6600.

Fig. 4. Same zone as fig. 3. Dividing trypomastigote form (tf). Nucleus (nu), kinetoplasts (↗), flagellar pockets (◀), × 6600.

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Figure 5: fl, hd, fp, cu

Figure 6: cb, fl, cu

Figure 7: fp, hd, cu

Figure 8: cb, pr, fm, hd, fp, cu

the cuticle, without forming hemidesmosomes. Only when they came laterally into contact with other flagella, hemidesmosomes were formed with the cuticle. This contact was often achieved by distinct foot-like processes (Figs. 5–8) from the flagella which gave rise to trypanosome tufts. The flagella grouped in this way often formed small projections towards their neighbours (Fig. 5). In this way they came into close contact. However, no specialized junctional structures such as desmosomes were observed.

Position of T. congolense on LCl sensilla

In zone 3, the trypanosomes were attached to either the cuticle of the labrum or to the basal cups, stalk-like collars and setae of the sensilla by means of hemidesmosomes (Figs. 9, 10). When the parasites were numerous and formed a compact layer, some of the sensilla were completely covered (Fig. 10).

Orientation of the trypanosomes in the food canal

In accordance with Evans et al. (1979), in the 3 zones the flagella of attached trypanosomes were generally orientated longitudinally with respect to the food canal (Figs. 2, 3, 5–9). This orientation of the flagella was also found when the cell bodies were disposed annularly in the lumen. The position of the arms of the subfibers A of the axoneme (DeRobertis et al., 1975) indicated whether the distal end of the flagella pointed towards the base or the tip of the labrum. At least 80% of the flagella pointed towards the tip of the proboscis in 6 flies, where the trypanosomes were orientated radially with respect to the food canal. In 2 labra, where the parasites formed an annular layer, 65 and 90% respectively of the flagellar tips were directed towards the base of the proboscis.


Fig. 5. Subhyoid level (zone 2). Anterior parts of long forms attached to the cuticle (cu) by flagella (fl). Flagella forming foot-like processes (fp), extensions between adjacent flagella (◀), hemidesmosomes (hd). Note grouped hemidesmosomes, ×24,000.

Fig. 6. Subhyoid level (zone 2). Long form attached to labral cuticle (cu). Transverse section of zone of adhesion between flagellum (fl) and cell body (cb). Several desmosomes (maculae adherentes, ⤢) present, ×51,000.

Fig. 7. LCl sensilla level (zone 3). Long forms attached to the labral cuticle (cu). Hemidesmosomes (hd) formed by foot-like processes (fp) in contact with other flagella (↗). Note the avoidance of foreign material (◀) by the cell membrane of the flagellum, and absence of a hemidesmosome in the vicinity of the axoneme. Microfilaments (▼), ×46,000.

Fig. 8. Subhyoid level (zone 2). Long form attached to the labral cuticle (cu). Foot-like processes (fp) formed laterally with respect to the axoneme. Attachment of the flagellum to the cuticle by hemidesmosomes (hd) typically facing doublets 8–2 of the axoneme (doublet 1 : 1→). Junctional gap between body (cb) and flagellum measuring c. 17 nm, maculae adherentes (⤢) less distinct on flagellar than on cell body side, paraxial rod (pr), extended flagellar matrix (fm).
Note dark deposits (▼) on the surface of the cell membrane and material of irregular thickness (◀) between cuticle and hemidesmosomes, ×61,000.

st

bc

cu

9

se

bc

cu

10

170
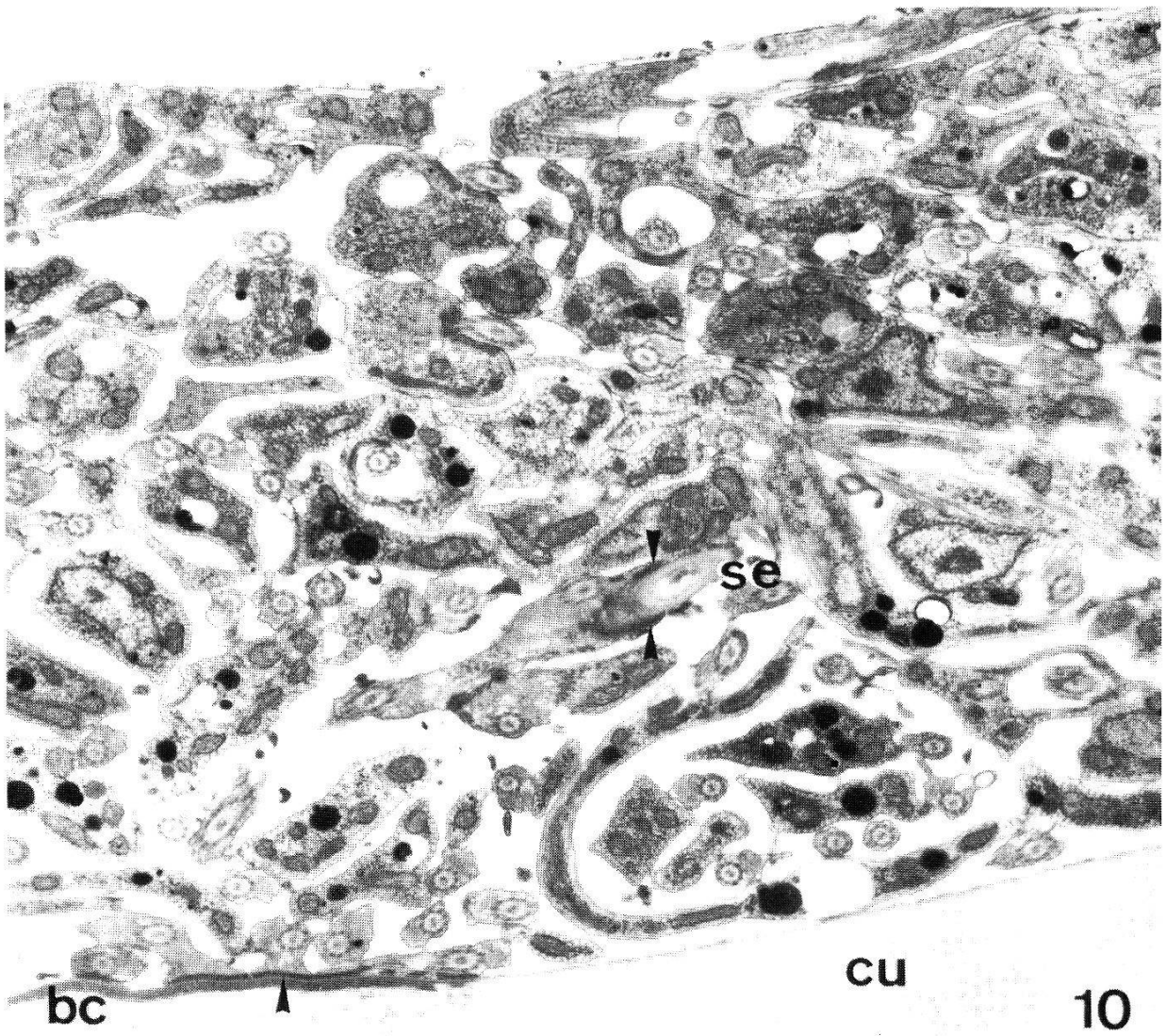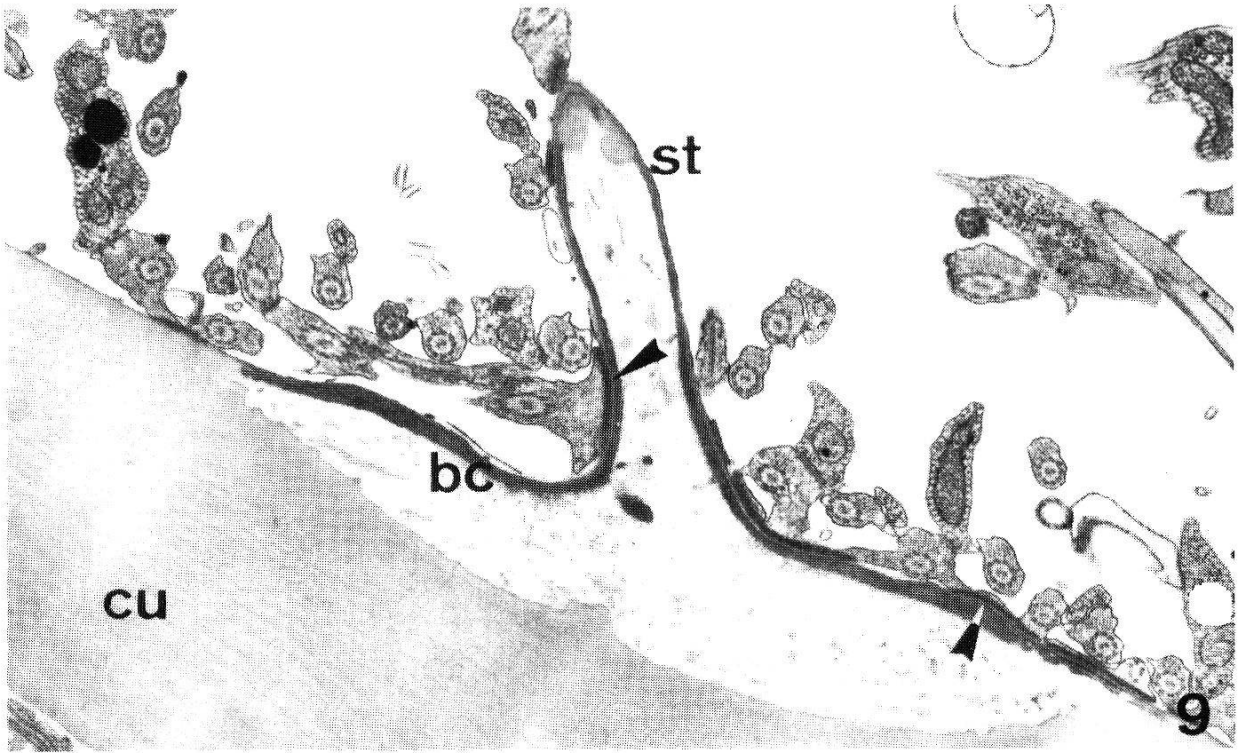
2. Hyphopharynx and common salivary duct

Trypanosomes were found in 14 of 20 infected flies in the hypopharynx. The density of the parasites was rather low and variable. However, a maximum of about 20 trypanosome profiles could be occasionally seen per sectioned plane. Long uncoated forms were sometimes present resembling those found in the food canal. In addition, forms bearing a coat were always encountered. Long forms were attached to the cuticle of the hypopharynx by hemidesmosomes. Coated forms exhibited a coat of thickness between 8 and 9 nm (Figs. 12, 13), the latter value being similar to that found for bloodstream forms (c. 9 nm). These coated forms were often attached to the cuticle by hemidesmosomes (Fig. 13). No formation of tufts and no distinct orientation of the parasites could be observed in the hypopharynx.

Coated and uncoated forms were present in the common salivary duct of 8 flies. Distribution and attachment of the forms were similar to the situation described in the hypopharynx (Fig. 11).

Discussion

The base of the labrum of *G. m. morsitans* seems to be more heavily infected by *T. congolense* than the distal part of the proboscis (Clarke, 1965). Therefore, the present investigation was concentrated on the proximal part of the labrum containing the highest densities of parasites.

Lloyd and Johnson (1924), among other authors, have already described the formation of trypanosomal tufts ("uncompact colonies") by the attachment of *T. congolense* to the labral cuticle. This seems not to be valid for all the zones of the proboscis, and especially not for heavy infections where the trypanosomes may form compact colonies. In the present paper results are presented which may explain to some extent the grouping of the trypanosomes into tufts or compact colonies. The contiguous position of attached flagella on the cuticle was evident and constant. The contact with the cuticle alone seemed not always to induce a flagellum to form hemidesmosomes. Contact with other flagella and / or existing hemidesmosomes seemed to favor the formation of new junctions between flagella and cuticle. This was supported by the observation of flagella which formed extensions towards flagella which were already attached. In contact with the latter and the cuticle this extension lead to the formation of hemidesmosomes. The attachment of single trypanosomes in the hypopharynx may be due to a loss of this "grouping-ability" or to the small density of the parasites present.

Fig. 9. Level of sensilla (zone 3). Long forms attached by hemidesmosomes (◄) to the cuticle (cu) of the labrum, and to the basal cup (bc) and stalk (st) of a sensillum, × 13,000.

Fig. 10. Level of sensilla (zone 3). Layer of long forms in the labrum including the obliquely cut seta (se) of a sensillum. Flagellates are attached to the seta and to the basal cup (bc) of sensilla by hemidesmosomes (◄), × 13,000.

lf

cu

11

mi

12

mi

cu   hd

13

14
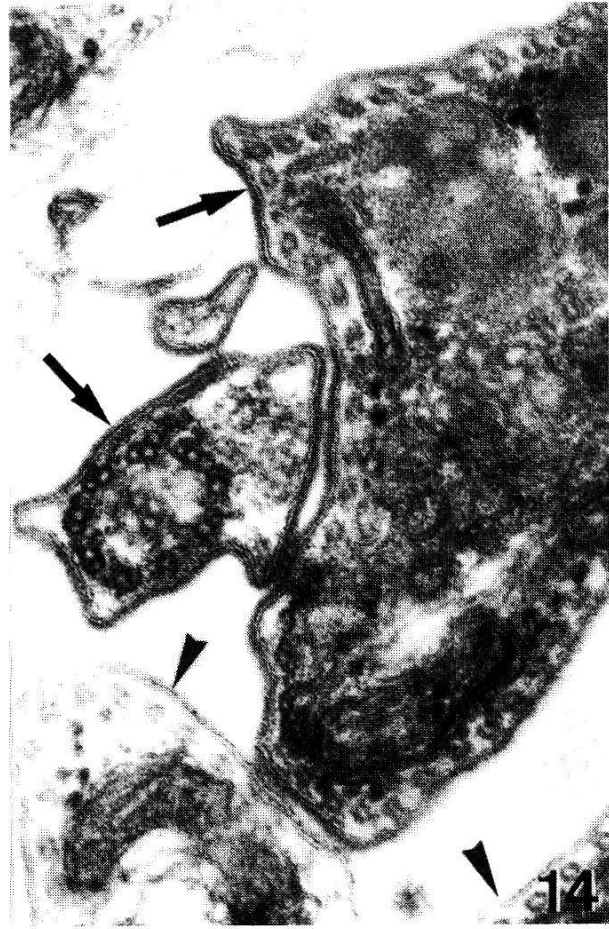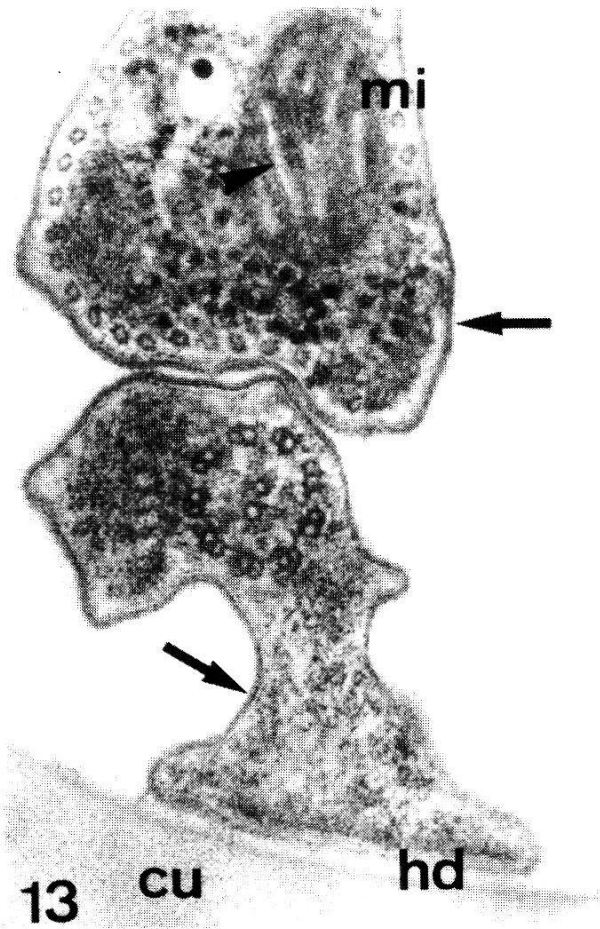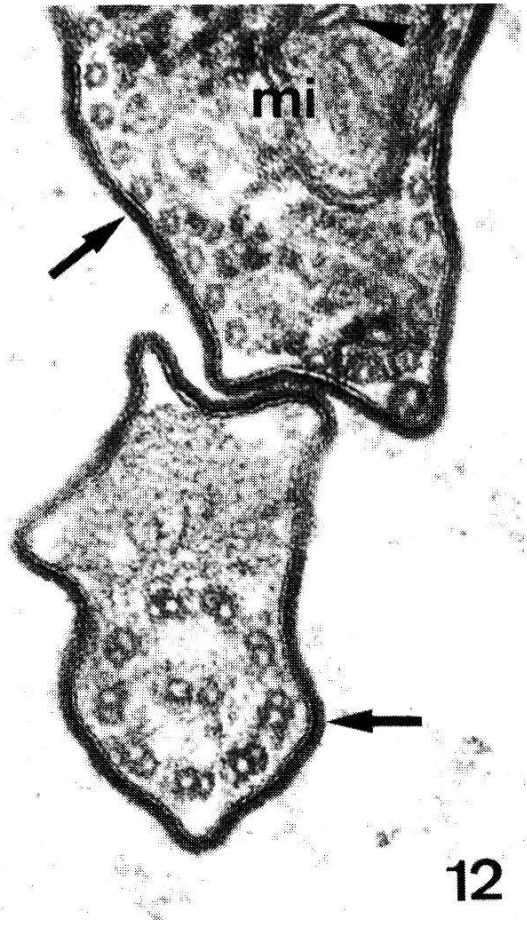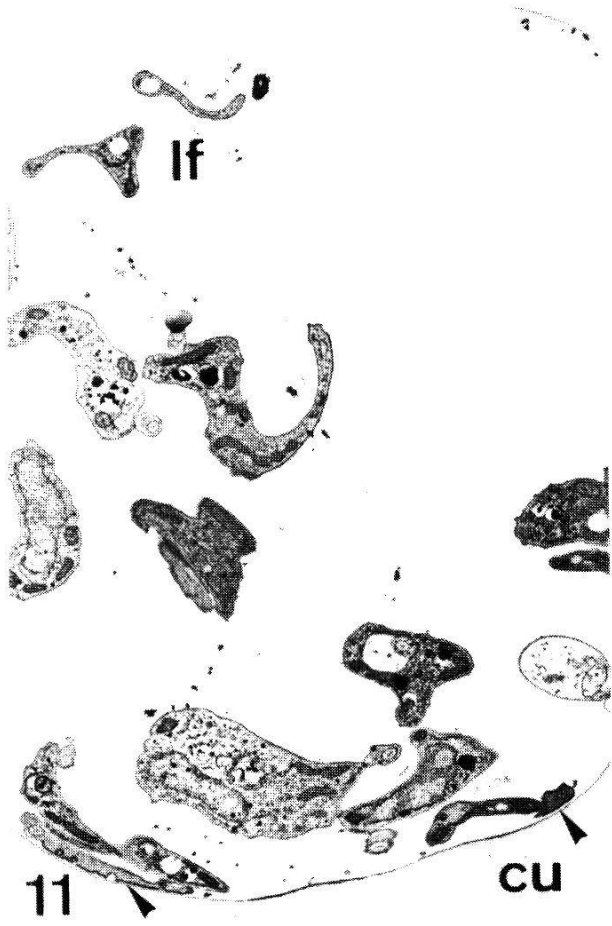
Büngener and Müller (1976) described the attachment of bloodstream forms of *T. congolense* to monocytes of the blood. Binding to erythrocytes and to the endothelia of small blood vessels by the anterior part of the flagellates was also demonstrated (Banks, 1978, 1979). In this way the parasites formed clusters resembling the tufts described in the present paper. However, the lack of detailed structural description of the attachment sites in the cited papers makes it impossible to compare directly the mode of attachment in the vertebrate host and in the fly.

Contacts between adjacent flagella by means of projections were described for attached forms in the labrum. As a result the contact zones were small and no distinct junctional structures were differentiated. No strong interdigitation of the cells involved took place as was shown for bloodstream forms by Büngener and Müller (1976). The significance of these interflagellar contacts and the part they play in the formation of the tufts in the labrum therefore remains unclear.

Hommel and Robertson (1976) stated that all the members of the family of the trypanosomatidae so far investigated undergo stages of development when they are fixed to the cuticle of the digestive tract of the invertebrate host. The mechanical function of the hemidesmosomes involved could be important for Nannomonas (e.g. *T. congolense)* and Duttonella *(T. vivax)*. Both genera are exposed to strong mechanical forces by the rapid flow of fluids in the labrum. They have to undergo structural and probably functional changes in the proboscis. Therefore, one can imagine that attachment structures in *T. congolense* are needed to prevent the developing stages from being evacuated. When blood is ingested the principal flow is directed from the tip of the food canal towards the pharynx. More than 80% of the trypanosomes were seen attached, with their anterior part directed towards the tip of the proboscis in most of the flies examined. A similar situation was observed in blood vessels of rats and rabbits, where the posterior end of attached *T. congolense* always pointed in the direction of the blood flow (Banks, 1978). The inverse position of the trypanosomes in 2 flies could not be explained.

The lateral accumulations of parasites at the zone of the LCl sensilla in the labrum could probably impair the function of the covered sensilla and reduced the diameter of the food canal. This may indeed influence the feeding behaviour of infected Glossina (Molyneux et al., 1979; Jenni et al., 1980).

Fig. 11. Hyoid level (zone 1). Distribution of trypanosomes in the common salivary duct. Posterior part of long forms (1f), and forms attached to the cuticle (cu) by hemidesmosomes (◄). Note variable contrast of trypanosomes, × 10,000.

Fig. 12. Hyoid level (zone 1). Trypanosome bearing a thick coat (↗) in the common salivary duct. Mitochondrion (mi) with a few cristae (◄) irregularly distributed, × 94,000.

Fig. 13. Hyoid level (zone 1). Coated trypanosome attached to the cuticle (cu) of the common salivary duct by a hemidesmosome (hd). Coat (↗), mitochondrion (mi) with regularly distributed cristae (◄), × 75,000.

Fig. 14. Subhyoid level (zone 2). Coated (↗) trypanosome in the labrum. Uncoated plasma membrane (◄) of surrounding trypanosomes, × 75,000.

It has been suggested until now that the transformation of the epimastigote forms to the metacyclics takes place in the hypopharynx (Wenyon, 1926; Hoare, 1972). In our study forms covered by a surface coat were also found attached in the proximal part of the food canal. This clearly demonstrated that the transformation to metacyclic forms may not strictly be limited to the hypopharynx.

The number of metacyclic forms deposited with saliva was low (usually less than 10 forms) when a fly was probing on a warmed glass slide. This could be due to the observed partial attachment of coated trypanosomes to the wall of the hypopharynx which were not liberated during the short time of probing.

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