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INTERACTION OF RICKETTSIAE WITH HAEMOCYTES OF *ALVEONASUS LAHORENSIS* TICKS

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Summary. — The development of different rickettsial species (*Rickettsia sibirica*, *R. conorii*, *R. acari* and *Coxiella burnetii*) in haemocytes of *Alveonasus lahorensis* ticks was compared by vital observations and time-lapse cine-micrography. The interaction of different rickettsial species with the haemocytes in vitro had specific characteristic features and reflected the pattern of interaction of the rickettsiae with tick cells in vivo.

Key words: rickettsiae; ticks; haemocytes

Comparative studies on different rickettsial species in the same type of cells from blood-sucking ticks make it possible to establish the species-specific features of their interaction with cells and to amend the current concept that interactions with ixodid ticks of all the rickettsiae studied from this aspect follow a similar pattern (Balashov and Daiter, 1973).

The aim of the present work was to study the development of different species of rickettsiae in tick haemocytes and their interactions with the cells by vital observations and time-lapse cine-micrography.

Adult *Alveonasus lahorensis* Neumann (*Argasidae, Ixodoidea*) ticks were inoculated with 0.01 ml volumes of rickettsial suspensions into the body cavities through the amputated distal part of the limb followed by coagulation of the wound opening with hot forceps (Sidorov, 1960). The rickettsial suspensions used for inoculation were an egg culture of *Rickettsia conorii* (strain M-1) in a dose of 100 ID_{50} for chick embryos and L-cell cultures of *R. sibirica* (strain Netsvetaev) and *R. acari* (strain M-3) in doses of 100 ID_{50} for L cells and *Coxiella burnetii* (vaccine strain M-44). At various intervals after inoculation, a drop of haemolymph was obtained from the ticks and placed in a microchamber for vital observations by phase contrast in MBB-IA and MBI-3 microscopes with a ×90 objective and ×5 Homal ocular. Time-lapse cine-micrography was done at 2—10 sec intervals with a KSR-I camera on a KN-2 negative film.

The bulk of haemocytes suspended in the haemolymph was represented by rounded cells filled with spherical vacuoles which gave the cells a morula-like appearance. Large vacuoles were polygonal due to their mutual compression (Fig. 1). Most haemocytes rapidly attached to the glass surface. As early as within the first hour some haemocytes began to spread on the glass surface (Fig. 2). Some cells showed a high motility. The spreading of the haemocytes was accompanied by dissolution of large vacuoles which

contained reserve nutrients in the cytoplasm. As a result of increased pinocytosis they were usually replaced by small vacuoles. The nuclei of the haemocytes were round and each contained 1–2 nucleoli.

The haemocytes infected with *R. sibirica* and *R. conorii* had the same appearance as uninfected cells. Vital observations of the infected cells confirmed the known fact that ticks infected with these rickettsial species show no manifest pathology (Grokhovskaya and Sidorov, 1967). The infected cells contained not many rickettsiae; they could usually be counted (up to several dozens, Fig. 3). As a rule, the rickettsiae were located directly in the cytoplasm and not in vacuoles, nor did they penetrate into the nuclei of argasid tick cells. These rickettsiae exerted no apparent cytotoxic effect on the cells. This rickettsial infection of ticks represents a balanced parasitic system. The motility of *R. sibirica* in tick cells was less intensive than in the cells of warm-blooded animals. The development of *R. sibirica* and *R. conorii* in tick cells was characterized by regular appearance in pairs connected end-to-end, the individual cells separating after some time. This phenomenon could represent conjugation (Kokorin *et al.*, 1969). We never observed such phenomenon in cells of warm-blooded animals.

R. acari-infected haemocytes appeared like those infected with *R. sibirica* or *R. conorii*. The rickettsiae were also located in the cytoplasm. *R. acari* showed no active motility in haemocytes in contrast to their very rapid movements in cells of warm-blooded animals (Kokorin and Chyong dinh Kyet, 1976). *R. acari* was highly toxic for *A. lahorensis* haemocytes. At the early stages of infection the cells in culture were stimulated and moved actively. Then the movement of haemocytes slowed down and necrobiotic processes accompanied by the appearance of numerous dendritic pseudopodia ended by cell death (Fig. 4).

Ticks infected with *C. burnetii* show marked, frequently severe pathology, particularly clearly demonstrable in long-term observations of the infected cells (Sidorov, 1978). Vital observations on infected haemocytes showed *C. burnetii* to be localized only in cytoplasmic vacuoles. Immediately after adding rickettsiae into a haemolymph drop, there started active phagocytosis of rickettsiae by haemocytes. In a few hours numerous clear phagocytic vacuoles containing single rickettsiae were seen in the cytoplasm (Fig. 5). As the rickettsiae multiplied and accumulated in vacuoles, the size of the primary phagocytic vacuoles increased; they sometimes fused and occupied most of the cytoplasm. Because *C. burnetii* is not toxic for tick cells, haemocytes retained their viability for a long time. As a result of fusion of the vacuoles the cell thus acquired the shape of a sphere fiilled with rickettsiae. The infection ended with rupture of the cell membrane and release of the rickettsiae into the environment.

The present results indicate that interaction of rickettsiae with tick cells in vitro reflects the pattern of their interaction with tick cells in vivo. The lack of pathological changes in connective tissue cells infected with *R. sibirica* and *R. conorii* is in complete correlation with transovarial transmission of these rickettsiae in ticks. The active movement of rickettsiae facilitates their

penetration through membrana propria of the organs. The rickettsiae readily penetrate into all organs, in particular, into the ovaries where they infect oocytes. Intrinsically immobile *C. burnetii* cannot overcome m. propria and cause a generalized process in infected argasid ticks. However, when *C. burnetii* persits for a very long time in ticks infected by engorgement of infected blood in the stage of larva (*Ornithodoros papillipes*) or 1st instar nymph (*O. moubata*), ovaries of these ticks may be infected in the process of ontogenesis. As a result of intensive multiplication, the rickettsiae fill the oocytes completely in the same way as in haemocyte infection. If in such instance occasional infected eggs are laid, they inevitably die for the same reason as oocytes (Sidorov, 1978). Consequently, here the data obtained in vivo and in vitro correlate as well. Finally, when *A. lahorensis* ticks were infected with *R. acari*, no transovarial transmission of the rickettsiae to the progeny of the infected ticks was observed: either the rickettsiae did not penetrate into the ovaries or the eggs died after oviposition. Further studies in this respect are required, as transovarial transmission of *R. acari* is known to occur in gamasid mites, hosts and vectors of this rickettsia (Kulagin, 1950).

Our results thus demonstrated characteristic features of the development and interaction of different rickettsial species with cells of *A. lahorensis* ticks. Each rickettsial infection in the ticks could be clearly characterized morphologically and by vital studies of rickettsia-cell interactions. Consequently, the development of different rickettsial species in ticks cannot be considered to proceed similarly.

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For micrographs 1—5 see Plate XIV.