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# Miscellanea

## Observations on the Ultrastructure of Various *Borrelia* Species (Blood and Tissue Forms)

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### Introduction

The following study is based on the work of previous authors, such as Babudieri & Bocciarelli (1943, 1948), Kawata (1957, 1961), and Pillot et al. (1964, 1965). The fine structure of four species of *Borrelia,* maintained at the Swiss Tropical Institute, has been examined. The aim was to demonstrate any ultrastructural differences between the four species as well as to show eventual dissimilarities between the corresponding blood forms in the mammalian host and tissue forms in the tick vector. We were able to study blood forms of *B. duttoni, B. tillae, B. crocidurae* and *B. hispanica.* As regards the tissue forms, only *B. duttoni* in *Ornithodorus moubata* could be examined, because for this species alone a sufficient number of vector ticks was available at the time.

### Material and Methods

The spirochetes used in our experiments were obtained as follows: several strains of *B. duttoni* isolated from ticks collected in the Ulanga District, Tanzania from 1949 onward and maintained in mice with frequent cyclical passages through *O. moubata, B. tillae* isolated in South Africa by Zumpt and sent to us 1962, *B. crocidurae* and *B. hispanica* received 1965 from the Bernhard-Nocht-Institut, Hamburg.

*Blood forms:* They were obtained from heavily infected Swiss mice by heart puncture, in sodium citrate-blood 1 : 3 centrifuged for 5 min at 1500 r.p.m. The fixation was carried out in $OsO_4$ 1% buffered with veronal-acetate for 1 hour or with $OsO_4$ 1,5% in phosphate buffer. Then followed dehydration with acetone and embedding into Epon. Between each step the material was centrifuged at 3000 r.p.m. for 5 to 10 minutes.

*Tissue forms:* Tick organs *(O. moubata)* were dissected in saline and controlled with the darkfield for the presence of a sufficient number of spirochetes. The material was fixed with glutaraldehyde 2.5% in cacodylate buffer for 1 hour, rinsed with buffer solution and postfixed for 2 hours with $OsO_4$ 1% in the same cacodylate buffer. Some preparations were fixed directly in $OsO_4$ 2%, buffered with phosphate, for 2 hours. Dehydration and embedding were carried out in the same way as for blood forms but without centrifugation.

Dissection and all fixations were made at 0–4°C, and the pH of the fixing solutions was kept at 7.2.

All sections were cut with glass knives on a LKB Ultrotome I, stained with uranyl acetate or lead citrate and the micrographs taken with a Zeiss EM 9.

### Results

*Blood forms:* Blood forms of all four species show the same typical pattern (cf. Geigy, 1968). An outer coat encloses the central cytoplasmic core and a lateral ridge (Fig. 1a). In this sort of elongated pocket, running along one

side of the body over its whole length, fibrils are located. The cytoplasmic cylinder itself is surrounded by its own unit membrane. In the cytoplasma we find ribosomes and in the centre the nuclear substance extending skein-like through the whole body. The latter is electron dense and consists of desoxyribonucleic acid (DNA). No nuclear membrane is visible.

No significant difference was found between the borrelias of the four species examined (Figs. 1 and 2). The diameter of all forms was approximately the same. Some little variations found for example in the degree of wrinkling of the outer envelope, in the width of the electron clear zone between outer coat and unit membrane, in the number of fibrils — between 12 and 25 are seen in our micrographs — in the appearance of the granulation of the cytoplasma and the compactness of DNA seem to be mainly due to the treatment of the material, e.g. fixation. However, in the case of *B. tillae* a piece of membrane could be found in the cytoplasma, probably part of a mesosome (Fig. 2 b).

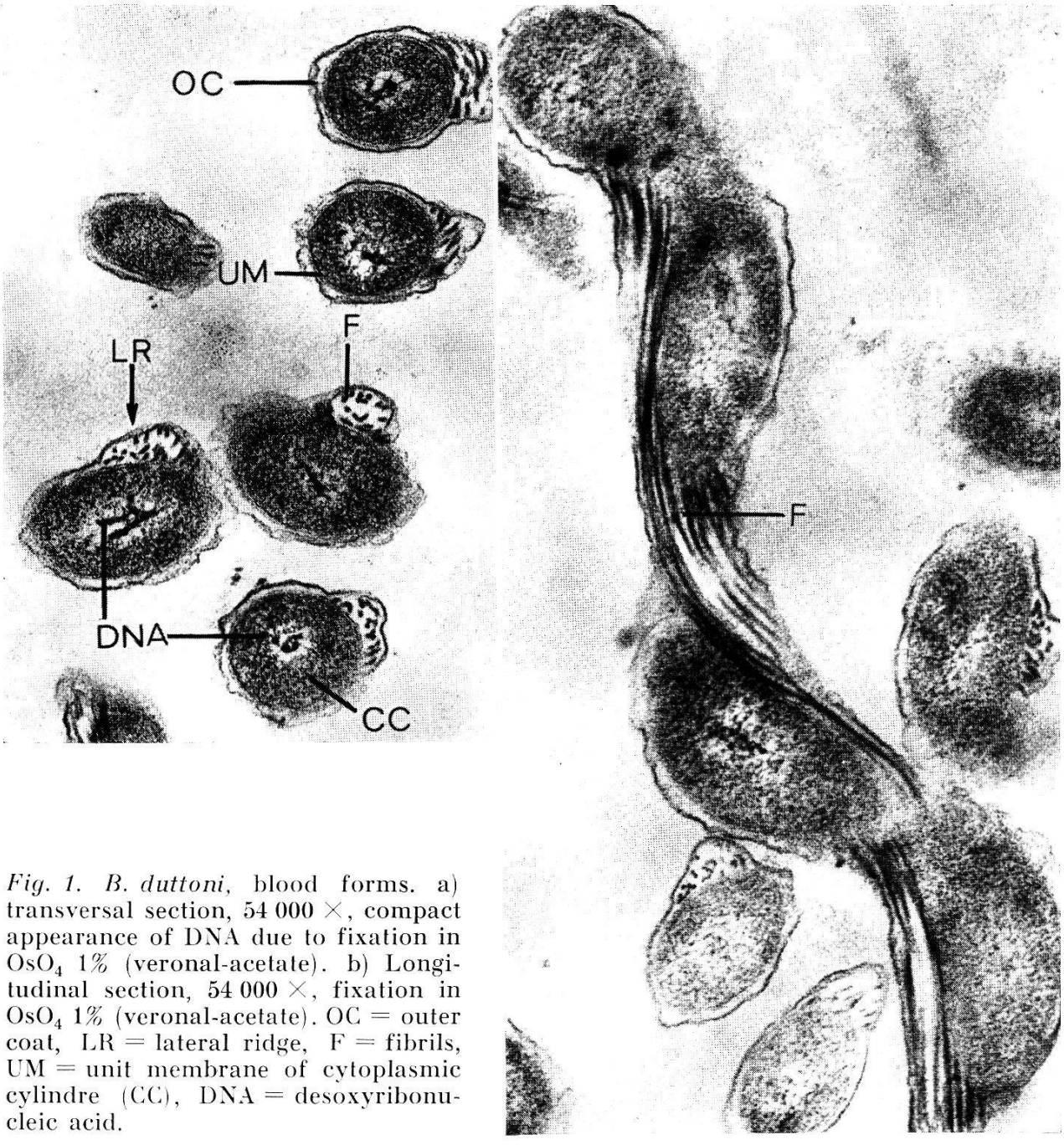


*Fig. 1. B. duttoni*, blood forms. a) transversal section, 54 000 ×, compact appearance of DNA due to fixation in $OsO_4$ 1% (veronal-acetate). b) Longitudinal section, 54 000 ×, fixation in $OsO_4$ 1% (veronal-acetate). OC = outer coat, LR = lateral ridge, F = fibrils, UM = unit membrane of cytoplasmic cylindre (CC), DNA = desoxyribonucleic acid.

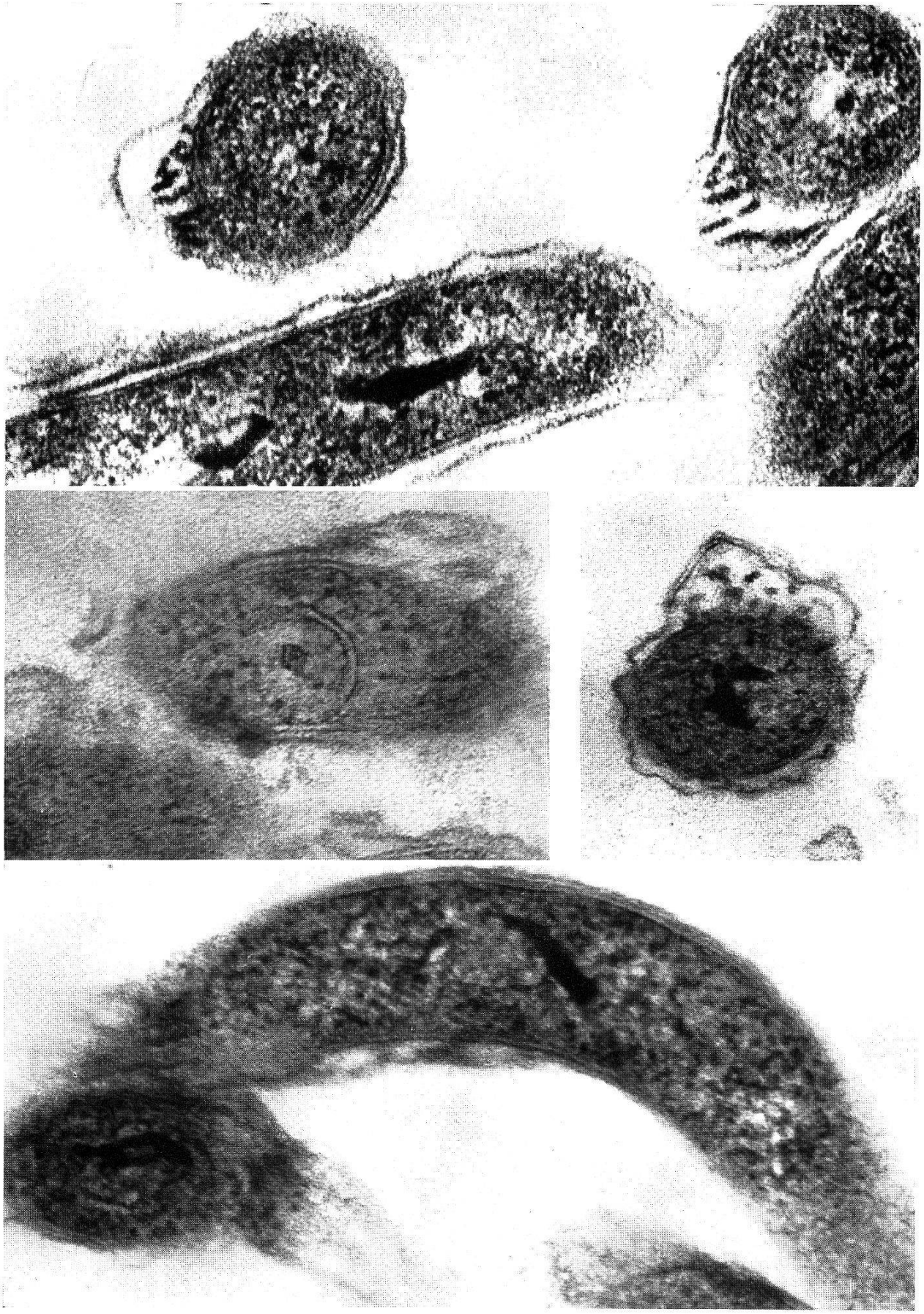


*Fig. 2.* Blood forms: a) and b) *B. tillae.* a) 120 000 ×, fixation in $OsO_4$ 1% (veronal-acetate), b) transversal section showing fragment of a mesome in the cytoplasmic cylindre, 120 000 ×, fixation in $OsO_4$ 1.5% (phosphate). c) *B. crocidurae,* transversal section, 100 000 ×, fixation in $OsO_4$ 1.5% (phosphate). d) *B. hispanica,* 100 000 ×, fixation in $OsO_4$ 1.5% (phosphate).

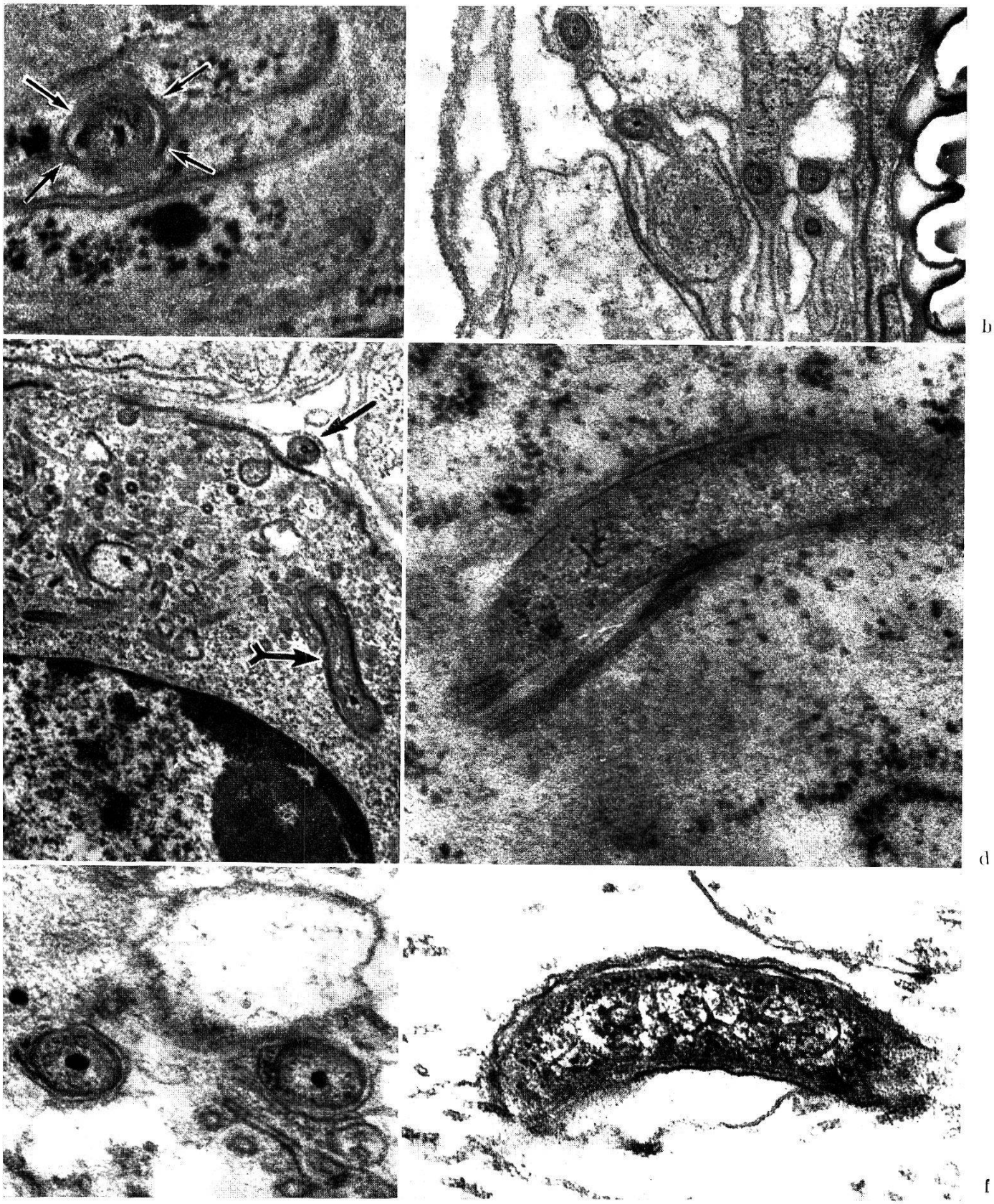


*Fig. 3.* Tissue forms of *B. duttoni* in *O. moubata.*

a) Transversal sections of intercellular *Borrelia* in coxal organ, 61 000 ×, fixation in $OsO_4$ 2% (phosphate). The cell membranes of the bordering cells surround the spirochete (→).

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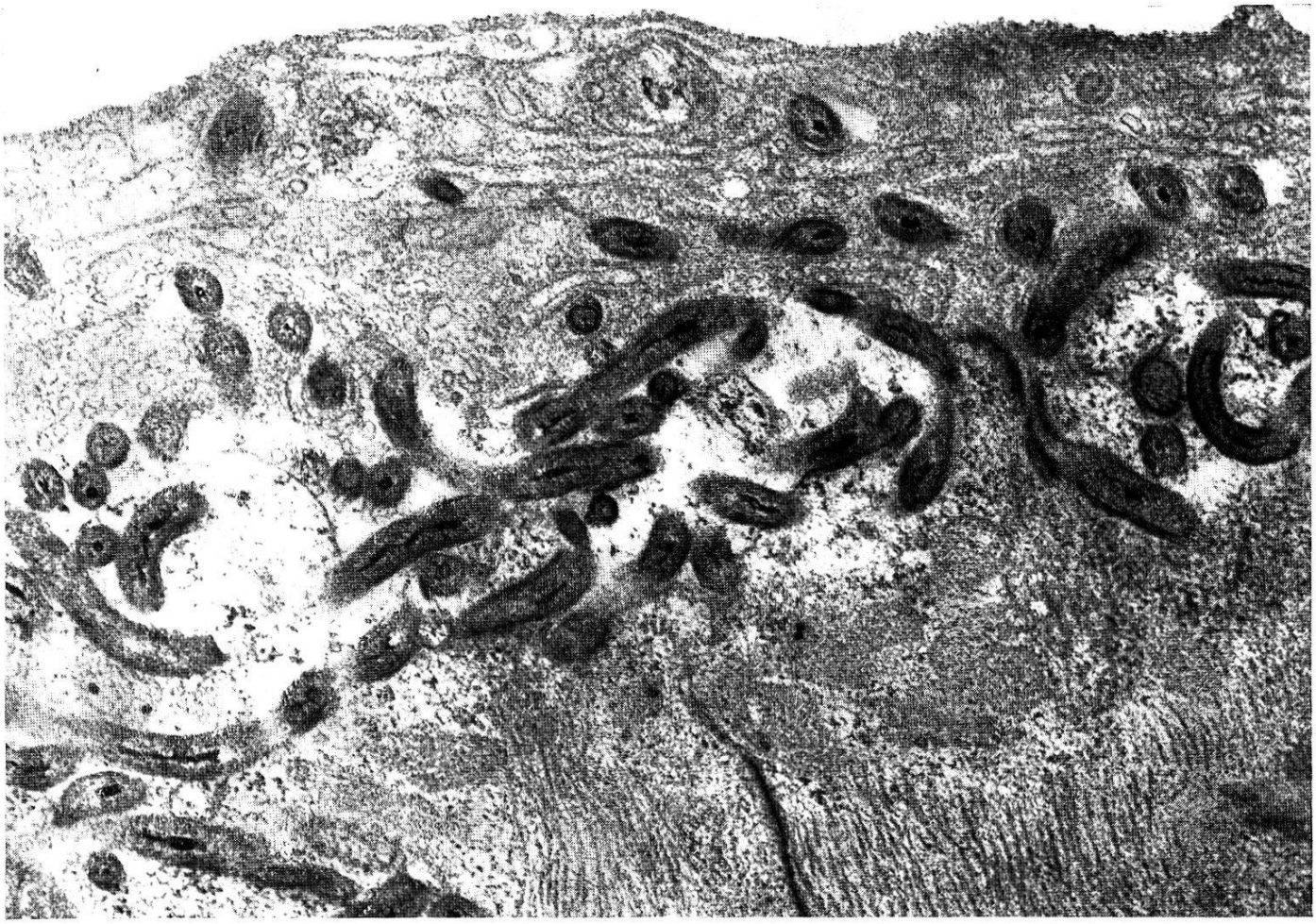

*Fig. 4.* Cluster of intracellular *Borrelia* in the coxal organ; degenerating tissue surrounding the intact spirochetes appears as a clear zone on the micrograph, 21 000 ×, fixation in $OsO_4$ 2% (phosphate).

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b) Inter- and intracellular forms in tracheal tissue, 21 000 ×, fixation in $OsO_4$ 2% (phosphate).

c) One intercellular (→) and one intracellular (➤→) form can be seen in the coxal organ, the intracellular form near a nucleus, 21 000 ×, fixation in $OsO_4$ 2% (phosphate).

d) An intracellular *Borrelia* between the ribosomes in the cytoplasma of a coxal cell, 61 000 ×, fixation in glutaraldehyde 2.5% (cacodylate) and $OsO_4$ 1% (cacodylate).

e) Intracellular forms in the CNS. The surrounding tissue is lysed, because the dissection was made in saline and not directly in the fixative, 61 000 ×, fixation in $OsO_4$ 2% (phosphate).

f) As e). Some rests of lysed neurofilaments are seen around the *Borrelia*, 54 000 ×, fixation in glutaraldehyde 2.5% (cacodylate) and $OsO_4$ 1% (cacodylate).

*Tissue forms:* Owing to the absence of suitable material, only *B. duttoni* was examined. As regards the fine structure, no difference was found between mammalian blood and tick tissue forms. Variations due to the procedure of fixation are even more frequent in the latter, as the fixative has first to penetrate the tissues of the vector before reaching the parasites. But interesting results were obtained about the localisation of the spirochetes in the tissues. They may be found either between the cells, i.e. *inter*cellular, or inhabiting the cells themselves, i.e. *intra*cellular. In Figs. 3a–d the two types are seen in preparations from coxal organs and tracheal tissue. The same was found in the central nervous system of the tick (Figs. 3e and f). The last two photographs show clearly the typical structure of *Borrelia*. Sometimes even clusters of intracellular forms can be seen (Fig. 4). In this case, the surrounding cells seem lysed, as shown by the clear zone surrounding the parasites.

## Discussion

The fine structure of various *Borrelia* (blood as well as tissue forms) were examined by electron microscopy. No relevant difference was found either between the different species of *Borrelia* obtained from the blood of Swiss mice, or blood and tissue forms of *B. duttoni*. However, one singularity was found in the blood forms of *B. tillae*, namely the presence of membrane fragments in the cytoplasmic cylinder which requires further examination.

On the other hand, the intracellular position of *B. duttoni* could be demonstrated in the coxal organ and tracheal cells as well as in the central nervous system of *O. moubata*, while most authors still insist on the purely extracellular localisation of tissue forms in the tick vector, except for the penetration of *B. duttoni* into the ovocytes allowing transovarial transmission. The question arises, if the spirochetes are phagocyted to be destroyed by the cells, or if they penetrate actively. The observation of whole groups of perfectly normal forms suggests rather that the intercellular and intracellular forms are capable to multiply freely. Furthermore, not the intracellular *Borrelia* degenerate but the surrounding tissue.

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