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MIXED RICKETTSIA-VIRUS INFECTION IN *DERMACENTOR RETICULATUS* IMAGO

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Summary. – Electron microscopic examination revealed replication and accumulation of *Rickettsia sibirica* in the fat body of experimentally infected *Dermacentor reticulatus* ticks. Rickettsia are released from the fat body cells by budding being surrounded with cytoplasm and plasmalemma of the host cell. Eukaryotic cell structures have been detected consisting of lamella layers whirled around the intact rickettsiae. In addition to rickettsia, microorganisms morphologically resembling *Francisella tularensis* and an orbivirus were found in tick tissues at morphological examination. The morphology of the virus and stages of its morphogenesis are described. Mixed viral and rickettsial infection has been shown to develop in the same ticks and even in the same fat body cells in a very close association.

Key words: Rickettsia sibirica; Dermacentor reticulatus; orbivirus; mixed infection; ultrastructure

Introduction

Infectious agents inducing diseases of man and animals under natural conditions usually occur in association. Therefore, much interest has been focused on their mutual interaction (including morphological aspects) and their interaction with representatives of nonpathogenic bacterial flora (Miller, 1980).

Ultrastructural studies on interaction of infectious agents with eukaryotic cells have brought new informations helpful for elucidating the development and assembly of a number of infectious agents in various animal models. One of the models deserving particular attention are ticks as natural vectors (Diehl *et al.*, 1980). At present, the investigations on the growth of several infectious agents in ticks are in the beginning (Gosteva *et al.*, 1988). Therefore, it appeared of interest to follow the patterns of relationship of rickettsiae with other agents infecting the tick organism under natural conditions. Although

ixodid ticks are the usual vectors of rickettsia (Řeháček and Tarasevich, 1988) for electron microscopic examinations, experimental infection of Dermacentor ticks was performed with *Rickettsia sibirica.*

Materials and Methods

Dermacentor reticulatus female imagoes were caught in nature in the vicinity of Bratislava. They were partially fed on rabbits in the laboratory 5–6 days prior to rickettsia inoculation. Netsvetaev strain of *R. sibirica* in the form of partially purified suspension of chick embryo infected yolk sacs containing about 10^2 rickettsia cells/ml was injected parenterally to half-fed ticks in the volume of approximately 0.01 ml.

After inoculation, the ticks were kept in a humid chamber at 25 ^{0}C; two groups on 10 and 27 days p. i. were taken for autopsy.

The tissues obtained at autopsy (the complex of a fat body, muscles, trachea, and Malpighian tubes) were fixed in the mixture of glutaraldehyde, formaldehyde, and picric acid (Ito and Karnovsky, 1968) and embedded in a low-viscosity epoxy resin (Spurr, 1969). Ultrathin sections cut on „Ultratom III LKB-8800" (Sweden) were stained with uranyl acetate and lead citrate by a conventional procedure; electron microscopic examination was carried out with a JEM-100B (Japan) at accelerating voltage 80 kV and magnifications of 5000, 15 000, 50 000, respectively.

Results

Ten days post-inoculation (p.i) the rickettsiae were detected in ultrathin sections of fat body cells obviously located free in host cell cytoplasm. Most frequently they were seen as single microorganisms in the parts of the fat body between the bands of muscle tissue.

By 27 days microcolonies of rickettsia were found in cell cytoplasm forming compact clusters of 600–700 μm in diameter, with more than 50 microorganisms per cell section (Fig. 1). As a rule, such microcolonies were seen to be located near to the basal membrane. In these agglomerations rickettsiae were in close contact with each other. The cells at the periphery of the agglomeration had a marked „halo".

Rickettsia were preserved to a different extent in terms of their morphology: together with anatomically intact forms some there were morphologically altered showing round-shaped defects at cross-section, condensation of the protoplast and its separation from the cellular wall. Some rickettsia with unaltered morphology had ring-shaped intracytoplasmic membrane structures. The rickettsiae agglomerations were frequently surrounded by parallel cisterns of granular endoplasmic reticulum frequently carrying a few ribosomes. Blebs and ring-shaped membrane structures usually occurred among the rickettsiae.

Sometimes in fat body cells adjacent to the basal membrane concentric lamellar structures of 200 μm in diameter were seen with lamels ranging from

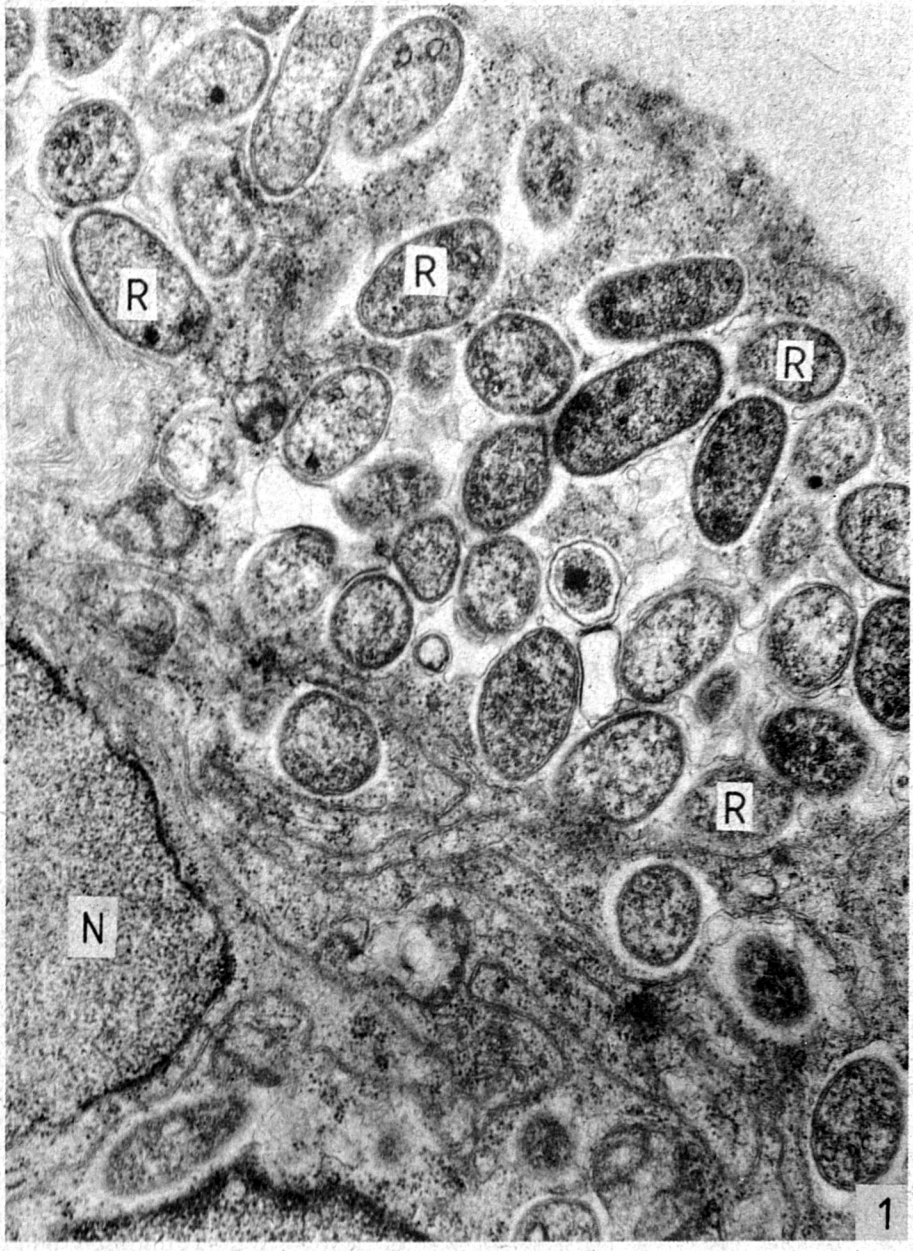


Fig. 1
Agglomeration of rickettsiae in the cytoplasm of tick fat body cell. x 32 000

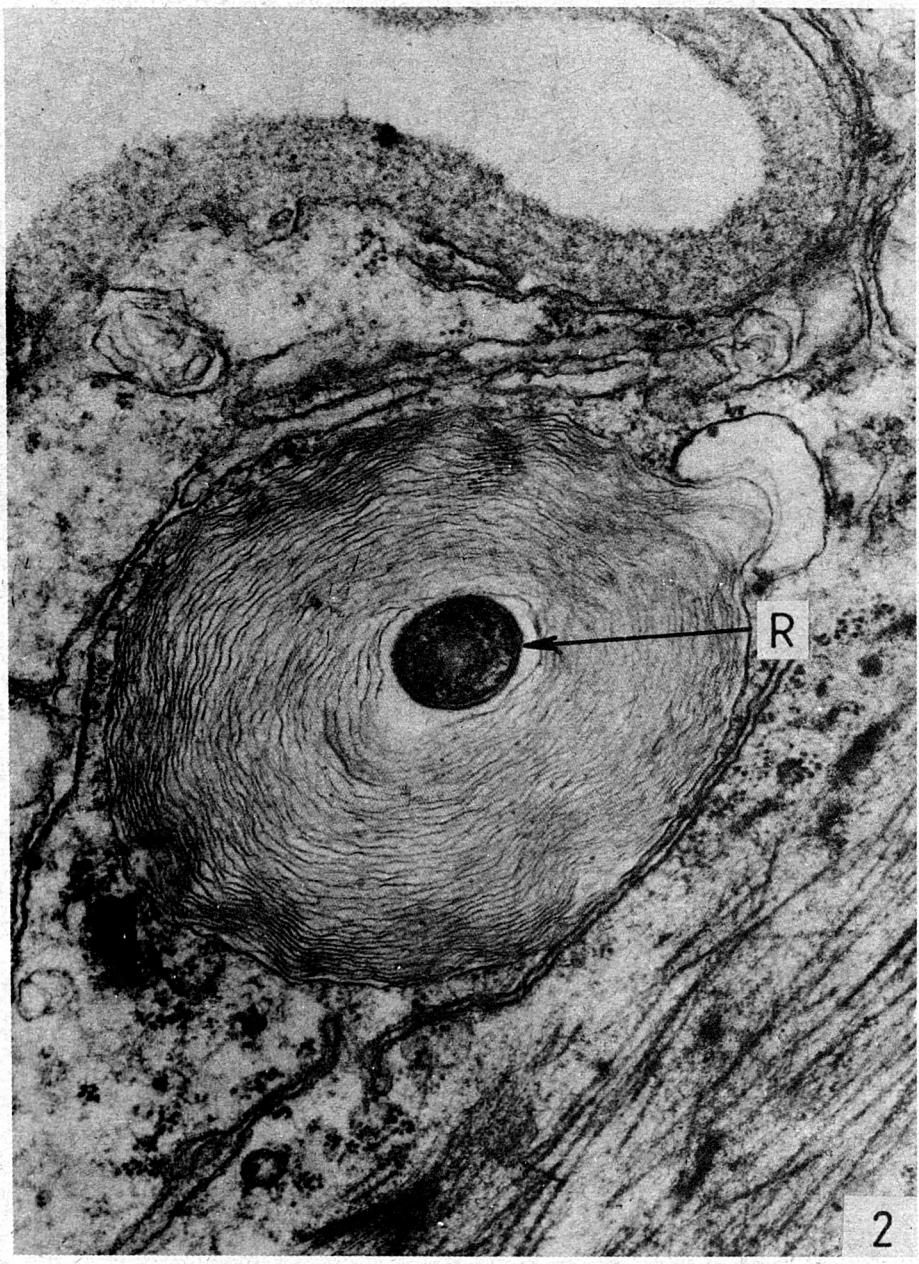
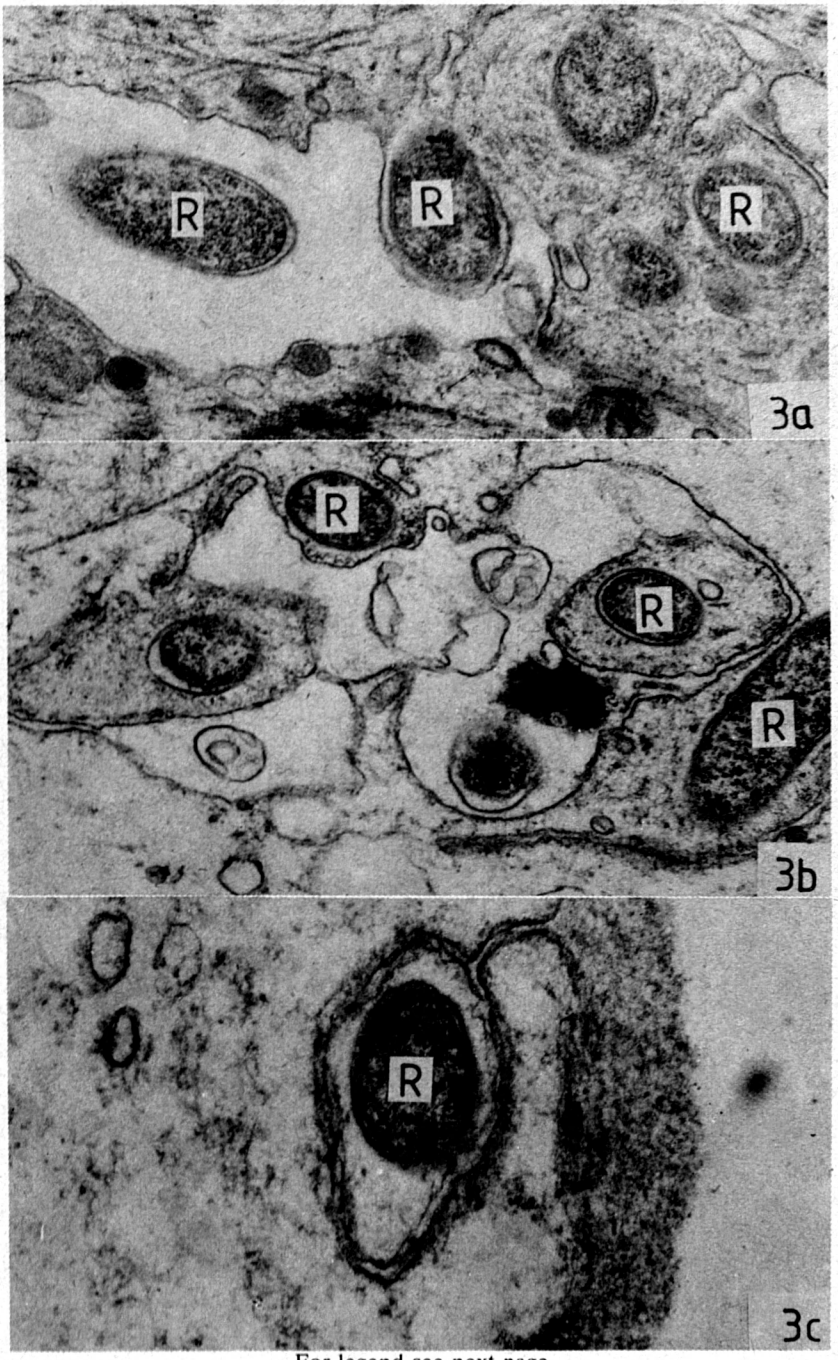


Fig. 2
Concentric lamellar structures surrounding rickettsiae in the host cell cytoplasm. x 72 000



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≈ 16 nm at the periphery to ≈ 31 nm in the centre, these structures contained a morphologically intact rickettsiae cell inside (Fig. 2).

Other variants of rickettsia location occurred frequently also in fat body tissues: single free lying rickettsiae in the cytoplasm of cells adjacent to trachea (tracheoli lumens) or rickettsial agglomerations lined along muscle bundles. The detailed examination of rickettsiae located within the fat body revealed that many rickettsia except the large agglomerations described above were surrounded to a certain extent by host cell membranes (Figs. 3a, b, c). Cytoplasmic membrane invaginations were ring-shaped thereby isolating rickettsiae from host cell cytoplasm (Fig. 3b). Surrounded by the membrane they apparently budded into extracellular space or into the vacuolar cavity (Fig. 3a).

In addition to rickettsia, viral inclusions were seen in ultrathin sections of tick fat body. By 17 days after inoculation, crystal-like accumulations of virus particles of hexagonal shape about 64 nm in diameter, with the core of 26 nm (Fig. 4a) were detected. Moreover, fibrillar matrices containing electron-dense, less frequently ring-shaped (with a light centre) particles of 60 nm in diameter, microtubules of 8 nm in diameter, and bundles of fibrillar substance were found (Fig. 4b). Intracellular rickettsiae could be noted both near crystal-like inclusions in cell cytoplasm (Fig. 5a) and in immediate vicinity of fibrillar matrices containing viral particles (Fig. 5b).

No organized viral crystals were found at later terms of the follow-up. The particles occurred in irregular accumulations surrounded by a zone cytoplasm lysis (Fig. 6a). In such zones virions and rickettsiae frequently occurred in the close proximity (Fig. 6b). No virion budding through the host cell plasmalemma was noted. The release of virus particles from the cell apparently took place in the course of eukaryotic cell lysis.

At the time getting nearer to oviposition, viral matrix changed considerably, especially in those cases when rickettsia were located in the vicinity. Decreased electron density of matrices and their vacuolization were registered, as well as changes in the ratio of electron-dense and ring-shaped particles, the latter increasing in number.

Occasionally large vacuoles were confined to the membrane and containing destructed rickettsiae as well as viral particles morphologically resembling those described inside matrix, and arch-curved membranes in the cytoplasm of host cells (Fig. 7). Those vacuoles appeared to be phagolysosomes in which destruction of cell-infecting parasites took place. Intact rickettsiae could occur in the immediate vicinity of phagolysosomes. Ultrathin sections of the Malpighian tubules in the same tick revealed colonies of round-shaped microorganisms with spare cytoplasm and cellular wall structure typical of gram-negative microorganisms (Fig. 8).

Fig. 3
Rickettsiae to a varying degree surrounded by the host cell membrane. Rickettsia budding into extracellular space (indicated by the arrow). a: x 38 000; b: x 38 000; c: x 56 000

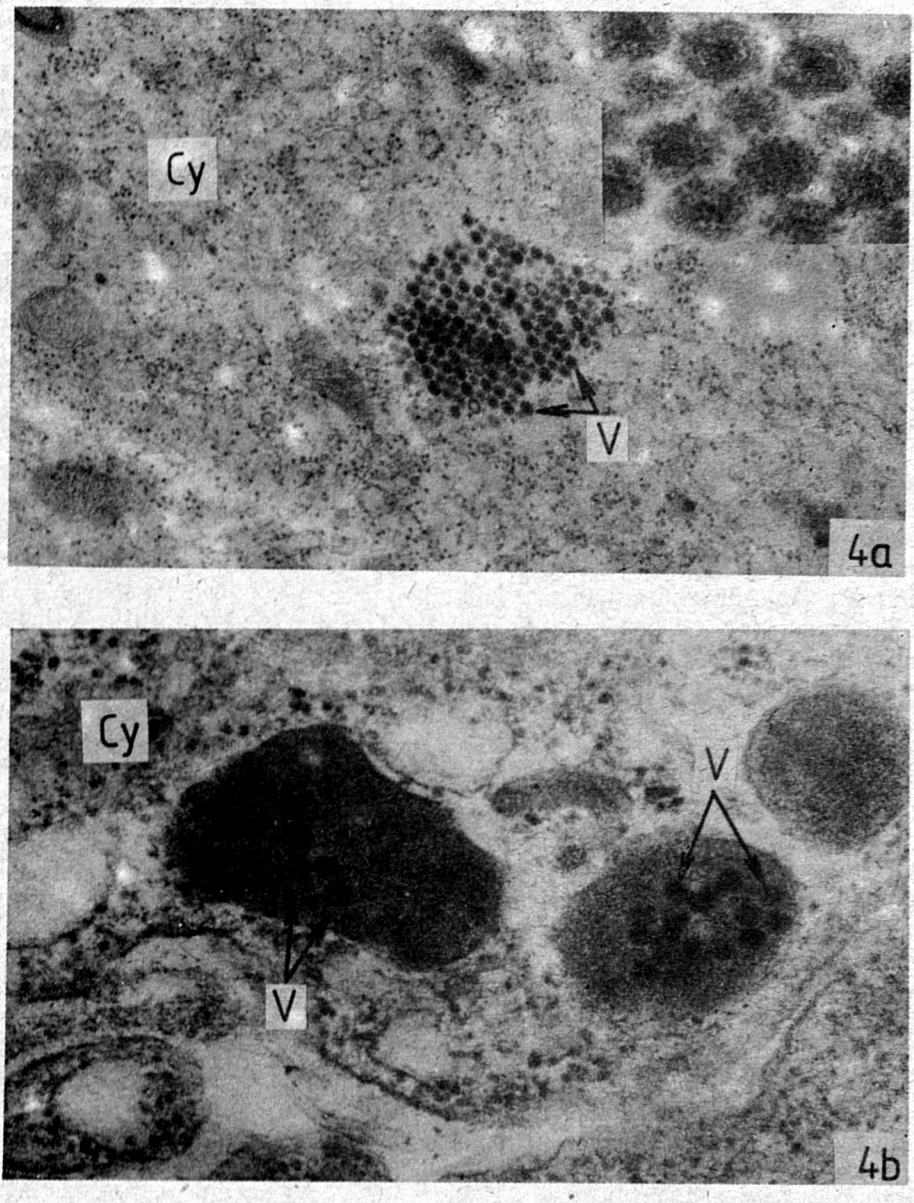


Fig. 4
Morphology and morphogenetic stages of viral particles in fat body cells. a: crystal-like inclusion of viral particles in the host cell cytoplasm. x 40 000, insert: x 150 00; b: fibrillar matrix containing viral particles in the host cell cytoplasm. x 58 000

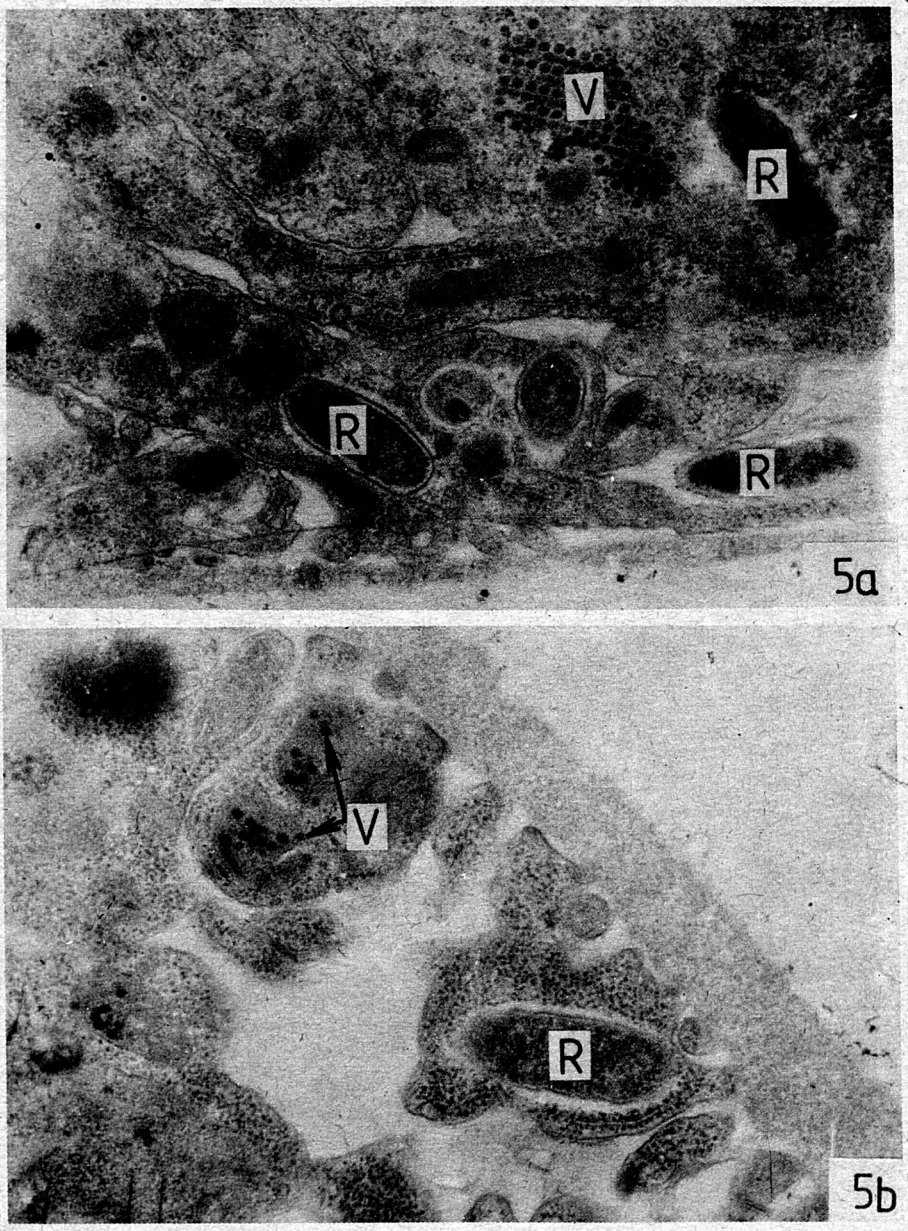


Fig. 5
Association of rickettsiae and virus in tick fat body cells. a: crystal-like inclusion of viral particles and rickettsiae. x 22 500; b: fibrillar templates containing viral particles and rickettsiae. x 27 000

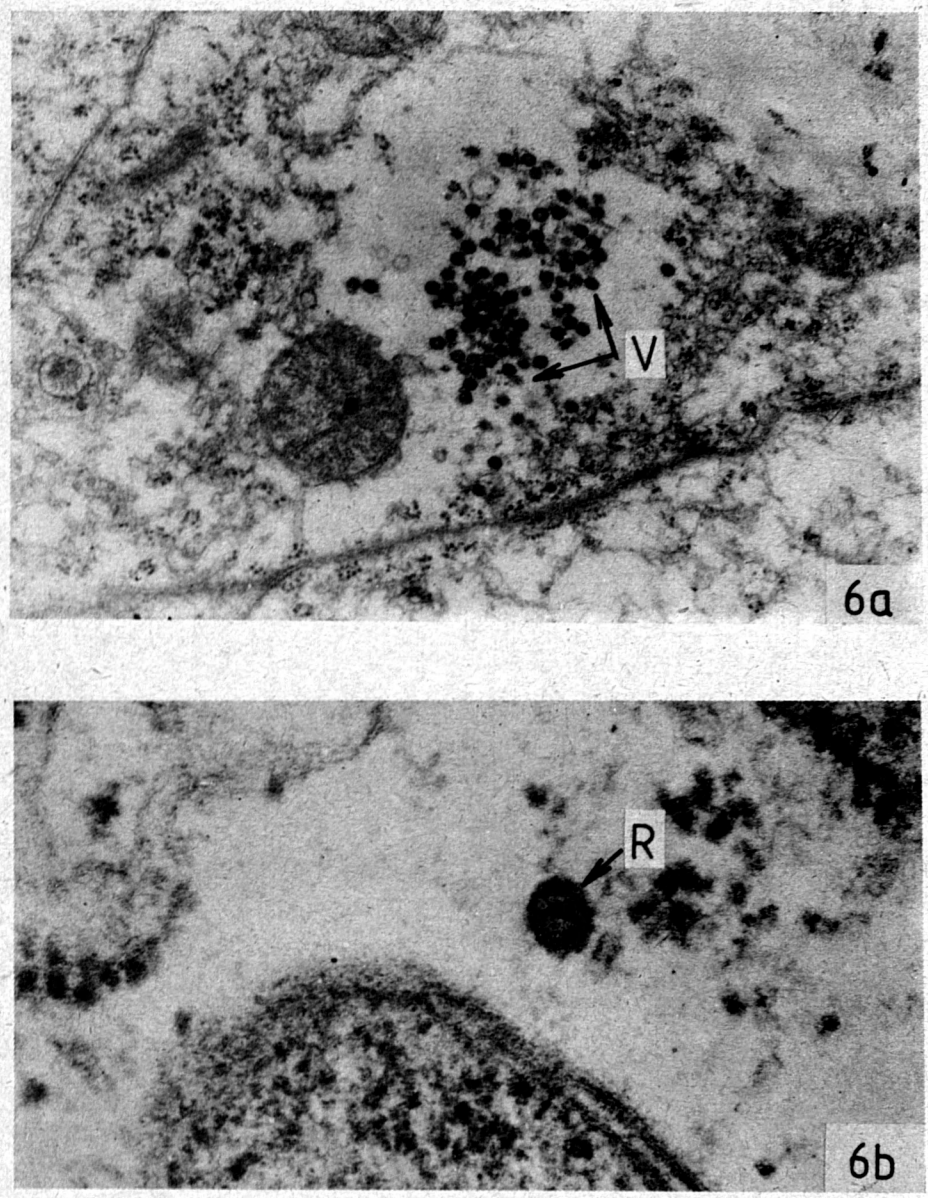
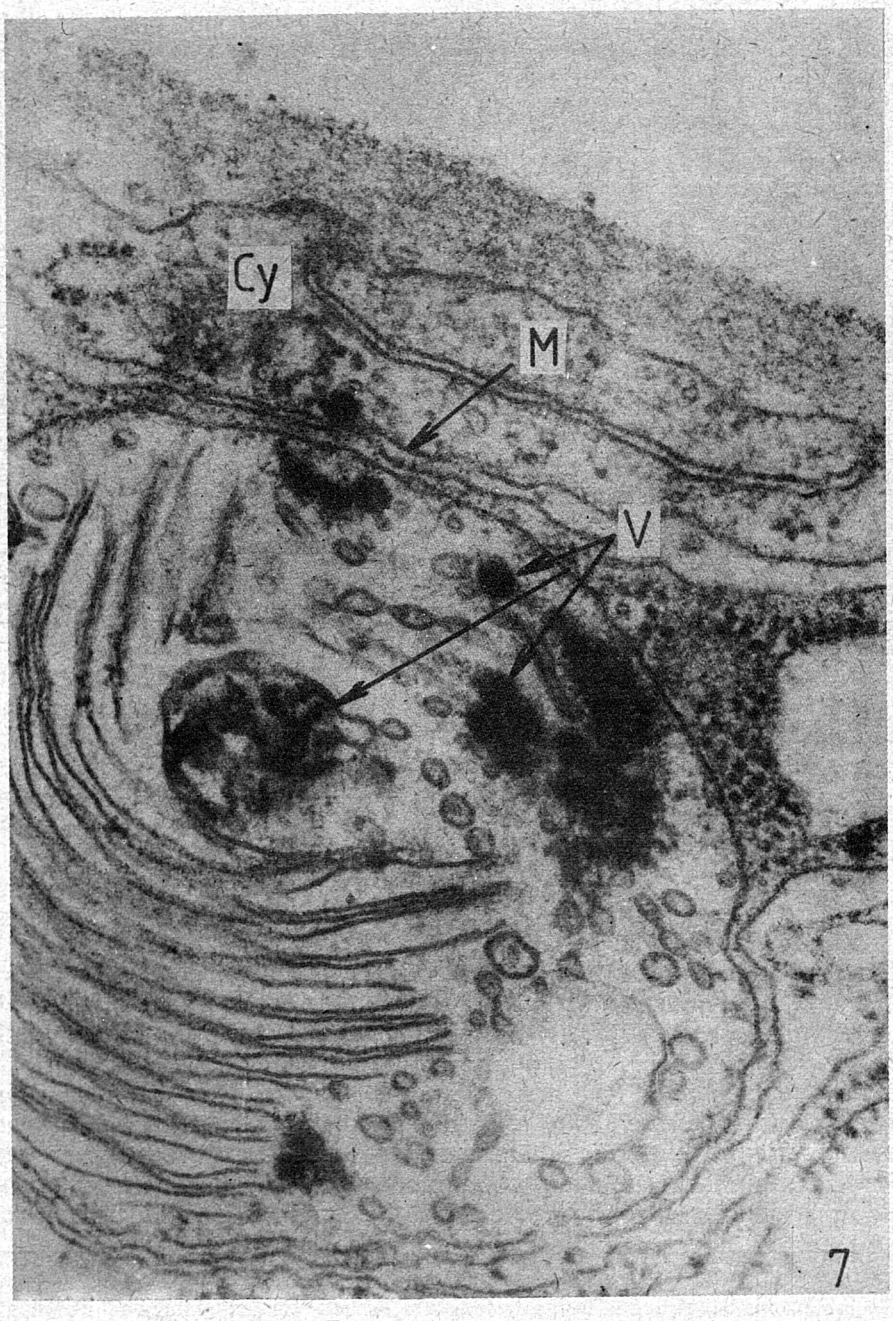


Fig. 6
Lysis of tick fat body cells. a: irregular accumulations of viral particles. x 36 000; b: the viral particle and rickettsia located close to each other. x 135 000



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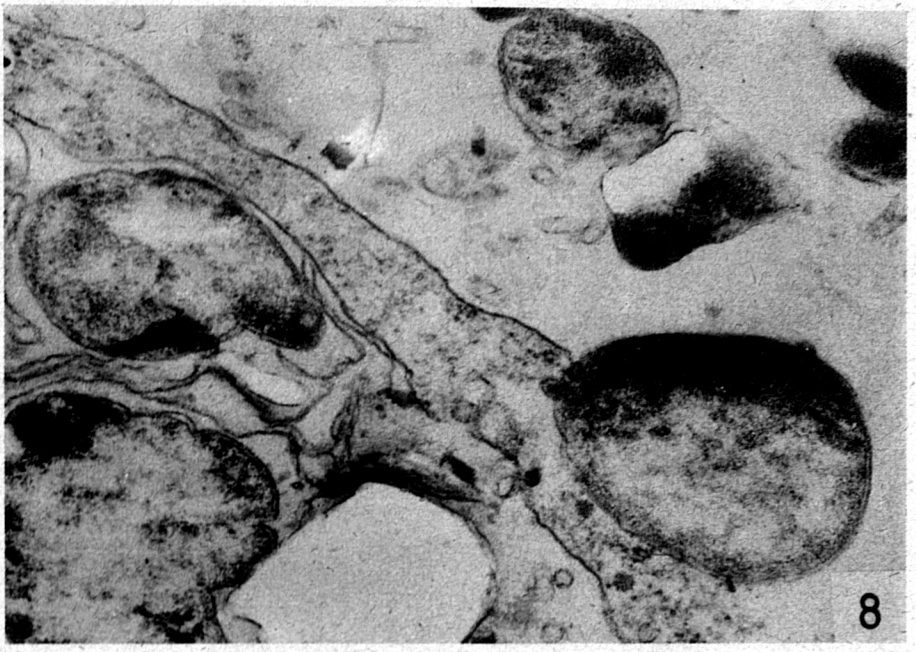

Fig. 8
Francisella tularensis-like microbe in the cells of Malpighian wall. x 41 000
Abbrevations: N – nucleus of the eukaryotic cell; C – cytoplasm of the eukaryotic cell; M – host cell membrane; V – viral particles; R – rickettsia.

Discussion

Electron microscopic investigations of rickettsial infection revealed replication and accumulation of *R. sibirica* in the fat body of experimentally infected *D. reticulatus* ticks as manifested by appearance of intracellular colonies which grow in size. It should be pointed out that large microcolonies frequently occurred near the basal membranes. Another line of evidence indicated that rickettsia can be released from cells into intercellular space by budding and subsequent redistribution in organs and tissues of the tick. On the other hand, we noted multilayer lamellar formations surrounding rickettsiae. Similarly piled up structures surrounding rickettsia-like symbionts in tick tissues were

Fig. 7
Phagolysosome-like structure in the cytoplasm of fat body cell containing viral particles, membranes, and possibly degenerated bacteria (arrow). x 90 000

described previously (Avakyan *et al.*, 1973) and proved to be significantly different as they consisted of eukaryotic cell membranes. The authors suggested that the physiological role of these structures was to isolate and protect symbionts from the effect of host cells, i. e. their conservation.

In our opinion, the structures detected are the manifestation of a specific defense mechanism promoting rickettsia preservation. It seems likely that multilayer lamellar structures protect rickettsia from the influence of cytoplasmic enzymes released in the course of cell lysis after ticks ovipostition, and from the effect of the environment to which the complex is exposed after the tick destruction.

The following associations were detected in the rickettsia infecting some tick tissues, the virus (in the fat body tissues), and microorganisms (in the cells of Malpighian tubules). Considering the combination of morphological features described in the literature (Pavlova and Mescheryakova, 1969; Kudelina and Popov, 1975) it is possible to classify the bacteria identified as *Francisella tularensis*. This is confirmed by frequent findings of *Francisella tularensis* in *D. reticulatus* collected in the vicinity of Bratislava (Řeháček, personal communication).

On the basis of data on virion morphology and some stages of its morphogenesis (2 types of particles, fibrillar matrix, and absence of budding) the virus detected in fat body tissues can be classified as Orbivirus belonging to *Reoviridae* family (Gaidamovich, 1982). The discovery of both rickettsia and virus in the same cell indicates that this double infection can develop both at the level of the tick body and its individual cells. None of the associants was subjected to morphological changes and both have the complete ontogenetic cycle of development.

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