Cultivation of Ultrafilterable Particles of *Coxiella burneti* in the Tick Organism

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Received April 3, 1959

Infection of cells with *Coxiella burneti* results in different morphological changes even when typical coxiellae are not found in the cells (Kordová, 1958). The infected material contains ultrafilterable particles which can pass through the pores of collodion filters with a mean porosity of 40 μμ and can be cultured in different media (Kordová, 1959a, b). ID<sub>50</sub> of these filtrates is over 10<sup>-10</sup>.

In the authors’ opinion, the different morphological changes observed in yolk sac cells (in vitro and in ovo) and in the spleen cells of animals infected with filterable particles of *Coxiella burneti*, before typical coxiellae appear, are associated with the process of proliferation of these micro-organisms. The authors wished to confirm this assumption by injecting filterable particles into ticks, which are known to be vectors and reservoirs of different rickettsiae.

The aim of the experiments was to test the suitability of the tick organism as a medium for cultivation of filterable particles of *Coxiella burneti*, to determine the time required for visible proliferation of the coxiellae to occur in the ticks, to determine whether the appearance of typical coxiellae was preceded by morphological changes in the cells of the infected tick organisms and to ascertain the character of such changes, and to investigate whether coxiellae which “develop” in an arthropod organism are capable of infecting warm-blooded organisms (and chick embryos).

**MATERIALS AND METHODS**

*Inocula (ultrafiltrates).* The ticks were inoculated with ultrafiltrates obtained by filtering a 10<sup>%</sup> suspension of chick embryo yolk sacs infected with *Coxiella burneti* (Nine Mile and Henzerling strains) in 10<sup>-4</sup> dilution through a collodion membrane with a mean porosity of 60 to 40 μμ. (For details on the filtration method v. Kordová, 1959a.)

Non-diluted filtrate (plus 500 units penicillin/ml.) was administered to the ticks by the intracoelomic route in doses of 0.02–0.3 ml., according to the size of the ticks.

*Species of ticks and examination method:* Partly engorged and completely engorged females of the species *Ixodes ricinus* L., *Dermacentor marginatus* Sulz. and *Hae-maphysalis inermis* Bir. were used. The ticks were allowed to feed on healthy guinea pigs which had been examined for complement-fixing antibodies against *Coxiella burneti* before the experiment. Groups of ticks were killed and dissected from the second day after inoculation and smear preparations were made of the individual organs, stained with Macchiavello or Giemsa and subjected to microscopic examination. The organs of the individual groups of ticks were dissected.

*) The collodion ultrafilter was prepared by Dr. J. Šmarda from the Institute of General Biology, Medical Faculty, Brno University, to whom the authors would like to take this opportunity to express their thanks.
out and suspended (pooled organs from ticks dissected on the same day); the suspension was then injected into chick embryo yolk sacs and administered intra-peritoneally to mice and guinea pigs.

Control ticks (non-infected, partly and completely engorged females of the same species) were inoculated by the intra-coelomic route with sterile broth (plus 500 units penicillin/ml.). The method of examining and treating the control ticks was the same as for the infected ticks. The complement-fixation reaction for determining antibodies in the animals was done in the modification of Siegert et al. (1951). The diagnostic antigen was prepared by the method of Craigie (1945) from the Nine Mile strain (Davis & Cox, 1938) and the Florian strain (isolated by Nizhnansky and Gmitter in 1956) of Coxiella burneti.

RESULTS

Cultivation of ultrafilterable particles of Coxiella burneti in the tick organism and detection of Coxiella burneti in the organs

Ninety female ticks (30 of each species) were inoculated with filtrate by the intra-coelomic route. Sterile broth was administered to the control groups. From the second to fifteenth day after inoculation, haemolymph was collected from the ticks, which were afterwards dissected. Smears from the organs were subjected to microscopic examination. Suspensions from pooled organs of ticks dissected on the fourth to fifteenth day after inoculation were injected into chick embryo yolk sacs and administered intraperitoneally to mice and guinea pigs. Thirty days later the animals were bled and antibodies were determined in the serum. Four successive passages with tick material were done in yolk sacs, which were subjected to microscopic examination.

In the cells of the organs (haemolymph, intestines, ovaries, Malpighian bodies, the fat body and hypodermis) of ticks inoculated with filterable particles, clearly discernible morphological changes were found two to three days after inoculation. Clearly defined, homogenous, red, rounded formations like inclusion bodies appeared, most of which were located in the cytoplasm (occasionally in the nucleus). Many cells were swollen, with vacuolated cytoplasm, and stained intensely. On the fourth to sixth day the number and size of the inclusion bodies increased, while their originally homogenous contents had the appearance of an aggregate of coarse or fine-grained particles. From the seventh to eighth day, occasional "microcolonies" of coccoid particles were observed. From the fourteenth to fifteenth day, groups of pleomorphic coxiellae were found in most organs; they were usually most numerous in the fat body (Fig. 1). Similar morphological changes in cells were also observed by Herzberg, Herzberg-Kremmer and Urbach (1950) in smear preparations from the testes of guinea pigs inoculated intratesticularly with Coxiella burneti. Herzberg et al. related these findings to the development and proliferation of the coxiellae in the host cells and drew attention to a certain similarity between these findings and morphological changes in cells infected with psittacosis and venereal lymphogranuloma.

Tab. 1 gives the results of inoculation with suspensions of the pooled organs of experimental ticks in chick embryo yolk sacs, mice and guinea pigs. After four successive passages of the organs of infected ticks in chick embryo yolk sacs, microscopic examination showed massive proliferation of morphologically typical coxiellae. Specific CF antibodies were found in four out of seven pooled sera from inoculated mice (each from four mice), but in only one guinea pig out of fourteen. No Coxiellae were found in control tick organisms. Microscopic examination of preparations from the spleens of guinea pigs inoculated with suspensions of infected ticks showed signs of cell injury.