NOTES

Intranuclear Growth of *Rickettsia canada*, a Member of the Typhus Group

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Intranuclear development of *Rickettsia canada*, a typhus-group rickettsia, in cells of tick tissues is described and illustrated.

The capacity for intranuclear growth is a characteristic of all spotted fever-group rickettsiae and, therefore, is considered an important criterion for taxonomic purposes (Bergey’s Manual, 7th ed., p. 934–957). Recently, a rickettsia was named *R. canada*. In a study to determine the behavior of this organism in laboratory-reared, rickettsiae-free *H. leporispalustris*, *Dermacentor andersoni*, and *D. variabilis*, growth of *R. canada* was noted not only in the cytoplasm but also in nuclei of infected cells. This paper illustrates this phenomenon, as observed by conventional and electron microscopy.

For light microscopy, infected ticks were dissected in Snyder I sucrose-phosphate-glutamate solution (6), and smears prepared from various tissues were stained by the method of Giménez.
For electron microscopy, the ticks were dissected in physiological saline (pH 7.2). Tissues were fixed in 3% glutaraldehyde (pH 7.4) for 1 to 1.5 hr and postfixed for 1 hr in 1% buffered OsO₄ (pH 7.4). They were then soaked in 0.5% uranyl acetate for 1 hr and dehydrated in ethanol. Infiltration was accomplished in a mixture of propylene oxide and Araldite 502 (Ciba). Tissues were embedded in Araldite 502 and sectioned on a Sorvall Porter-Blum MT-1 microtome. Thin sections stained with uranyl acetate and Reynolds's lead citrate (8) were examined with a Perkin-Elmer Hitachi HU-11 E electron microscope.

Intranuclear proliferation of *R. canada* (Fig. 1-3) was detected in adult ticks of all three species, occasionally in hemocytes, but more frequently in cells of hypodermal tissues and gut epithelium. It was also readily observed in tissues of engorged larval *D. andersoni* that acquired infection transovarially from infected female ticks. Appearance of intranuclear *R. canada* in ultrathin sections was similar to that of the spotted fever agent, *R. rickettsii* (2); individual organisms invariably were surrounded by clear spaces, a phenomenon the nature of which remains to be determined.

Ability of rickettsiae to grow in cytoplasm as well as in nuclei of infected cells has not previously been associated with species other than those belonging to the spotted fever group. We have repeatedly noted that *Coxiella burnetii*, the etiological agent of Q fever, is capable of invading cell nuclei when grown by the agar-tissue culture method of Zinsser et al. (9). However, this has not yet been confirmed histologically.

*R. canada* shows a strong serological relationship to the typhus group, but its pattern of infection in the aforementioned species of ticks is strikingly similar to that of spotted fever-group rickettsiae. It produces a generalized infection of all tissues, exhibits intranuclear as well as intracytoplasmic growth, and, in some instances, is passed via eggs to the progeny (1; W. Burgdorfer, unpublished data).

French rickettsiologists (4, 5) have long...
hypothesized that rickettsiae may undergo biological and physiological modifications to the extent that a strain representing one group eventually acquires the characteristics of the other group. Accordingly, strains of *R. conorii* of the spotted fever group, after adaptation to mouse lung, were reported to behave like typhus-group rickettsiae. The organisms became more slender, grew more abundantly, stained less intensively, and ceased growing in cell nuclei. Rabbits inoculated with such modified organisms produced antibodies against typhus-group antigen. Since these observations have not been confirmed by other workers, the French hypothesis is not widely accepted. Indeed, it is doubtful that *R. canadensis* represents a modified strain of either spotted fever or typhus rickettsiae. On the other hand, its peculiar behavior in ticks does suggest that, in nature, rickettsiae exist which biologically behave like those of the spotted fever group but serologically react like typhus-group agents.

**LITERATURE CITED**


