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The Occurrence of Intracellular Rickettsia-like Organisms in the Tsetse Flies, *Glossina morsitans*, *G. fuscipes*, *G. brevipalpis* and *G. pallidipes*

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Abstract

Various tissues of the tsetse flies, *Glossina morsitans, G. fuscipes, G. brevipalpis* and *G. pallidipes* were examined with the electron microscope. Intracellular rickettsia-like organisms were observed in midgut epithelium, in cells associated with the fat body and in developing oocytes. The possible role of these organisms in tsetse flies is discussed.

Introduction

The presence of microorganisms within the midgut mycetocytes of the tsetse fly has been established for a considerable amount of time (ROUBAUD, 1919; WIGGLESWORTH, 1929). Recently an electron microscope study by REINHARDT et al. (1972a) described the microorganisms found in the midguts of *Glossina morsitans, G. fuscipes* and *G. brevipalpis*. In addition to the bacteroids reported in light microscope studies they reported the presence of sparse rickettsia-like microorganisms within the midgut mycetocytes of *G. morsitans* and *G. fuscipes*. In *G. brevipalpis* these organisms were found in the cytoplasm of surrounding muscle cells only.

The presence of endosymbiotic microorganisms within other tissues in many insects has also been established (BUCHNER, 1965; MILBURN, 1966; ROSHDY, 1968; REINHARDT et al., 1972a, b), and it has been suggested by LANHAM (1968) based on BUCHNER's survey (1965) that well over 10% of the living species of insects regularly have intracellular microorganisms. Therefore, it was not unreasonable to assume that the symbiotes of the tsetse fly are not confined to midgut tissue. Since some symbiotes are transferred transovarially (WIGGLESWORTH, 1965; BURGDORFER & VARMA, 1967) and rickettsial organisms as well as bacteria are sensitive to antibiotics (PERKINS & ALLISON, 1963), the presence of these rickettsia-like microorganisms within other tissues, particularly the ovary, could well lead to a method of aposymbiotic control of tsetse fly. It is the purpose of this study to report on the presence of these microorganisms in other tissues of the tsetse fly.

Materials and Methods

Tsetse flies for this study were obtained from three different sources. The first set, with a total of 40 flies, was obtained from Uganda and from Tanzania. All wild flies were slit along the abdomen and immersed whole in 4% glutaraldehyde buffered to pH 7.2 with 0.1 M phosphate-buffer. Upon arrival they were

dissected, rinsed in buffer and postfixed for one hour in 1 % OsO_4 (phosphate-buffered, pH 7.2).

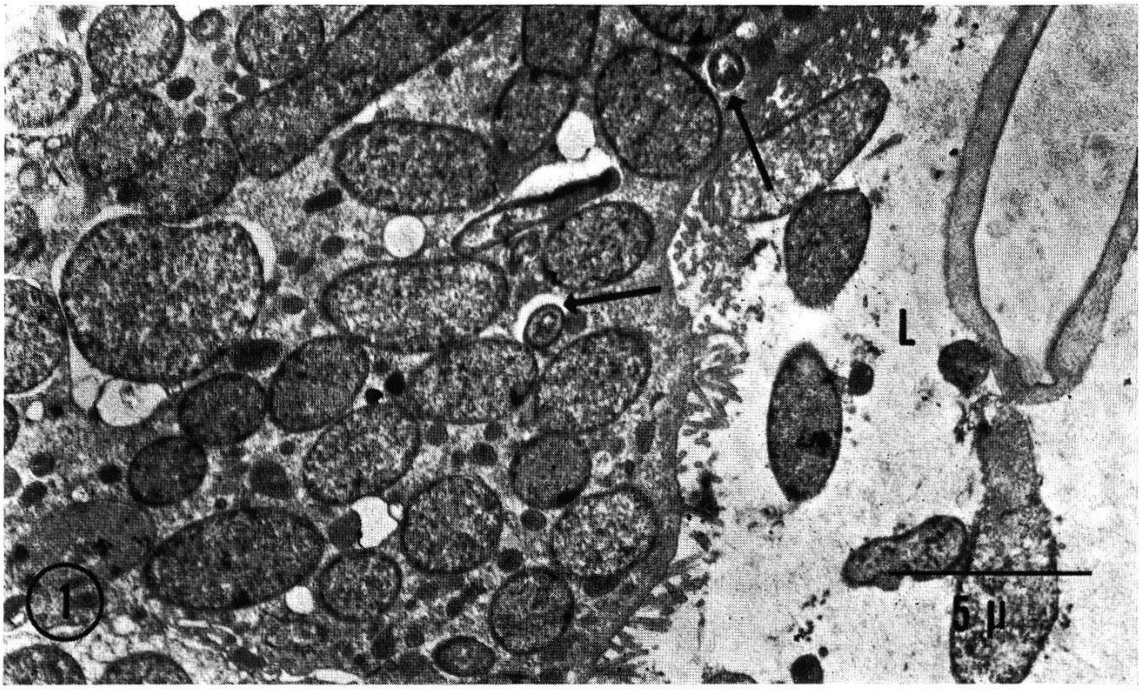
Eleven female flies obtained from the Tsetse Research Laboratory (Bristol University, England) were immersed whole in 12.5 % phosphate-buffered glutaraldehyde with the wings and legs removed and the abdomen slit dorsally. These flies were transferred to buffer, dissected and postfixed as above. All tissues were dehydrated in a graded ethanol series and embedded in Araldite 6005. Sections were obtained on a Porter Blum MT2 ultramicrotome and stained with uranyl acetate followed by lead citrate stain (REYNOLDS, 1963). Observations were made on a RCA-EMU 3F or Philips EM 300 electron microscope. The following species were investigated: *Glossina morsitans, G. fuscipes, G. brevipalpis* and *G. pallidipes.*

Results

The midguts of the tsetse flies were examined to determine whether the smaller bacteroids or rickettsia-like microorganisms described by REINHARDT et al. (1972a) were uniformly present in all sources. The mycetome described by WIGGLESWORTH (1929) was easily visible as an enlarged whitish ridge in the first bend of the anterior midgut. In the electron microscope the midgut cells were observed to contain large bacteria in the cytoplasm (Fig. 1). The bacteria filled the cells from the apical to basal regions. Bacteria were also observed within the gut lumen on occasion (Fig. 1). In the basal regions of midgut mycetocytes which were partially filled with bacteria, the "midgut cells" limiting membrane was observed to be ruptured, and bacteria were present in the space between the epithelial cells and the surrounding muscle layer. The bacteria were similar in structure to those described by REINHARDT et al. (1972a) being rod-shaped and measuring up to 9.5 μ in length and 1.8 μ in width.

Also present within the midgut cytoplasm were smaller rickettsia-like organisms. These organisms were also rod-shaped, 1.9 μ in length and 0.56 μ in width with a double membrane. The outer membrane of the organisms was scalloped or wavy and appeared to be layered, perhaps due to the angle of sectioning (Figs. 7, 8, 9, 10). The organisms were separated from the cytoplasm by a clear zone that may be lytic (arrows, Fig. 1; also Figs. 7, 8, 10). In addition to those within the mycetome, organisms were found to be present in the general midgut epithelium (Fig. 2). The organisms were not confined to any region of the cytoplasm but were situated randomly. The frequency of these organisms in midgut cells other than the mycetome was very low and they were not found in every sample. The morphology and size of the organisms present in the midgut epithelial cells were similar to those found in the mycetomes (Fig. 7). No organisms were observed within the muscle-cell cytoplasm that basally envelops the midgut epithelium.

Cells closely associated with the fat body were observed to contain



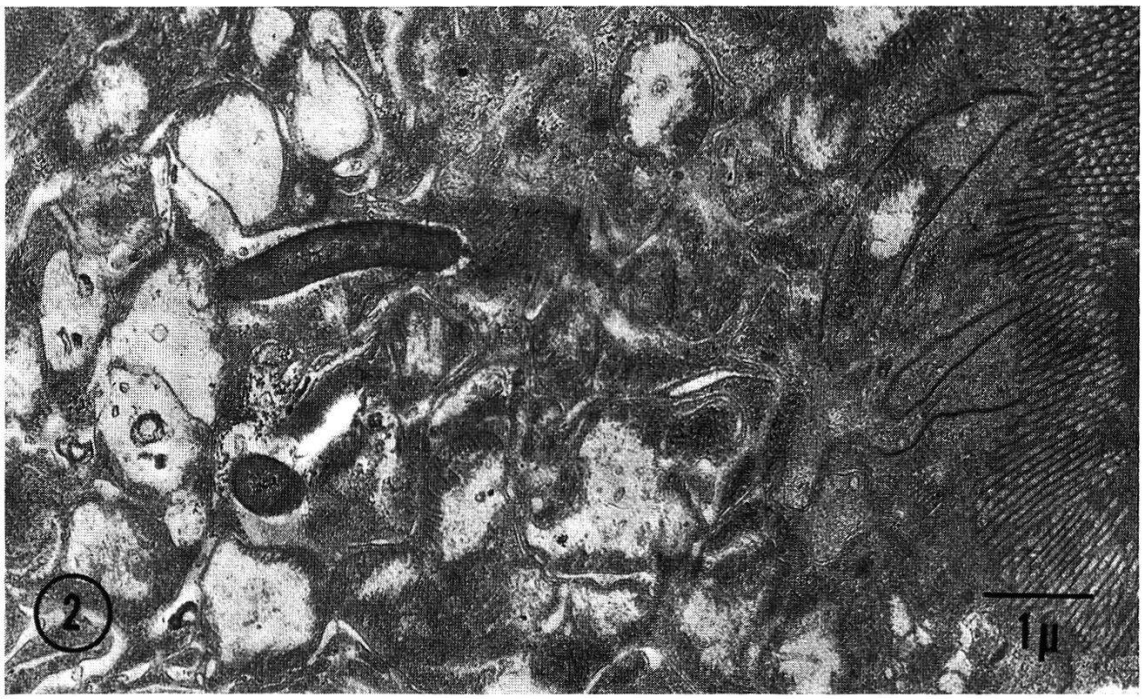
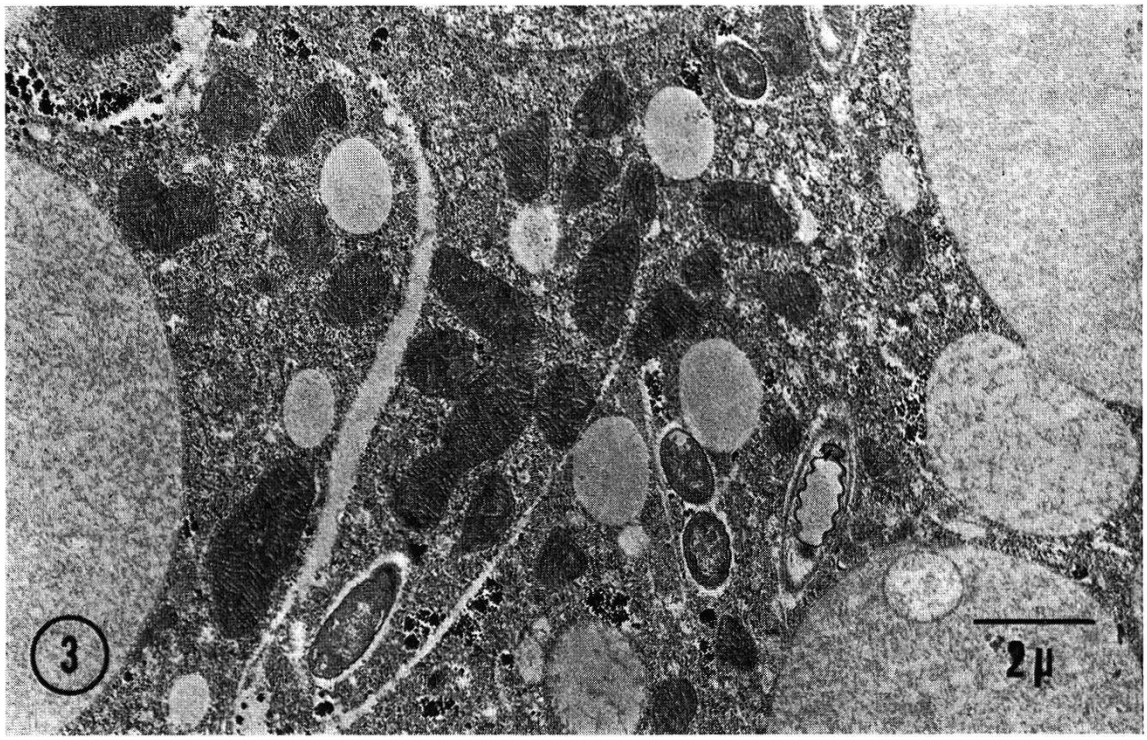


Fig. 1. Midgut mycetocyte of *G. morsitans* (♂, Tanzania) showing large symbiotes and smaller rickettsia-like organisms with lytic zone (arrow). Note that some of the large symbiotes appear free in the midgut lumen (L).

Fig. 2. Midgut epithelial cell of *G. morsitans* (♀, England) containing smaller organisms only.



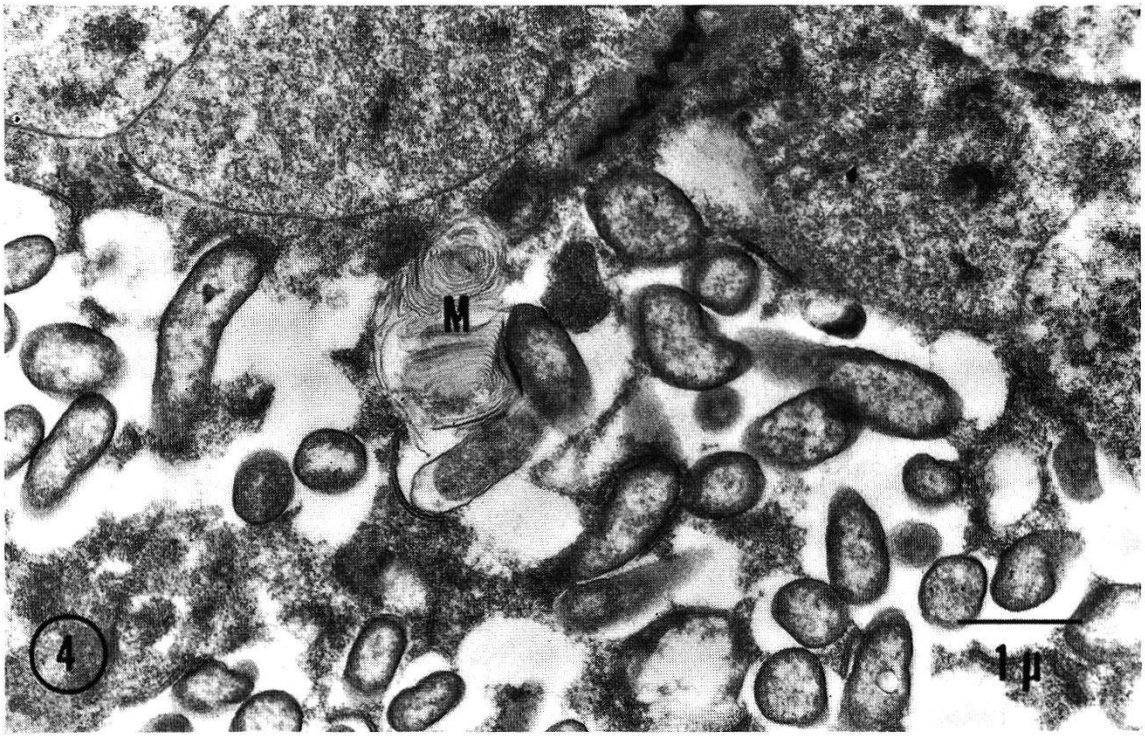
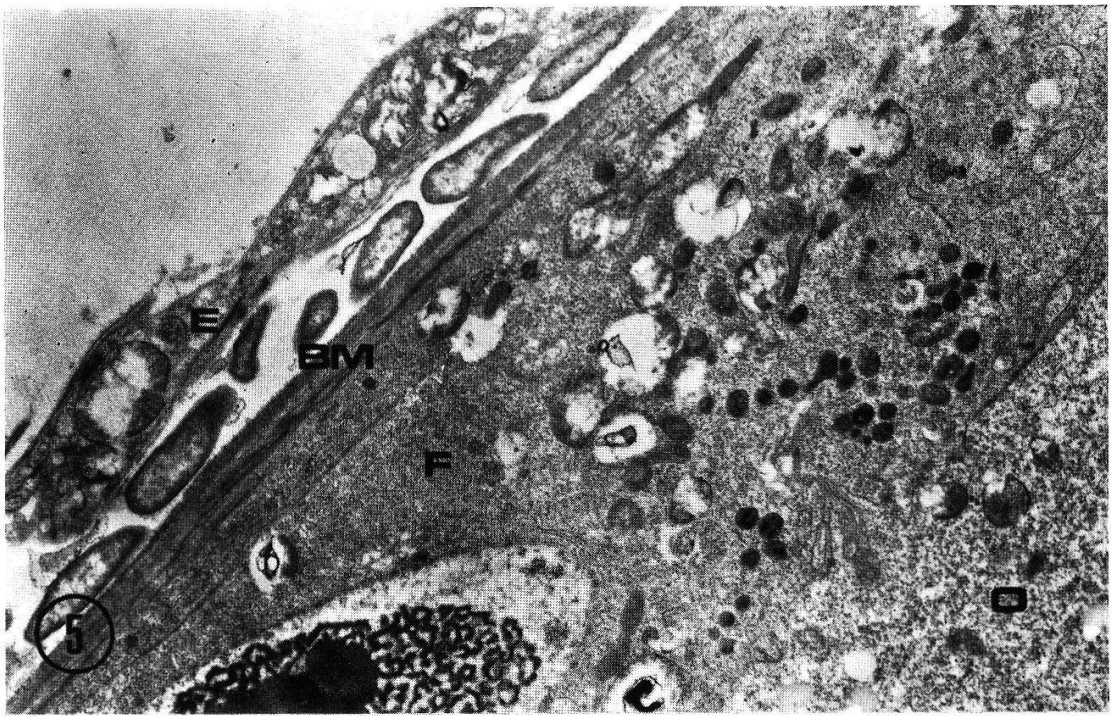


Fig. 3. Fat body of *G. morsitans* (♂, Tanzania) with sparse rickettsia-like organisms present.

Fig. 4. Fat body of *G. pallidipes* (♀, Uganda) with large populations of organisms. Note tissue damage and myelin-like membranes (M).



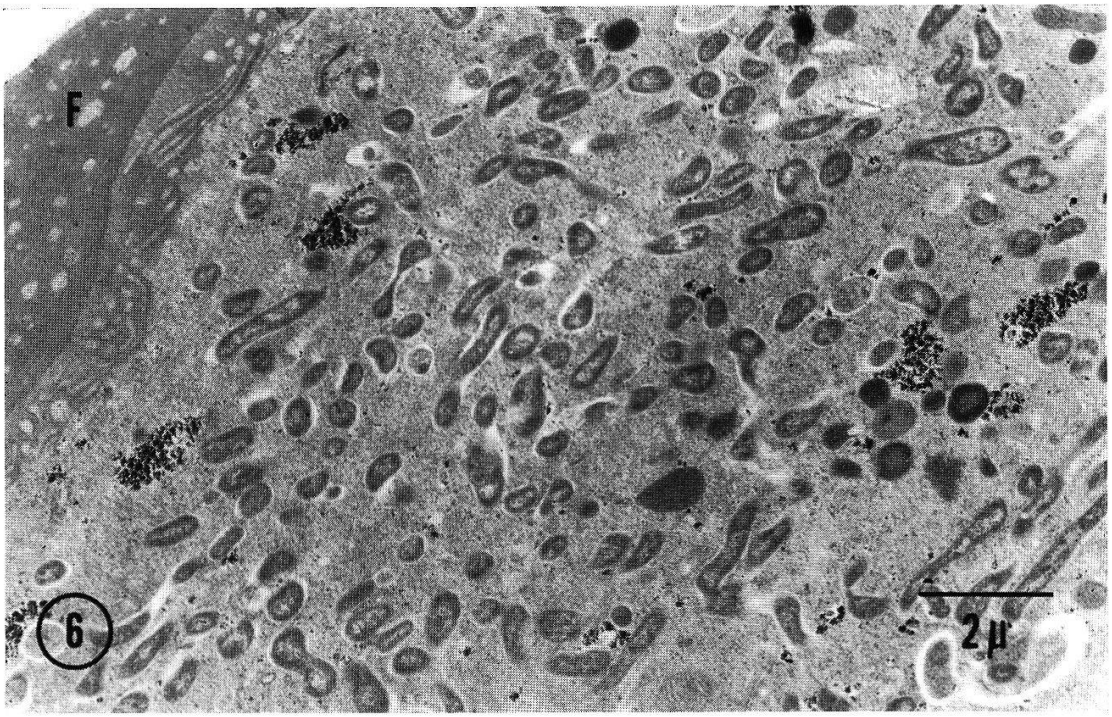
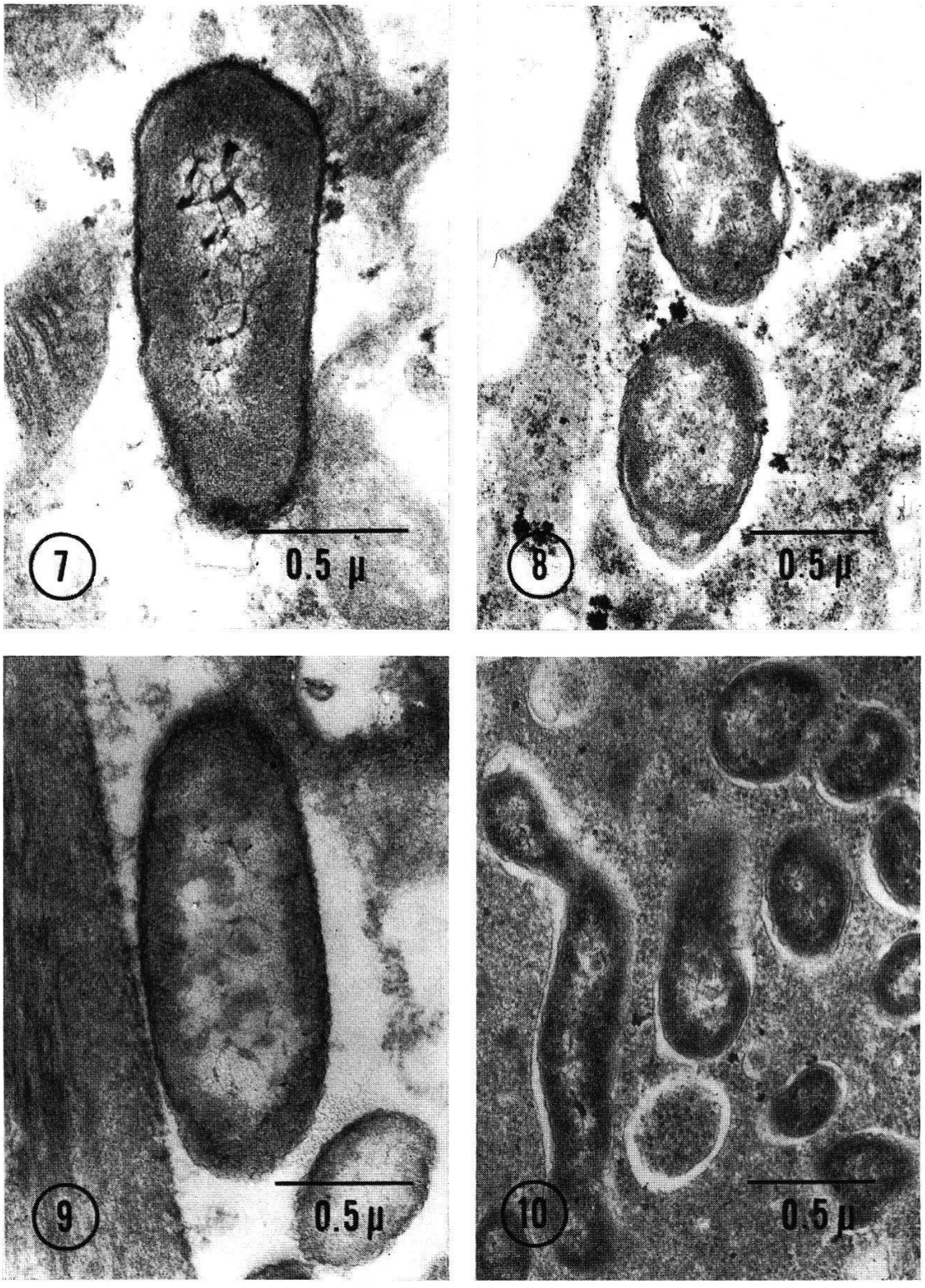


Fig. 5. Developing oocyte of *G. pallidipes* (♀, Uganda) with organisms between epithelial sheath (E) and basement membrane (BM). Follicle cells (F) and oocyte (O) are also shown.

Fig. 6. Developing oocyte of *G. morsitans* (♀, England) with organisms well below microvillar surface and follicular layer (F).



Figs. 7–10. Higher magnification of organisms from midgut epithelium (Fig. 7), fat body (Fig. 8), and outside (Fig. 9) and inside (Fig. 10) the developing oocyte showing similarity in ultrastructure. Apparent dividing forms are seen in the organisms within the developing oocyte (Fig. 10). Sources: Fig. 7, ♀, England; Fig. 8, ♂, Tanzania; Fig. 9, ♀, Uganda; Fig. 10, ♀, England.

organisms similar to those present in the midgut. These organisms were of the same size distribution, possessed scalloped membranes and were surrounded by a clear or "lytic" zone (figs. 3, 8). The frequency of these organisms in the fat body tissue was also extremely low. One such group of cells sampled, however, contained a large number of organisms (Fig. 4). Many of the cells were undergoing lysis and signs of cell disruption were evident. The cells in which the organisms occurred were within the fat body lobules and cells containing lipid deposits were immediately adjacent. The cells containing the organisms generally contained abundant glycogen and storage granules and did not appear to contain lipid. The granules had a fine flocculent structure very different from that found in adjoining cells, and appeared ultrastructurally similar to protein or carbohydrate reserves.

Organisms of similar morphology to those found in the midgut and fat body were also observed closely associated with the developing oocytes (Fig. 5) or within the oocyte cytoplasm (Fig. 6). The organisms found associated with the oocyte lay between the enveloping epithelial sheath, consisting of muscle and tracheal cells, and the basement membrane which lies outside the follicle cells (Fig. 5). At this stage of oocyte development the egg itself had not yet produced the abundant microvilli associated with protein synthesis, although some microvilli were present at the oocyte surface. The follicle layer, however, had passed to the syncytium stage described by MOLOO (1971). Figure 6 shows a developing oocyte with a large number of organisms well beneath the microvillar surface. In some sections where the numbers of organisms were small they were in isolated clumps of 10–15. These perhaps represent organisms which had recently penetrated through the surface of the egg, although the clear or lytic zone was already associated with them. Unfortunately no organisms were observed between the follicle cells and the oocyte, nor in any position that could be associated with the process of penetration into the oocyte.

In addition to the tissues in which the organisms were observed, the following tissues were sectioned in a few insects; spermatheca, salivary glands, undeveloped ovary, flight muscle, Malphigian tubules and thoracic ganglion. Although organisms were not observed in these tissues, it does not necessarily mean that they were not present, but that the frequency was as low or lower than that of other tissues.

Discussion

The purpose of this study was to determine whether intracellular microorganisms were present in tsetse flies in the same tissues that symbiotes had been described for in other insects, and present in tsetse flies from a variety of sources. Because of the difficulty in obtaining

tsetse fly samples, the fixation procedures were not ideal. However, the results indicate that small organisms of uniform size and morphology occur infrequently in the fat body, midgut, and ovary of tsetse flies from all sources sampled. The organisms described here would appear to be similar to the rickettsia-like organisms described in other insects. They fall within the general morphology and size of rickettsia and were very close in size to the rickettsial organism in saturnids (ENTWISTLE & ROBERTSON, 1968). These organisms were 1.49 μ long and 0.67 μ wide with those measuring greater than 1.8 μ long clearly dividing. In addition they are surrounded by a clear zone, possibly a lytic area. Since it is well established that members of the Rickettsiae produce toxins (RHODES & VAN ROAGEN, 1962), this clear zone could be the result of toxin secretion. The possibility that it is an insect cellular reaction, however, cannot be ruled out. The organisms observed here, however, were not enclosed in groups within the limiting membranes described in the ticks (ROSHDY, 1968) or roach (MILBURN, 1966).

The various reproductive stages that have been postulated in rickettsiae or rickettsia-like organisms (HUGER & KRIEG, 1967; REINHARDT et al., 1972a, b) were not observed in the tissues examined. MILBURN (1966) described mycetome bacteroid transformations which included disorganization and alteration of structure and shape. These changes may have occurred in the specimens examined, but due to the methods of fixation and study could have been altered and not be recognizable. In fact, forms similar to those described by others were noted, but attributed rather to cell degeneration than to symbiote reproduction. The long filamentous forms found within the oocyte could represent a stage in reproduction by binary fission.

The presence of these organisms in the oocyte suggests that they are passed from generation to generation. WIGGLESWORTH (1929) states that the bacterial symbiotes of the midgut are transmitted through the milk glands although this theory seems to be based only on ROUBAUD's (1919) finding of bacteria in the milk gland and ducts. Since in this study these symbiotes were not observed associated with the developing oocyte, it is possible that with double endosymbiosis, the migration into the developing oocyte by the bacteria occurs at a later stage in the differentiation of the larva.

The occurrence of these organisms within a variety of tissues does not seem surprising. It has been stated that well over 10 % of the living species of insects regularly have intracellular microorganisms or microorganism-like particles (LANHAM, 1968). With such a frequency, more than one tissue may well be infected and more than one symbiote could occur. Ample literature exists describing "bacteroids" in more than one tissue and the multiple occurrence of more than one symbiote in a single species. The recent electron microscope study by REINHARDT

et al. (1972b) on the soft tick describes long rickettsia-like microorganisms that occur in all organs of both sexes with the exception of the spermioduct and testicules and of coccoid microorganisms in the Malphighian tubules and oocytes. GRINGER & MUSGRAVE (1966) report the presence of both large and small microorganisms in mycetomes of *Sitophilus granarius*. KORNER (1969) described two microorganisms in the embryos of the leafhopper. REINHARDT et al. (1972a) mentioned rickettsia-like microorganisms as occurring sparsely in the midgut mycetome of the tsetse fly, together with the bacteria reported by ROUBAUD (1919), and WIGGLESWORTH (1929). In *Glossina brevipalpis* the rickettsia-like organisms occurred only in the cytoplasm of the muscle cells surrounding the midgut.

The role these rickettsia-like organisms play in the development and physiology of the tsetse fly can only be speculated on. From the observations in this study the rickettsia-like organisms may be parasitic rather than conferring any benefit to the host, and would appear to be close to a moderate parasite, although the evidence at present is only circumstantial. The frequency of occurrence of the organism appears to be too low to produce any by-product that may be regarded as beneficial to the insect. The organisms were not observed in the majority of the insects or tissues sampled and even when found, generally occurred as only a few organisms per host cell. The clear or "lytic" zone found surrounding the organisms and reported also by ENTWISTLE & ROBERTSON (1968) may indicate an adverse reaction of the infected cytoplasm. This zone was not observed surrounding the bacterial symbiotes in the midgut mycetocytes. In addition, cells where multiplication of the rickettsia-like organisms may have occurred were observed to be disrupted and undergoing degeneration that could not be attributed to fixation procedures (Fig. 4). These factors and the close ultrastructural resemblance to rickettsia, suggest existence of a parasitic association.

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Zusammenfassung

Elektronenmikroskopisch wurden verschiedene Gewebe von Tsetsefliegen, *Glossina morsitans, G. fuscipes, G. brevipalpis* und *G. pallidipes,* untersucht. Intrazelluläre rickettsienähnliche Mikroorganismen wurden sowohl in Mitteldarmepithelzellen als auch im Zusammenhang mit dem Fettkörper und mit den sich entwickelnden Ovocyten gefunden. Es wird diskutiert, welche Rolle diese Organismen in Tsetsefliegen spielen könnten.

Résumé

Différents tissus de mouches tsé-tsé *(Glossina morsitans, G. fuscipes, G. brevipalpis* et *G. pallidipes)* ont été examinées au microscope électronique. Des organismes intracellulaires de type rickettsien ont été observés dans des cellules épithéliales de l'intestin moyen, en connection avec le corps gras, ainsi que dans des ovocytes en voie de développement. Le rôle possible de ces organismes pour les mouches tsé-tsé est discuté.