Ultrastructural study of the midgut mycetome-bacteroids of the tsetse flies "Glossina morsitans, G. fuscipes," and "G. brevipalpis" (Diptera, Brachycera)

Autor(en): **Reinhardt, C. / Steiger, R. / Hecker, H.**

Objekttyp: **Article**

Zeitschrift: **Acta Tropica**

Band (Jahr): **29 (1972)**

Heft 3

PDF erstellt am: **22.07.2024**

Persistenter Link: https://doi.org/10.5169/seals-311804

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Ultrastructural Study of the Midgut Mycetome-Bacteroids of the Tsetse Flies *Glossina morsitans, G. fuscipes,* and *G. brevipalpis* (Diptera, Brachycera)*

C. REINHARDT, R. STEIGER and H. HECKER

Introduction

Endosymbiotic bacteroids are known to occur within cells of a wide variety of insects (BROOKS 1956, BUCHNER 1965, MILBURN 1966, LANHAM 1968). Symbiosis of microorganisms with blood-sucking arthropods is so common that one may imagine that essential advantages for the host result from it (GEIGY et al., 1953, WIGGLESWORTH 1965). In view of these facts it is remarkable that there is very little accurate knowledge of the endosymbiotes in the tsetse-fly, the vector of trypanosomes (GEIGY & HERBIG 1955, MULLIGAN 1970).

ROUBAUD (1919) and WIGGLESWORTH (1929) have found a giant-cell zone in the anterior segment of the midgut of several *Glossina* species. These cells appear hypertrophied and get infected by rod-shaped bacteroids during metamorphosis. WIGGLESWORTH (1929) classified these bacteroids as being gram-negative. In the present paper we want to describe the fine structure of the mycetome in the midgut of *Glossina morsitans, G. fuscipes* and *G. brevipalpis* and to prove the gram-negative character of their symbiotes.

Material and Methods

Males of teneral and non-teneral tsetse-flies *(Glossina morsitans, G. brevipalpis* and *G. fuscipes)* were used. *G. morsitans* were derived from pupae collected in Singida (Tanzania), *G. brevipalpis* were caught in the South Busoga District (Uganda) and *G. fuscipes* emerged from pupae collected at the same place in Uganda. The pupae were kept in the EATRO laboratories up to emergence. The flies were dissected in insect Ringer solution and the fore-part of the midgut including the mycetome was prepared for electron microscopy by standard methods (AESCHLIMANN & HECKER 1967). The means and standard deviations of the bacterial cell wall dimensions were calculated using 40–70 measurements from electron micrographs (× 350,000).

Results

The midgut mycetome of *Glossina morsitans* forms an annular structure stretched along the axis of the fore-part of the midgut (WIGGLESWORTH 1929). In a cross section one can see the double bulge of hypertrophied epithelial cells almost filling up the gut lumen (Fig. 1). The cylindrical cells of the mycetome (= mycetocytes) are very high. Their large nucleus with a darkly contrasted nucleolus lies mostly in the

* This publication is dedicated to Professor R. Geigy on the occasion of his 70th birthday.

apical part of the cell. The nucleolus is clearly visible in the nucleoplasm, which shows inconspicuous heterochromatin (Fig. 4). The basal lamina of the mycetocytes borders directly on the longitudinal and ring muscle cells of the midgut (Fig. 2). The mycetome is lined by normal midgut epithelial cells, which do not contain bacteroids (Fig. 1, 2). Tracheoles often extend into intercellular spaces between mycetocytes (Fig. 1, 4). The mycetocyte's cytoplasm is flocculent containing vesicles, mitochondria, microtubules and free ribosomes (Fig. 3). Its electron density is lower compared with that of the normal cytoplasm (Fig. 2). In opposition to the normal epithelial brush border shorter microvilli line the mycetocyte's cytoplasm towards the lumen.

Usually the bacteroids lie intracellularly between the cell organelles, often very compact and somewhat oriented in the longitudinal axis of the mycetocyte (Fig. 1). They are mostly rod-shaped up to 8 μ in length and 1–1.4 μ in width. The bacterial cytoplasm contains ribosome-like granules and diffuse filamentous structures (Fig. 3, 6), which may represent the microorganism's DNA (HECKER et al. 1968, HECKER 1970). Sometimes an electron dense agglomeration exists close to the symbiote's wall and membranous structures can be found in connection with the plasma membrane (Fig. 3, 6).

The plasma membrane (p: 76 ± 8 Å), a poorly contrasted intermediate layer (i) of 125 ± 40 Å and an outer unit membrane (c) of 82 ± 10 Å form the complete wall of a total thickness of about 250 to 300 Å (Diagram 1). The two dark layers of the plasma membrane

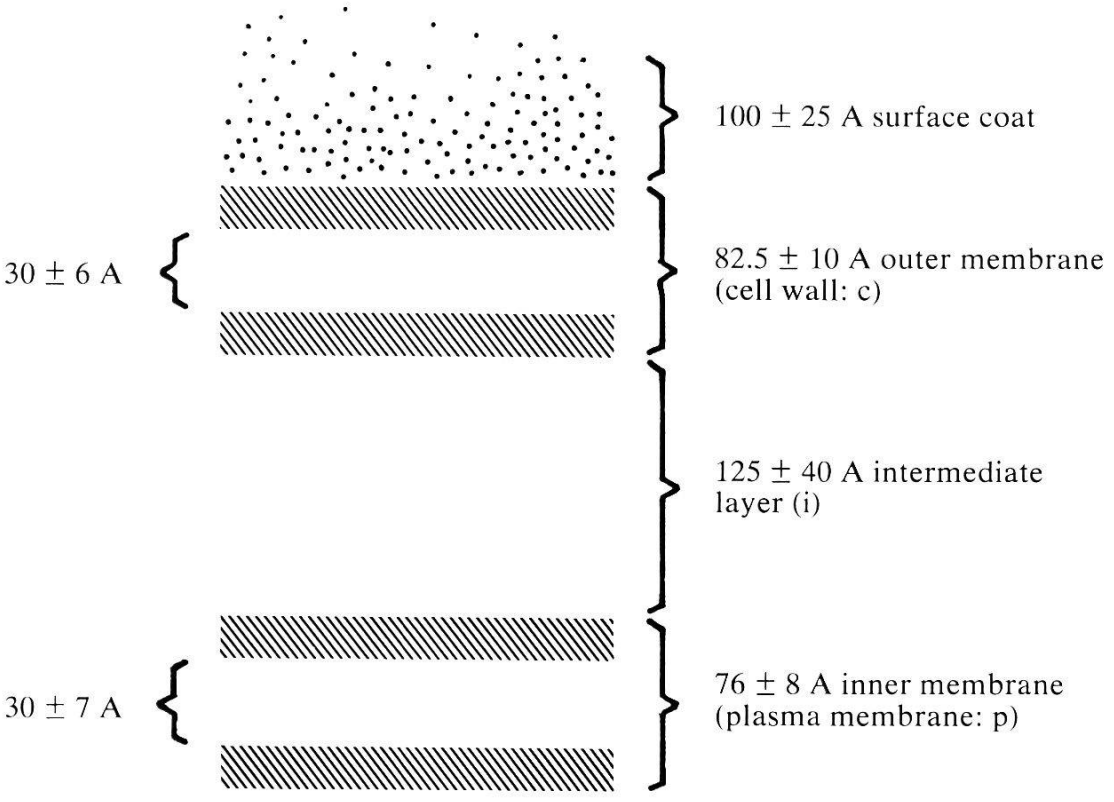


Diagram 1

show less contrast and seem to be thinner than the corresponding layers of the outer membrane (Fig. 5). Sometimes an additional electron dense surface coat (100 ± 25 Å) is present (Fig. 6). When two symbiotes are lying in close contact their two coats, at places, seem to fuse irregularly. The distance between adjacent symbiotes measures then 135 ± 20 Å (Fig. 3, 5).

Moreover, sparse rickettsia-like microorganisms occur showing a dense fibrillary cytoplasm (Fig. 4). They are surrounded by a lytic zone described for similar organisms in the oocytes of the tick *Ornithodorus moubata* (HECKER 1970).

G. fuscipes and *G. brevipalpis* show similar mycetomes containing similar bacterial symbiotes as *G. morsitans*. In addition, *G. brevipalpis* is lodging a greater number of rickettsia-like organisms than *G. morsitans*. In *G. brevipalpis* these organisms occur in the cytoplasm of the surrounding muscle cells only.

Discussion

According to light microscopical investigations by ROUBAUD (1919) and WIGGLESWORTH (1929) the mycetome is perfected during pupation of the tsetse flies. Preformed larger midgut cells are infected by bacteroids from the larval gut. These epithelial cells get much more expanded possibly due to intensive dividing activity of the bacteroids. The finally hypertrophied cells, the mycetocytes, retain, however, their original epithelial character (Fig. 1).

Fig. 1. G. morsitans (♂, 2 days after bloodmeal), view of cross sectioned midgut mycetone (my): bulge of high cylindrical mycetocytes (dotted lines indicate cell boundaries), marked nucleus (nu), normal midgut epithelial cells (ep), gut lumen (lu), peritrophic membrane (pm), longitudinal and ring muscle cells (mc), tracheoles (tr), hemocoel (he). 660 ×.

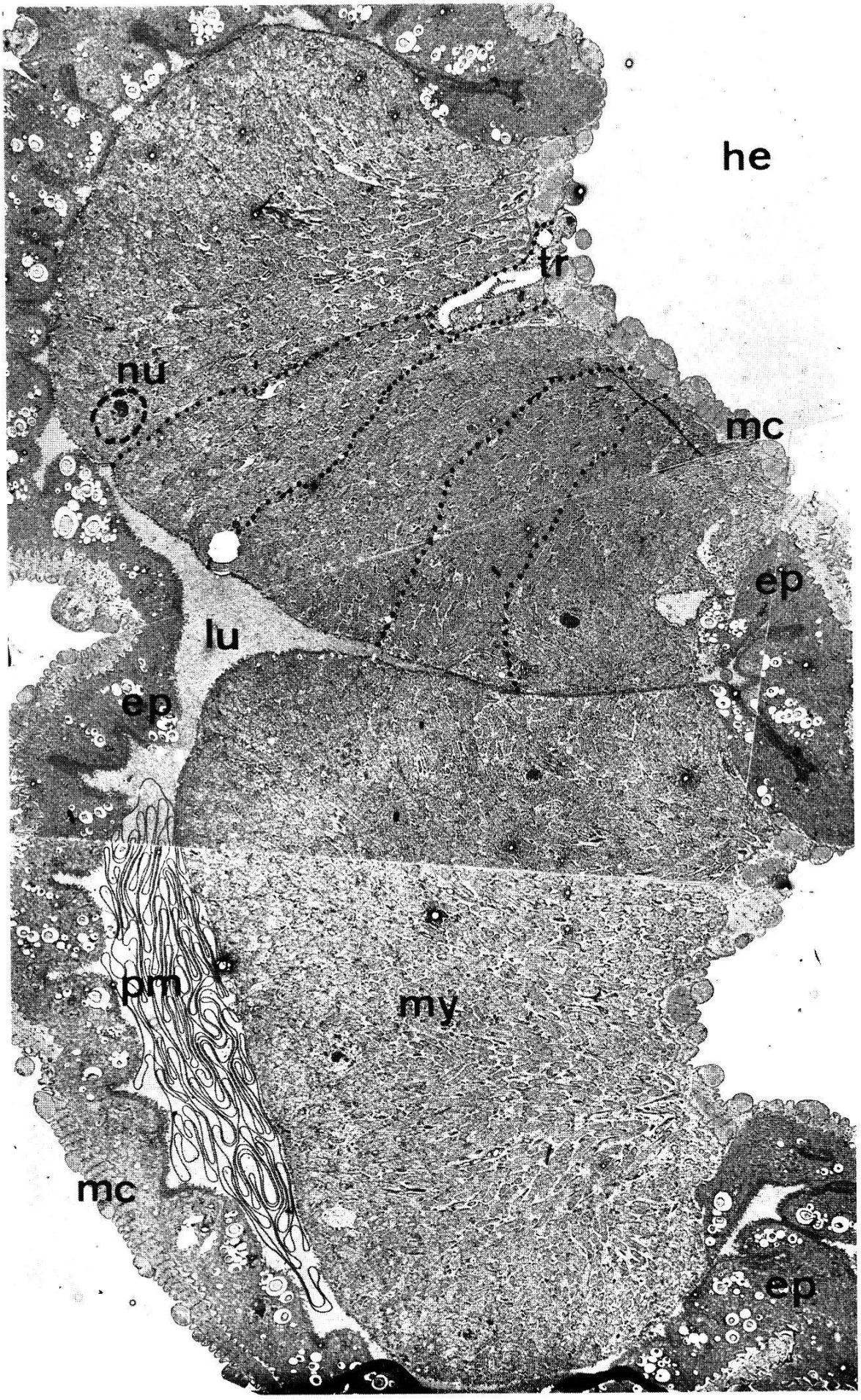
Fig. 2. ♂, teneral: basal part of a mycetocyte bordering to a normal epithelial cell (ep): symbiotic bacteria (sb), basal lamina (→), muscle cell (mc). 6,880 ×.

Fig. 3. ♂, teneral: two symbiotic bacteria (sb) in close membrane contact: electron dense agglomeration (ag), mesosome-like vesicles (→); cytoplasm of mycetocyte (cy): mitochondria (mi), microtubules (mt), ribosomes (ri) and many vesicles (ve). 34,400 ×.

Fig. 4. ♂, teneral: mycetocyte with symbiotic bacteria (sb) and rickettsialike microorganisms (rlm), note lytic zone (→); nucleus (nu), nucleolus (nl), intercellular tracheole (tr). 12,000 ×.

Fig. 5. Close membrane contact of two symbiotic bacteria (sb): interbacterial space (ii), outer membrane (cell wall: c), intermediate layer (i), inner membrane (plasma membrane: p). 350,000 ×.

Fig. 6. Part of a symbiotic bacterium (sb) with a vesicle (*) continuous with the plasma membrane of the bacterium (→); filamentous structures (→ →), ribosome-like grana (rg), surface coat (▲). 81,300 ×.



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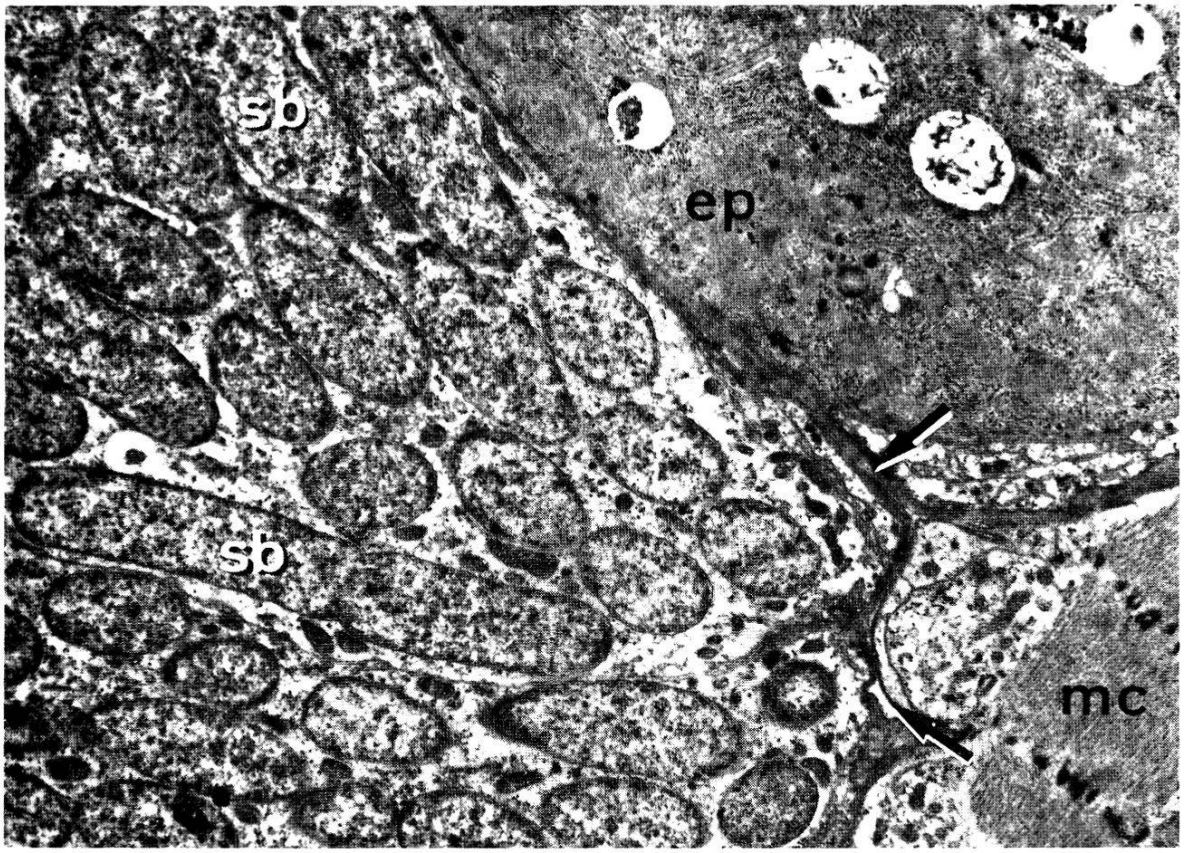

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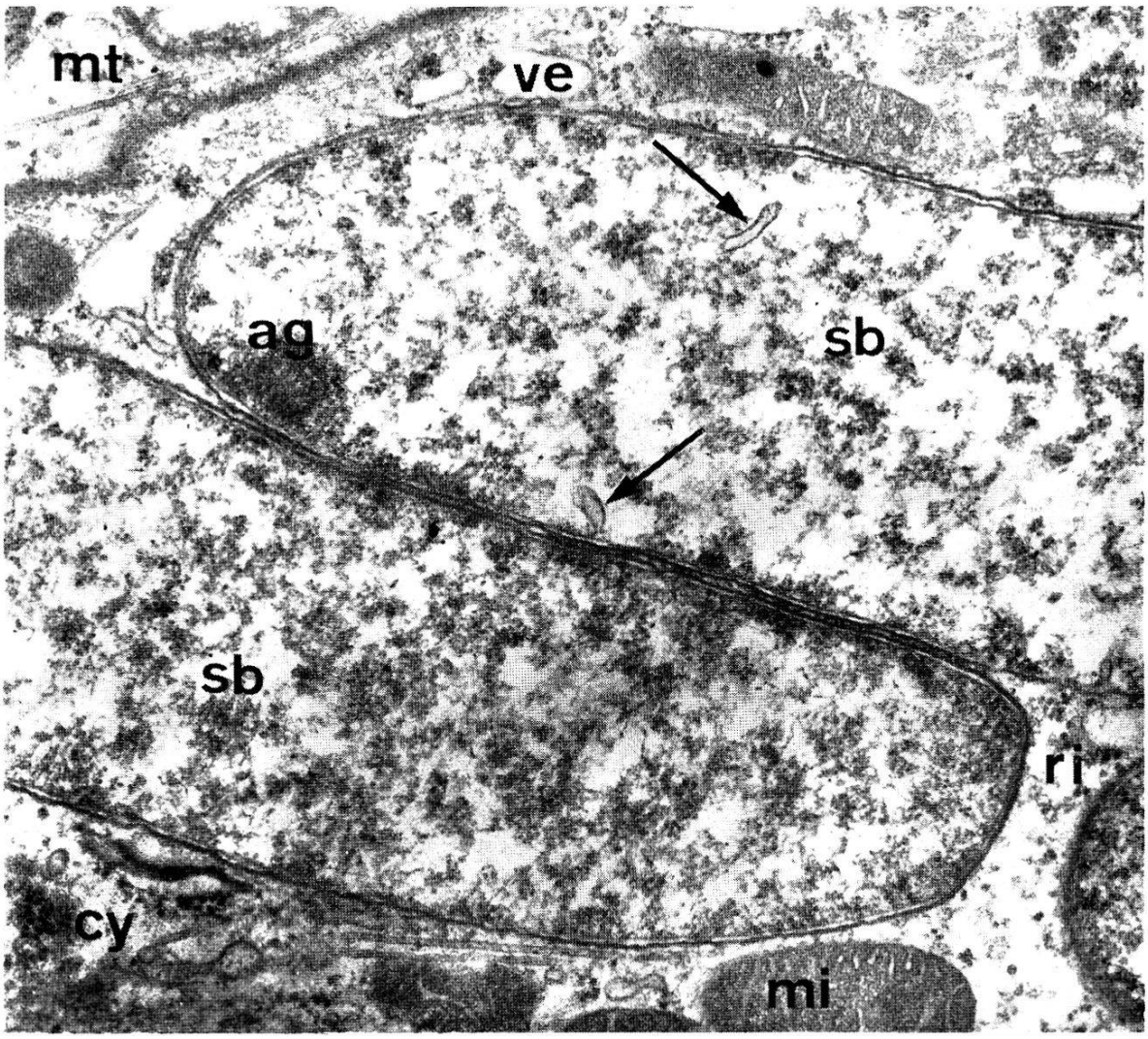

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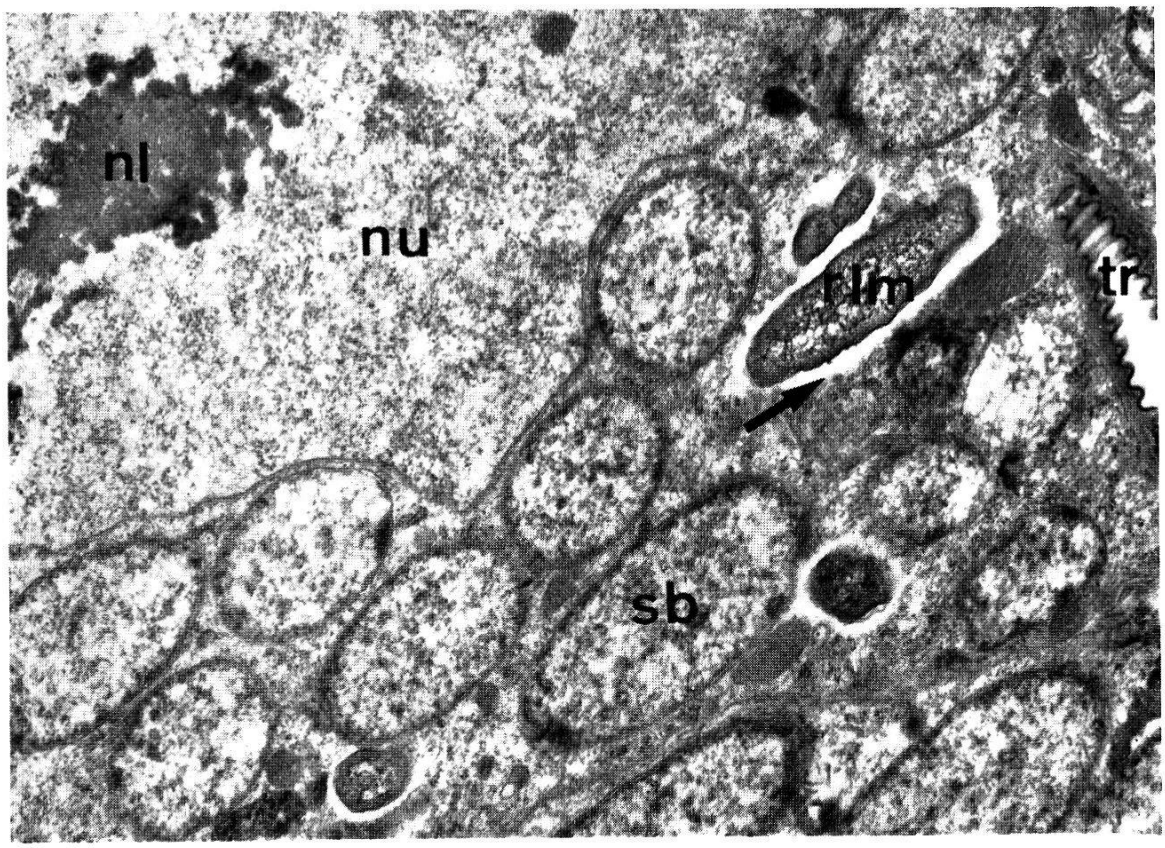

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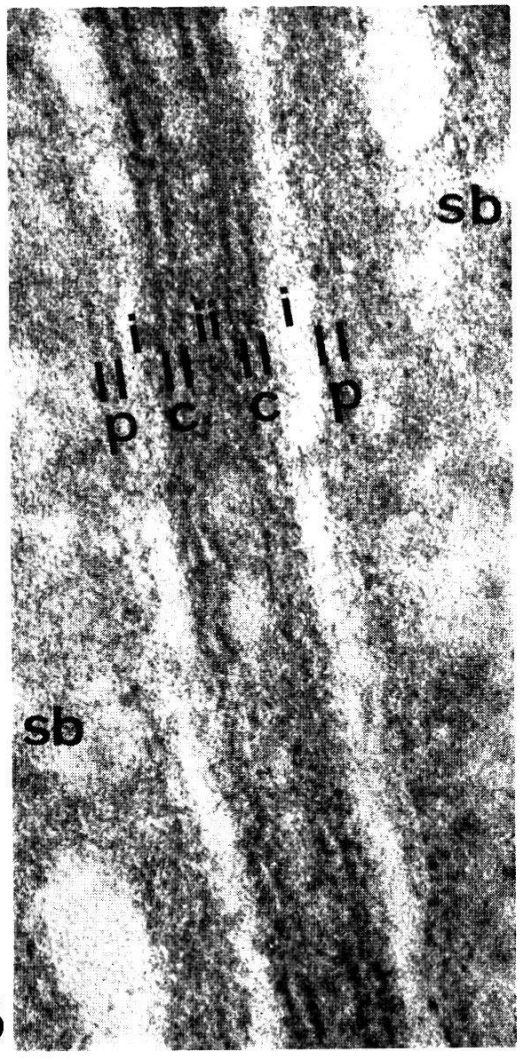

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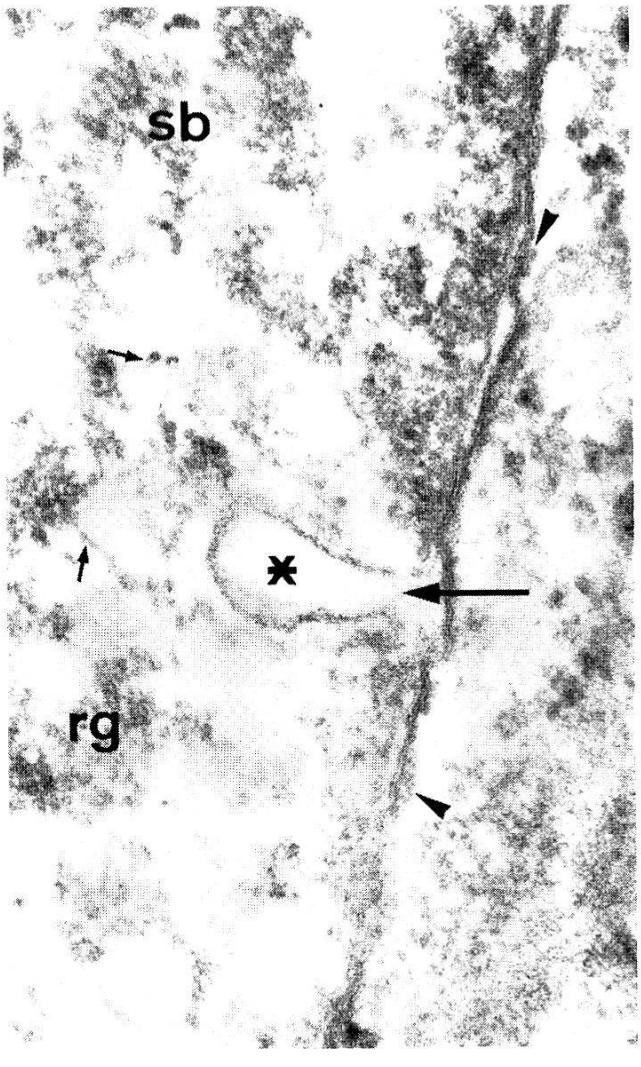

6

As regards the shape and ultrastructure of the cell wall of the microorganisms, they can clearly be classified as gram-negative bacteria. The cell wall thickness of about 80 Å (c, Diagram 1) coincides with that of gram-negative bacteria (GLAUERT 1962, CLAUS & ROTH 1964, BURGE & DRAPER, 1967, GRANBOULAN & LEDUC 1967, JAWETZ et al. 1968).

They seem not to be rickettsiae because rickettsiae are throughout smaller (ANDERSON et al. 1965, ENTWISTLE et al. 1968, JAWETZ et al. 1968, REINHARDT et al. 1972). The symbiotic Blochmann bodies, especially the bacteroids of fat-body mycetomes, which are ovarially transmitted, are also much smaller than the tsetse symbiotes and are clearly classified as gram-positive (BROOKS 1956, MILBURN 1966, LANHAM 1968).

The intracellular membranous elements of our bacteria can be interpreted as mesosome-like bodies or as vacuoles, both in facultative connection with the plasma membrane (DREWS & GIESBRECHT 1966).

Cultured human bacteroids described by BLADEN & WATERS (1963) are, in their structure, comparable to our bacteria, even though they seem to be smaller. A precise classification cannot be given because all culture experiments with the intracellular *Glossina* bacteria have failed (WIGGLESWORTH 1965). For the same reason there is very few knowledge of the function of these symbiotes.

WIGGLESWORTH (1929, 1965), in opposition to ROUBAUD (1919), denies any role of mycetome-bacteria in the digestion of the bloodmeal. He rarely observed them in the gut lumen, which corresponds to our observations. Finally, membrane destruction of mycetocytes is not to be excluded, which would explain the presence of some bacteria in the lumen.

Yet, symbiotes of many blood-sucking insects may be responsible for vitamin synthesis (GEIGY et al. 1953, BUCHNER 1965, WIGGLESWORTH 1965). Such a symbiote-host relation has been demonstrated in *Triatoma infestans*, which harbours gram-positive coccoid symbiotes (*Nocardia* spec.) in the gut lumen (GEIGY et al. 1953, 1954, HALFF 1956). The synthesis of folic acid and some other pteridine derivatives of the vitamin B complex by bacteria (KARLSON 1967) are not only necessary for their own use but also for their host; a symbiote-free bug is not viable (GEIGY et al. 1953, 1954; HALFF 1956). Moreover, folic acid is an essential substrate for the growth of microorganisms as cofactor of the C_1-metabolism (KARLSON 1967), especially shown by TRAGER (1969) for *Leishmania tarentolae* and *Crithidia fasciculata*. Supposing the bacteria of the tsetse fly mycetocytes also synthesize folic acid for their host, it would be very interesting to know whether eventually carried trypanosomes profit from this vitamin, too. By means of a high level dosis of antibiotica in the bloodmeal it is pos-

sible to harm tsetse fly's symbiotes (F. K. DAR, personal communication). This may lead to new aspects for analysing and affecting the infection rates of trypanosomes in their vectors.

Acknowledgements

This work has partly been supported by the "Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung". The authors acknowledge the cooperation of Dr. F. K. Dar, E.A.T.R.O. Tororo, who provided some of the Glossina material for our examinations. We are grateful for the technical assistance of Miss S. Bleiker.

References

AESCHLIMANN, A. & HECKER, H. (1967). Observations préliminaires sur l'ultrastructure de l'ovocyte en développement chez *Ornithodorus moubata* Murray *(Ixodoidea: Argasidae).* – Acta trop. *24,* 225–243.

ANDERSON, D. R., HOPPS, H. E., BARILE, M. F. & BERNHEIM, B. C. (1965). Comparison of the ultrastructure of several rickettsiae, ornithosis virus and Mycoplasma in tissue cultures. – J. Bact. *90,* 1387–1404.

BLADEN, H. A. & WATERS, J. F. (1963). Electron microscopic study of some strains of bacteroides. – J. Bact. *86,* 1339–1344.

BROOKS, M. A. (1956). Nature and significance of intracellular bacteroids in cockroaches. – Proc. 10th Int. Congr. Ent., Montreal *11,* 311–314.

BUCHNER, P. (1965). Endosymbiosis of animals with plant microorganisms. – Intersci. Publishers New York, London, Sydney. (review).

BURGE, R. E. & DRAPER, J. C. (1967). Structure of the cell wall of the gram-negative bacterium *Proteus vulgaris.* – J. molec. Biol. *28,* 189–204.

CLAUS, G. W. & ROTH, L. E. (1964). The fine structure of the gram-negative bacterium *Acetobacter syboxydans.* – J. Cell Biol. *20,* 217–233.

DREWS, G. & GIESBRECHT, P. (1966). In: "Die Zelle", chapter: "Der Aufbau der Bakterien- und Cyanophyceenzellen", ed. by H. METZNER. – Wissenschaftl. Verlags-GmbH, Stuttgart.

ENTWISTLE, P. F., ROBERTSON, J. S. & JUNIPER, B. E. (1968). The ultrastructure of a rickettsia pathogenic to a saturnid moth. – J. gen. Microbiol. *54,* 97–104.

GEIGY, R., HALFF, L. A. & KOCHER, K. (1953). Untersuchungen über die physiologischen Beziehungen zwischen einem Träger der Chagas-Krankheit *Triatoma infestans* und dessen Darmsymbionten. – Schweiz. med. Wschr. *83,* 928–936.

GEIGY, R. & HALFF, L. A. (1954). L'acide folique comme élément important dans la symbiose intestinale de *Triatoma infestans.* – Acta trop. *11,* 163–166.

GEIGY, R. & HERBIG, A. (1955). Erreger und Überträger tropischer Krankheiten. Acta trop. Suppl. 6. – Verlag für Recht und Gesellschaft, Basel.

GLAUERT, A. M. (1962). The fine structure of bacteria. – Brit. med. J. *18,* 245–250.

GRANBOULAN, P. & LEDUC, E. H. (1967). Ultrastructural cytochemistry of *Bacillus subtilis.* – J. Ultrastr. Res. *20,* 111–126.

HALFF, L.-A. (1956). Untersuchungen über die Abhängigkeit der Entwicklung der Reduviide *Triatoma infestans* Klug von ihren Darmsymbionten. – Acta trop. *13,* 225–253.

HECKER, H., AESCHLIMANN, A. & BURCKHARDT, M. J. (1968). Contribution à la connaissance des symbiotes chez *Ornithodorus moubata (Ixodoidea).* Etude au microscope éléctronique. – Acta trop. *25,* 256–262.

HECKER, H. (1970). Ultrastruktur der Symbionten in Ovocyten von *Ornithodorus moubata,* Murray *(Ixodoidea: Argasidae)* nach simultaner Glutaraldehyd-Osmium Fixierung und Nachbehandlung mit Uranylacetat (Triple-Fixation). – Experienta *26,* 874–877.

JAWETZ, E., MELNICK, J. L. & ADELBERG, E. A. (1968). Medizinische Mikrobiologie. – Springer Verlag, Berlin.

KARLSON, P. (1967). Kurzes Lehrbuch der Biochemie. – Thieme Verlag, Stuttgart.

LANHAM, U. N. (1968). The Blochmann bodies: Hereditary intracellular symbionts of insects. – Biol. Rev. *43,* 269–286.

MILBURN, N. S. (1966). Fine structure of the pleomorphic bacteroids in the mycetocytes and ovaries of several genera of cockroaches. – J. Insect. Physiol. *12,* 1245–1254.

MULLIGAN, H. W. (Editor) (1970). The African Trypanosomiases. – G. Allen & Unwin Ltd. London.

REINHARDT, C., AESCHLIMANN, A. & HECKER, H. (1972). Distribution of rickettsia-like microorganisms in the organs of a laboratory strain of *Ornithodorus moubata,* Murray *(Ixodoidea: Argasidae).* – Z. Parasitenk. in press.

ROUBAUD, E. (1919). Les particularités de la nutrition et de la vie symbiotique chez les mouches tsétsé. – Ann. Inst. Pasteur *33,* 489–537.

TRAGER, W. (1969). Pteridine requirement of the hemoflagellate *Leishmania tarentolae.* – J. Protozool. *16,* 372–375.

WIGGLESWORTH, V. B. (1929). Digestion in the tsetse-fly: A study of structure and function. – Parasitol. *21,* 288–321.

WIGGLESWORTH, V. B. (1965). The principles of insect physiology. – Methuen & Co. Ltd., London.

Zusammenfassung

Die Ultrastruktur der Bakterien im Mitteldarm-Mycetom von Tsetsefliegen *(Glossina morsitans, G. fuscipes* und *G. brevipalpis)* wird beschrieben. Stäbchenförmige, bakterienähnliche Mikroorganismen sitzen dicht gedrängt, in grosser Zahl, in hypertrophierten Epithelzellen (= Mycetocyten) des vordern Mitteldarms. Die Zellwand der Symbionten wurde ausgemessen und mit den Hüllen verschiedener Mikroorganismen (Rickettsien, gram-positive und gram-negative Bakterien, Blochmann Bodies) verglichen. Diesbezüglich können die Mycetom-Symbionten eindeutig zu den gram-negativen Bakterien gezählt werden.

Mögliche Einflüsse der Symbionten auf die Tsetsefliege und auf die von ihr übertragenen Trypanosomen werden diskutiert.

Résumé

On décrit l'ultrastructure de bactéries trouvées dans le mycétome intestinal de mouches tsé-tsé *(Glossina morsitans, G. fuscipes* et *G. brevipalpis).* Des microorganismes de type bactérien en bâtonnets s'observent en grand nombre, serrés dans des cellules épithéliales hypertrophiées (mycétocytes) de l'intestin moyen. La paroi cellulaire des symbiotes a été mesurée et comparée avec les enveloppes de microorganismes différents (rickettsies, bactéries gram+ et gram–, corps de Blochmann). Les résultats obtenus nous permettent de conclure que les symbiotes du mycétome intestinal appartiennent au groupe des bactéries gram–.

Les influences possibles de ces symbiotes sur la mouche tsé-tsé et les trypanosomes qu'elle transmet sont discutées.