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COXIELLA AND *RICKETTSIELLA:* COMPARISON OF ULTRASTRUCTURE WITH SPECIAL REFERENCE TO THEIR ENVELOPE

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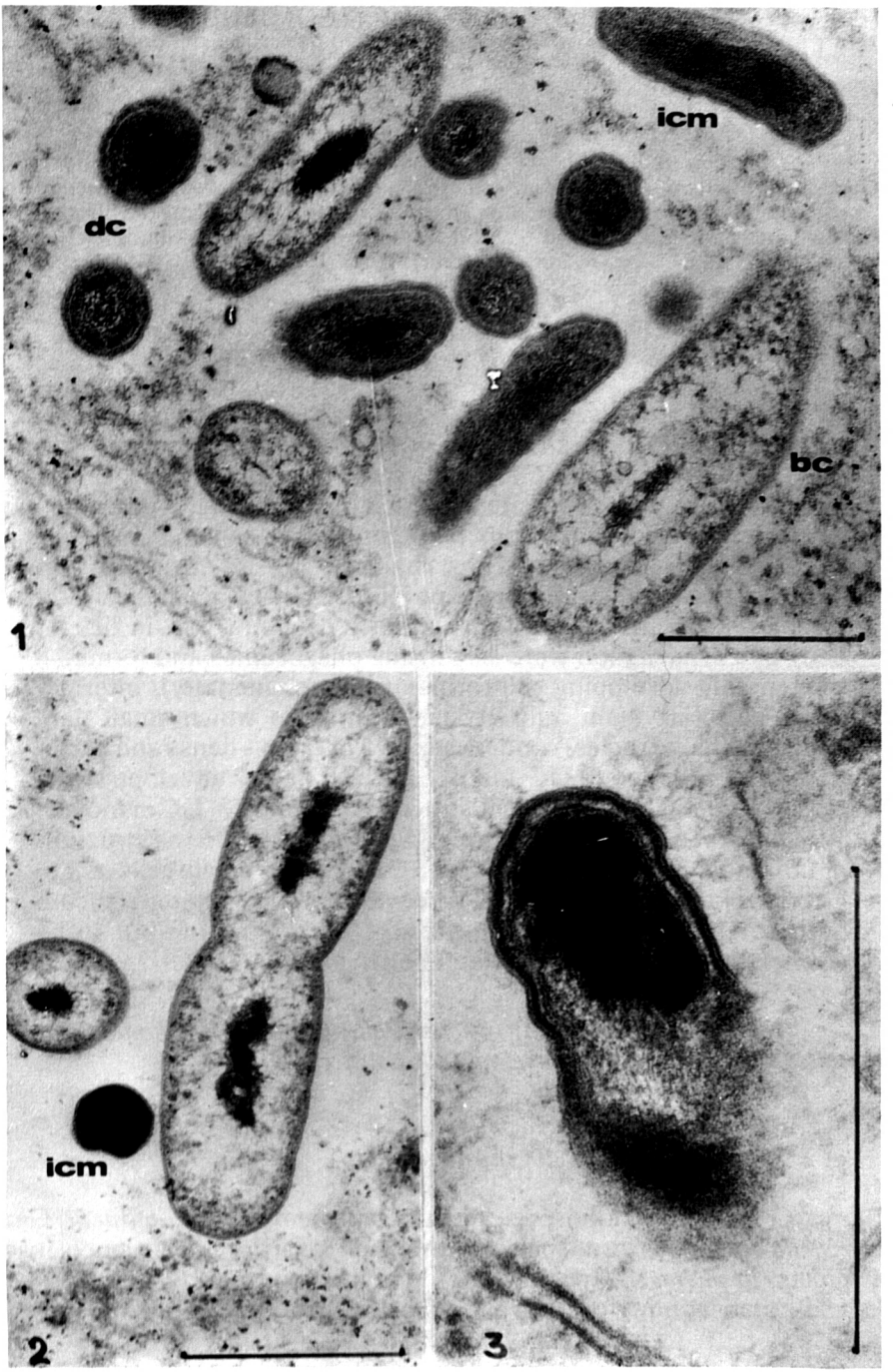
Summary. - Ultrastructure of *Rickettsiella phytoseiuli (R.p.)* multiplying in female ticks *Dermacentor reticulatus* was compared with that of *Coxiella burnetii (C.b.)* in the same ticks and in mice. *C.b.* in ticks and mice were always represented by 2 main cell types: small dense round or rod-like cells (DC) and larger bacteria-like cells (BC). DC were surrounded with a characteristic five-layered 20 nm thick envelope. Under the envelope DC had a stack of parallel intracytoplasmic membranes with a periodicity 5-6 nm. *R.p.* in tick fat body and synganglion were also inside phagosomes and formed 6 sequentially developing cell forms: dense (elementary), intermediate, bacterial, giant, and crystal-forming in which small dark bodies (initial particles) condensed. Two of them - dense and bacterial - corresponded to DC and BC of *C.b.* The DC envelope structure of *R. p.* was strikingly similar to that of some *C.b.* DC in mouse. We confirmed the general morphologic similarities in the structure of *C.b.* and *R.p.* DC and that of *C.b.* BC and intermediate cells of *R.p.* The envelope structure of DC type was found in other gracilicute bacteria and is supposed to have no taxonomic value but to be a reflection of population hetergeneity.

Key words: Coxiella burnetii; Rickettsiella phytoseiuli; Dermacentor reticulatus; mouse; cell envelope; ultrastructure

Introduction

Genus *Coxiella* is a monospecies genus represented by *C. burnetii.* Genus *Rickettsiella* comprises three species pathogenic for arthropods, namely insects and spiders. *R. phytoseiuli* was discovered in predator mite *Phytoseiulus persimilis* and mites, although massively infected survive the infection.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In both *Coxiella* (McCaul and Williams, 1981) and *Rickettsiella* (Devauchelle *et al.*, 1972) complex developmental cycles were described, and some morphological similarities between their cells were observed (Avakyan *et al.*, 1983; Šuťáková and Řeháček, 1988).

The subject of the present study is to compare in detail the ultrastructure of the cells of these genera cultivated in different model systems.

Materials and Methods

Rickettsiae. C. burnetii (C.b.), strain Nine Mile, phase I, 3rd passage in chicken embryo yolk sac was used for the infection of ticks. *C.b.*, strain *Apodemus flavicollis* - Luga, phase I, 3rd passage in chicken embryo yolk sac was used to infect laboratory mice.

R. phytoseiuli (R.p.) 1st passage of its isolate from mites *P. persimilis* in *Dermacentor reticulatus* ticks (Šuťáková and Řeháček, 1988; Řeháček and Šuťáková, 1989) stored at -70 °C was used for the inoculation of ticks.

Animals. C3HA mice (14±1 g) were inoculated intraperitoneally (i.p.) with *C.b.* suspension (1 ml, 2 x $10^{6.2}$ ID_{50} per mouse). Female *D. reticulatus* ticks collected near Bratislava and found free of rickettsiae in haemocyte tests were half-engorged on rabbits and inoculated intracoelomally with *C.b.* (about $10^3 EID_{50}$ per tick) or with *R.p.* was comparable with that of *C.b.* as estimated in the light microscope.

Electron microscopy. Mice were sacrificed 7 and 14 days post-infection (p.i.) and pieces of spleen, omentum, and liver were fixed on ice. Synganglion and fat body were dissected from ticks 21 days p.i. with *C.b.* or *R.p.* and also fixed in a mixture of glutaraldehyde, formaldehyde, and picric acid (Ito and Karnovsky, 1968) in 0.2 mol/l cacodylate buffer pH 7.4, postfixed in 1 % OsO_4 and embeded in Spurr low viscosity resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in JEM 100B electron microscope.

Results and Discussion

C.b. inside the intracellular phagosomes or free in the host cell cytoplasm in organs of ticks and mice were always represented by 2 main cell types: small dense round or rod-like cells (DC) and more large bacteria-like cells (BC) morphologically similar to conventional gram-negative bacteria. These cells corresponded to small cell variants (SCV) and large cell variants (LCV) described earlier (McCaul and Williams, 1981) in chicken embryo.

DC had the diameter 200 nm or the dimensions 200 x 600 nm (Fig. 1). In tick tissues rod-like DC predominated but some of round profiles in ultrathin

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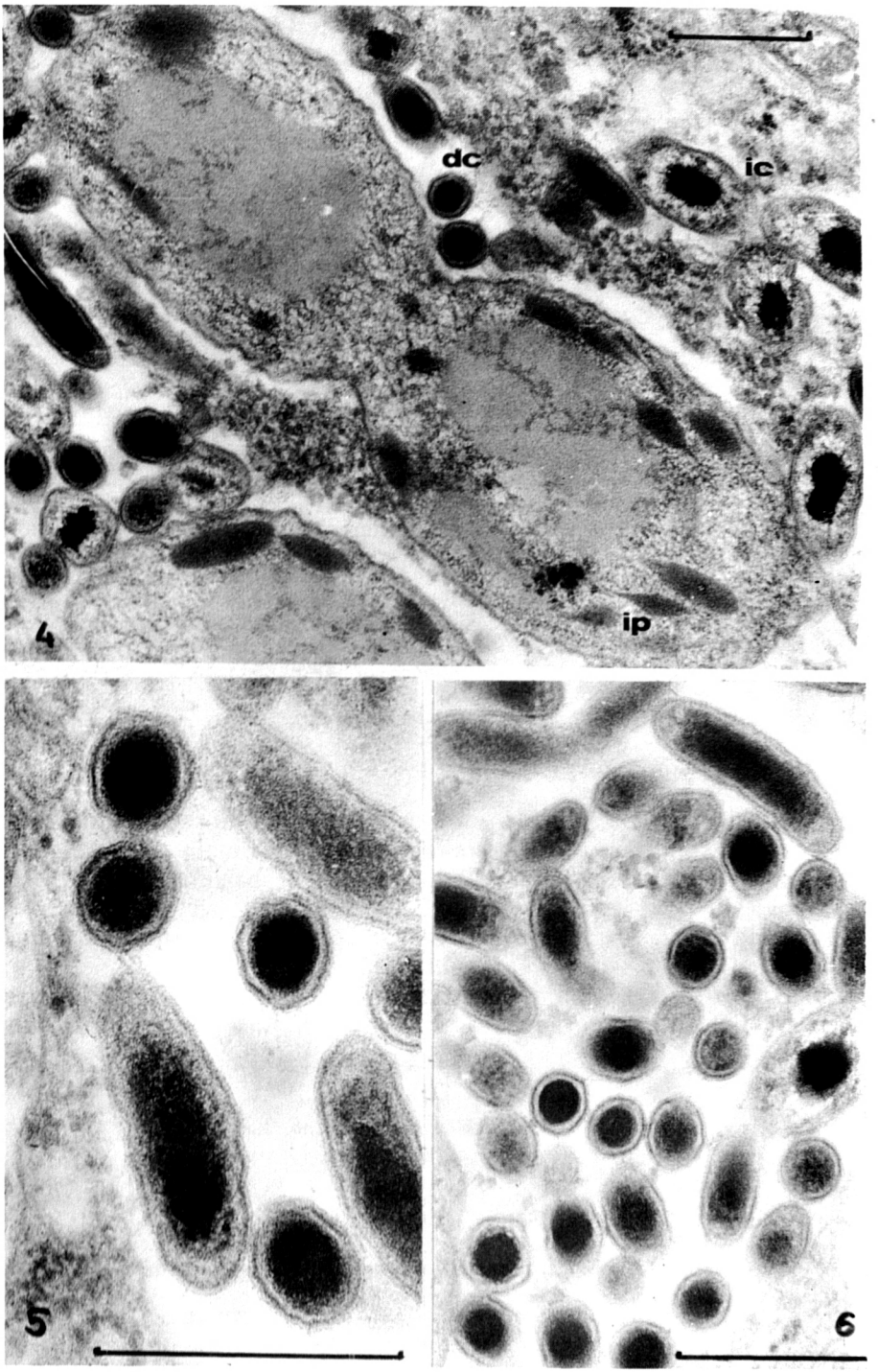
Figs. 1-3
C. burnetii in ticks *D. reticulatus* and mice

Fig. 1. DC and BC of C.b. in fat body cell.

Fig. 2. DC with circular ICM and dividing BC in fat body cell.

Fig. 3. Cell envelope structure of DC in mouse spleen macrophage (14 days postinfection).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sections could also correspond to spheroid DC. DC were surrounded with a characteristic envelope 20 nm thick with a prominent electron-dense inner layer. In sections the envelope was represented by a five-layered structure (electron dense-lucent-dense-lucent-dense, measuring correspondingly 3-4-8-2-3 nm). The inner layer was usually masked by dense cytoplasm. Under the envelope some DC could have a stack of parallel intracytoplasmic membranes (ICM) with a periodicity 5-6 nm (Fig. 2). It is remarkable that in tick cells rod-like DC could have ICM on the both sides of the nucleoid (Fig. 1), and in transverse sections of such cells ICM surrounded it in almost all the circumference.

BC had the dimensions 300-400 x 700-900 nm in both ticks and mice. It tick tissues their cytoplasm was usually looser and DNA fibers radiating from the central electron dense body were clearly seen.

BC in mice were always surrounded by 2 unit membranes with a lucent periplasmic space between them. In tick these membranes were usually separated by a periplasmic space of moderate density and 6-8 nm thick ICm were never seen in BC.

R.p. in tick organs existed also in phagosomes and formed 6 sequential developmental cell forms (Šuťáková and Řeháček, 1988): dense (elementary) cells (DC), intermediate cells (IC), bacterial cells (BC), giant, and crystal-forming cells in which small dark particles (initial) were condensed. Two of them, i.e. DC and BC corresponded to DC and BC of *C.b.* These DC nucleoid of DC were very dense, and only in some oblique sections the ribosomes were differentiated. BC had a typical gracilicute protoplast sometimes with a central dense zone of nucleoid (Figs. 4-6).

IC had a transitory morphology between DC and BD: their nucleoid gradually became more dispersed and ribosomes became visible, and thus they resembled some BC of *C.b.* (Fig. 4).

DC of *R.p.* were surrounded by an envelope consisting of the cell wall membrane with a dense inner layer 7 nm thick (total thickness of the cell wall was 12 nm), rather large and lucent periplasmic space (15 nm) and a unit cytoplasmic membrane. The DC envelope structure of *R.p.* was strikingly similar to that of some *C.b.* DC in mice (Figs 5 and 6).

The envelope of BC of *R.p.* consisted of two unit membranes separated by a periplasmic space of moderate density. Its inner membrane could often be masked by the cytoplasm.

Figs. 4-6

R. phytoseiuli in ticks *D. reticulatus.* Bar in all figures 0.5 μm

Fig. 4. Different developmental cycle cell types in R.p.: DC, intermediate cells (i.c.), and dividing crystal-forming cell with dark initial particles (i.p.).

Figs. 5-6. Envelope structure in DC: Fig. 5 in fat body. Fig. 6 in synganglion.

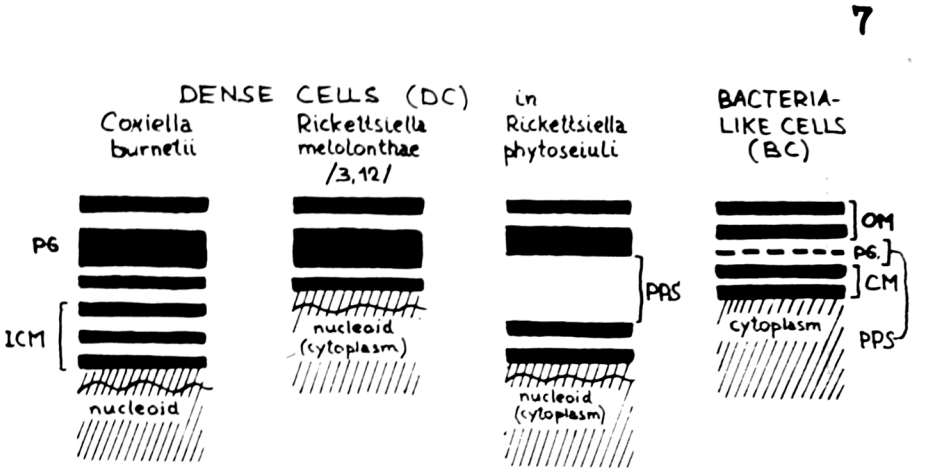

Fig. 7
Schematic representation of the structure of the cell envelope of *Coxiella* and *Rickettsiella* cells CM, cytoplasmic membrane; ICM, intracytoplasmic membranes; OM outer membrane (cell wall membrane); PG, peptidoglycan; PPS, periplasmic space (periplasm)

In intermediate cells the cell wall was 10 nm thick having a dense inner layer, and their cytoplasmic membrane usually was not clearly visible in our preparations.

Some differences in details of the envelope structure of the two types of *C.b.* cells (i.e. presence of the slime layer, thickness and density of periplasmic space) may be dependent of their host (vertebrate or invertebrate) but principal organization of the envelope is similar in mice and ticks.

Our data confirm the general morphologic similarities in structure of DC in *C.b.* and *R.p.*, and BC in *C.b.* with BC and IC in *R.p.* Nevertheless, the DC envelope of these rickettsiae was differently organized: in *C.b.* the periplasm was dense and had a uniform thickness. The same structure of the envelope was described earlier in DC of other *Rickettsiella* species (Devauchelle *et al.*, 1972; Louis *et al.*, 1977; Yousfi *et al.*, 1979) and could be seen even in *Rickettsia tsutsugamushi* in mites *Leptotrombidium arenicola* (Wright *et al.*, 1984).

This structure is not unique for *Rickettsiaceae,* it can be regularly observed in some enterobacterial colonies, i.g. *Shigella sonnei* (Kaminsky *et al.*, 1989). The organization of gram-negative (gracilicute) cell envelope is studied best in *Enterobacteriaceae* (Hobot *et al.*, 1984; Lambert *et al.*, 1988; Leduc *et al.*, 1989).

It is presumed that their periplasmic space is completely filled by peptidoglycan gel (Hobot *et al.*, 1984) or peptidoglycan can form there a multilayered structure (Leduc *et al.*, 1977).

Hence we propose the structure of *Coxiella* and *Rickettsiella* cell envelopes as depicted in Fig. 7. We can assume that it has no taxonomic value but is merely a reflection of microbial population heterogeneity.

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