Intranuclear Growth of *Rickettsia rickettsii*

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The ability of the spotted fever agent, *Rickettsia rickettsii*, to grow not only in the cytoplasm but also in the nucleus of infected cells was first noted by Wolbach (12) in epithelial cells of the gut, hypoderm and salivary gland of infected ticks; it has subsequently been established for all spotted fever group rickettsiae investigated, especially when they were maintained on mammalian tissue cultures. Therefore, this phenomenon has been considered an infallible criterion for the differentiation of *R. rickettsii* from nonpathogenic rickettsia-like organisms in ticks (8) and for the differentiation between the spotted fever and typhus groups of rickettsiae (7).

At the Rocky Mountain Laboratory, intensive studies have been carried out in an effort to clarify the parasite-vector relationships of *R. rickettsii*. During these studies, thousands of either wild-caught or experimentally infected ticks were examined microscopically. Although intranuclear localization of rickettsiae could occasionally be recorded, our consensus is that this phenomenon in tick tissues does not occur as readily and frequently as in mammalian tissue culture cells. Conventional staining procedures, such as those of Giemsa and Macchiavello, did not enable us to determine unequivocally whether the rickettsial organisms were located within the nucleus or in the overlying cytoplasm only.

Recently, in addition to electron microscopy, by which intranuclear growth of *R. rickettsii* in tissue cultures has already been demonstrated (2), fluorescent antibody procedures and the staining method of Giménez (5) were applied to studies of the development of the spotted fever agent. It is the purpose of this note to illustrate intranuclear proliferation of *R. rickettsii*, as demonstrated by these techniques in infected *Dermacentor andersoni* ticks and in tissues of infected chick embryos. Thus, rickettsiae in smears of hemolymph and tissues of experimentally infected ticks were stained with Giménez stain or with specific guinea pig antibody conjugated with fluorescein isothiocyanate (4). For electron microscopy, ticks were dissected in Rhodin-Zetterqvist buffer, fixed in a 1% solution of buffered osmium tetroxide and processed according to Bertram and Bird (3). Sections were viewed on a Zeiss E.M. 9 electron microscope. Fragments of infected yolk-sac tissue were fixed in phosphate-buffered saline (pH 7.4), containing 5% Formalin, and subsequently in 1% buffered osmium tetroxide. Dehydration, embedding, and sectioning were performed as outlined by Anacker et al. (1). Thin sections were treated with the lead stain of Venable and Coggeshall (10) and then were examined with a Siemens Elmiskop I electron microscope.

Figures 1 and 2 illustrate Giménez-stained *R. rickettsii* in the nuclei of a hemocyte and an epithelial cell from infected Malpighian tubules, respectively. Fluorescent antibody-treated smears of the same tick specimens are shown in Fig. 3 and 4. In areas of massive rickettsial growth, fluorescence was so intense that individual organisms could no longer be detected. The morphological outline of *R. rickettsii* can still be observed in the moderately infected nuclei of the two lower cells, but it is no longer apparent in the heavily infected nucleus of the upper left cell (Fig. 4). Figures 5 and 6 show appearance of intranuclear rickettsiae in ultrathin sections of tick and yolk-sac tissues, respectively. In both preparations, the individual rickettsiae are surrounded by clear spaces. This phenomenon, which has been described for other rickettsial agents (6, 9, 11), was consistently observed in our studies. Whether this clearing is due to enzymatic or toxic activities of the rickettsiae, subcellular response of the host, or to some other factors has yet to be determined.

The above-mentioned observations confirmed the intranuclear proliferation of *R. rickettsii* in tick tissues and provided evidence that this phenomenon occurs in both arthropod and vertebrate hosts.

**LITERATURE CITED**


FIG. 1. *R. rickettsii* in the nucleus of a blood cell of infected *Dermacentor andersoni* (Giménez stain, × 3,150).

FIG. 2. Massive rickettsial infection in the nucleus of an epithelial cell from Malpighian tubule of infected *Dermacentor andersoni* (Giménez stain, × 3,150).

FIG. 3. *R. rickettsii* in cytoplasm and nucleus of tick hemocyte. (Fluorescent antibody-staining, × 2,500).

FIG. 4. *R. rickettsii* in epithelial cells of Malpighian tubule of infected *Dermacentor andersoni*. Moderate rickettsial infections in the nuclei and cytoplasm of the two lower cells; heavy infection in the nucleus and cytoplasm of the upper left cell. (Fluorescent antibody-staining, × 2,500).
FIG. 5. Ultrathin section of *R. rickettsii* in nucleus of epithelial cell (Malpighian tubule) of *Dermacentor andersoni* (× 6,100).

FIG. 6. Ultrathin section of intranuclear *R. rickettsii* in chick yolk sac (× 12,900).

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