Electron Microscopic Observations of *Coxiella burnetii* in the Guinea Pig

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The ultrastructure of the rickettsial agent of Q fever, *Coxiella burnetii*, has been studied in the yolk sac (R. L. Anacker, K. Fukushi, E. G. Pickens, and D. B. Lackman, J. Bacteriol. **88:** 1130, 1964; M. Rosenberg and N. Kordová, Acta Virol. **4:**52, 1960), in an established tissue culture cell line (M. Rosenberg and N. Kordová, Acta Virol. **6:**176, 1962), and in purified suspensions (M. G. P. Stoker, K. M. Smith, and P. Fiset, J. Gen. Microbiol. **15:**632, 1956), but no studies on the ultrastructure of this rickettsial organism within an infected animal have been reported. In this note, some results of a study of *C. burnetii*-infected guinea pigs are reported.

Four 450-g guinea pigs (Hartley strain) were infected intraperitoneally with *C. burnetii* (phase I), Nine Mile strain, and four control animals were inoculated, as previously described (D. Paretsky, C. M. Downs, and C. W. Salmon, J. Bacteriol. **88:**137, 1964). Animals were sacrificed 84 hr after infection. Impression smears of infected liver and spleen, stained by a modified Machiavello method, were 4+, i.e., too numerous to count.

Samples of tissue from the liver, spleen, and adrenal glands were immediately removed from each of two animals in both the control and infected groups. The tissues were fixed in buffered osmium tetroxide (*p*H 8) for 1 hr at 0 C, and then were dehydrated and embedded in Epon 812 (Shell Chemical Co., San Francisco, Calif.), as described by J. H. Luft (J. Biophys. Biochem. Cytol. **9:**409, 1961). Thin sections were cut on a Porter-Blum MT-1 ultramicrotome, picked up on uncoated copper grids, and stained in a 25% solution of uranyl acetate in absolute methanol for 15 min (J. G. Stempak and R. T. Ward, J. Cell Biol. **22:**697, 1964). Sections were examined with an RCA EMU-3F2 electron microscope at 50 kv.

Examination of the infected liver and splenic tissues consistently revealed numerous large clusters of *C. burnetii* in the cytoplasm, surrounded by a limiting membrane (Fig. 1–3) which was sometimes multi-layered (Fig. 3).

Many rickettsiae were also found free in the cytoplasm and sinusoids (Fig. 1, 2). N. Kordová (Arch. Ges. Virusforsch. **15:**697, 1964) reported light-microscopic observations of *Rickettsia prowazekii* in cytoplasmic vesicles of L cells 2 hr after infection. D. Anderson et al. (J. Bacteriol. **90:**1387, 1965) found that *R. sennetsu* was intravacuolar in kidney cells, whereas other rickettsial species remained free in the cytoplasm. J. Tuomi and C. -H. von Bonsdorff (J. Bacteriol. **92:**1478, 1966) recently reported the occurrence of the tick-borne fever agent (tentatively placed in the family *Rickettsiaceae*) within cytoplasmic vacuoles of neutrophil and eosinophil granulocytes. Rosenberg and Kordová (Acta Virol. **6:**176, 1962) reported one or two *Coxiella*-like particles enclosed in a vesicle in Detroit-6 cells. These particles were oval and possessed a single limiting membrane surrounding a dense amorphous cytoplasm. The vesicle wall appeared as a single- or double-layered structure.

In Fig. 2, the rickettsiae are shown to be enclosed in a limiting membrane within the cytoplasm of a parenchymal cell of the liver. It is evident from this that *C. burnetii* is multiplying in the liver cells. Although liver involvement in Q fever is not considered to be as common as pulmonary involvement, several human cases have been reported in which marked pathological changes were observed in the liver (J. Picchi et al., Ann. Internal Med. **53:**1065, 1960; O. W. Powell, Australasian Ann. Med. **10:**52, 1961).

Within the vacuoles are found various rickettsial forms, including short bacillary and ovoid types (Fig. 1, 3). A cell wall surrounding a cytoplasmic membrane is clearly evident in many of the organisms (Fig. 3), and there is a well-defined internal structural organization (Fig. 3, arrows). The cytoplasm contains large areas of a dense fibrillar structure and lighter areas of lower density material. These forms of *C. burnetii* closely resemble those from yolk sac material reported by Anacker et al. J. Bacteriol. **88:**1130, 1964).

Organisms were also observed in adrenal tissue. It is possible that such adrenal invasion and con-

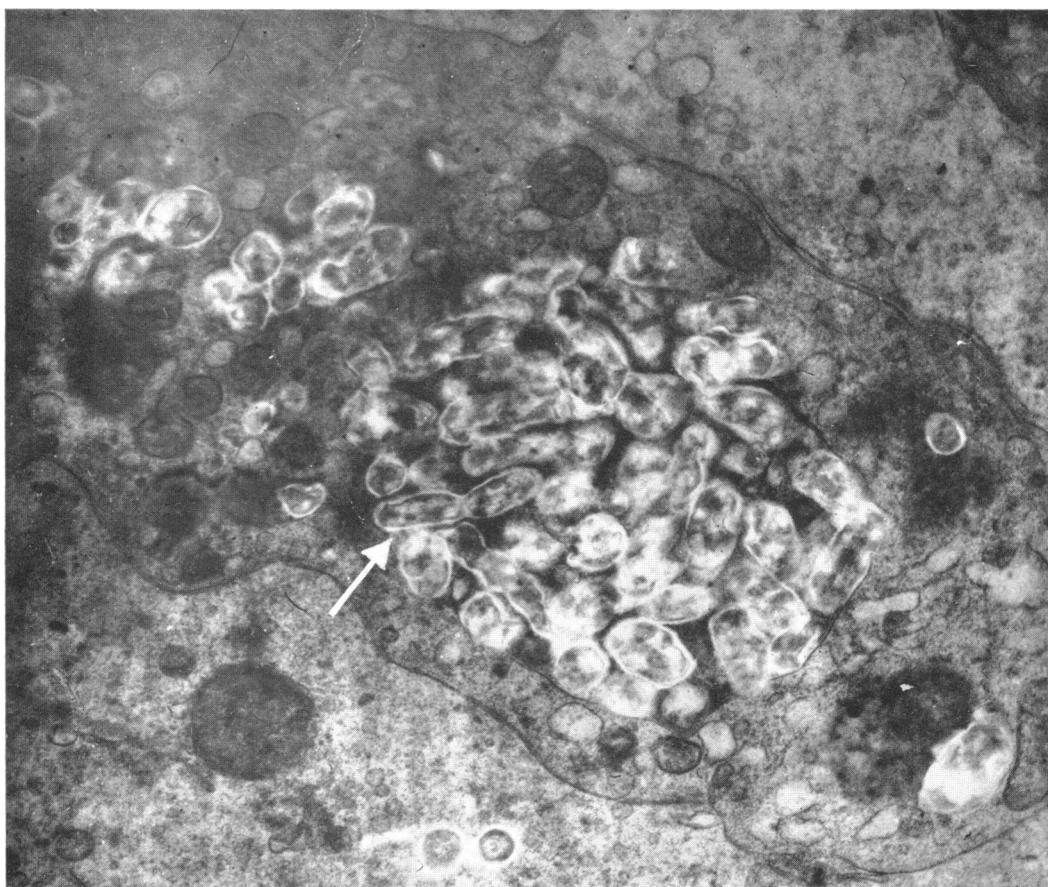FIG. 1. *Coxiella burnetii, both free in the cytoplasm and within a vacuole in guinea pig liver. Arrow indicates C. burnetii undergoing binary fission.* × *17,800.*

comitant hormonal alterations may influence the host biochemical activities reported by Paretsky et al. (J. Bacteriol. **88**:137, 1964), D. Paretsky and J. Stueckemann (Bacteriol. Proc., p. 116, 1966), and L. P. Mallavia and D. Paretsky (J. Bacteriol, **93**:1479, 1967).

While it is generally accepted that rickettsiae multiply by binary fission, it has also been proposed (Rosenberg and Kordová, Acta Virol. **4**: 52, 1960; **6**:176, 1962) that *C. burnetii* possesses a method of reproduction resembling that of the large viruses. In studies of infected Detroit-6 cells, these workers found no evidence of binary fission. In the present study, several examples of binary fission such as that shown in Fig. 1 (arrow) were observed, consistent with the report of Anacker et al.

Despite the multiplicity of organisms in the tissues, little cytopathological change was ob-

served. The cytoplasm and nuclei of the infected cells in all tissues were apparently normal (Fig. 2, 3 inset). The mitochondria were numerous and unchanged (Fig. 2, 3 inset). The only difference noted was the highly vacuolated appearance of the cytoplasm of the infected liver as compared with the uninfected controls (Fig. 2). Increases in total liver lipids have been reported in *Coxiella-* infected guinea pigs (Paretsky et al., J. Bacteriol. **88**:137, 1964), and this vacuolated effect may be due to lipid droplets. It is also possible that it is due to the dilated endoplasmic reticulum, such as was shown in human fatty livers (M. E. C. Thorpe and C. D. Shorey, Australasian Ann. Med. **15**:4, 1966). In contrast, Rosenberg and Kordová (Acta Virol. **6**:176, 1962) demonstrated marked pathological changes in the nuclei, mitochondria, and cytoplasm of *C. burnetii-* infected Detroit-6 cells as early as 48 hr after
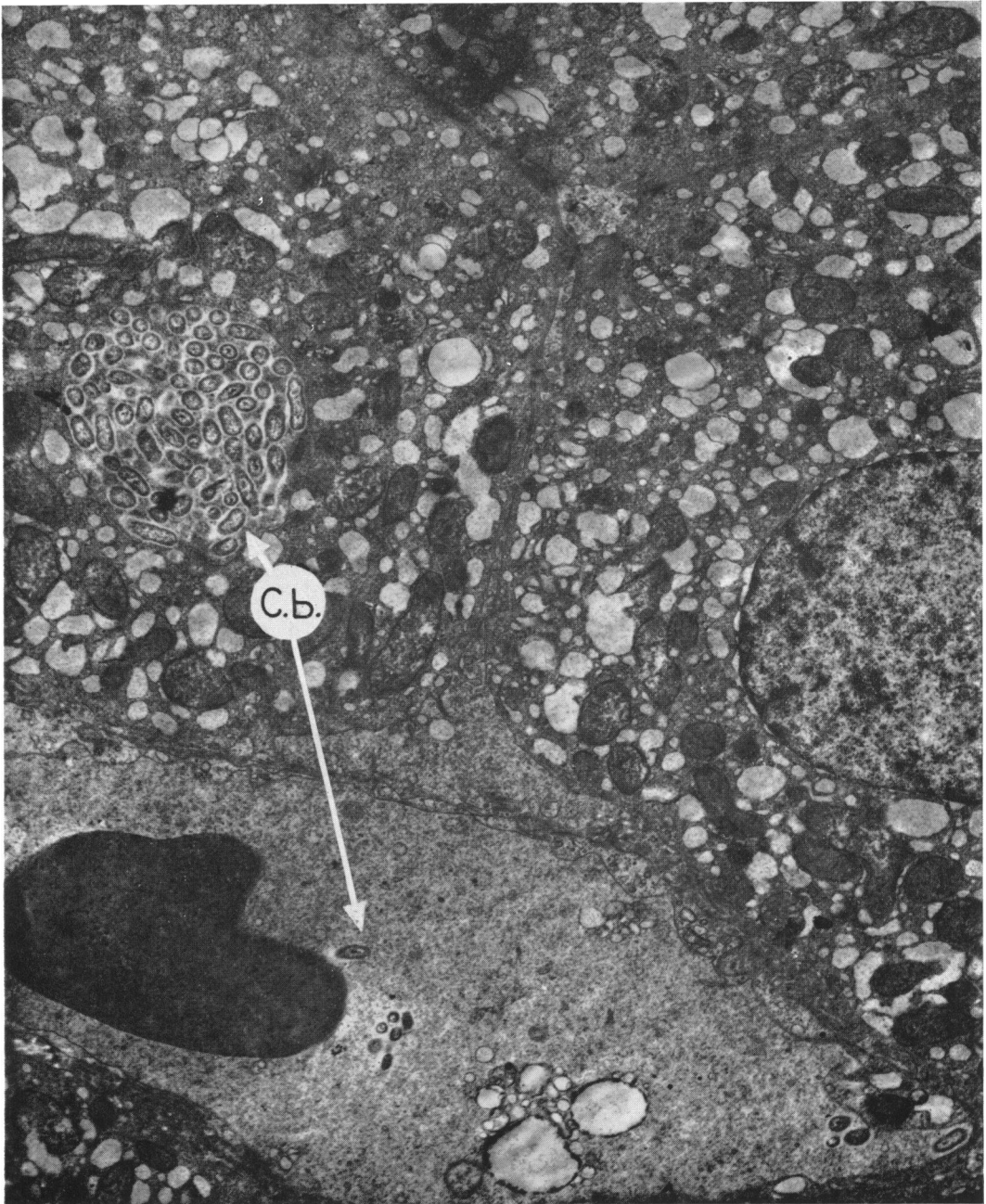
FIG. 2. *Coxiella burnetii (C.b. arrow) within a vacuole in a parenchymal cell and free in a sinusoid. The parenchymal cytoplasm is highly vacuolated.* × *11,000.*
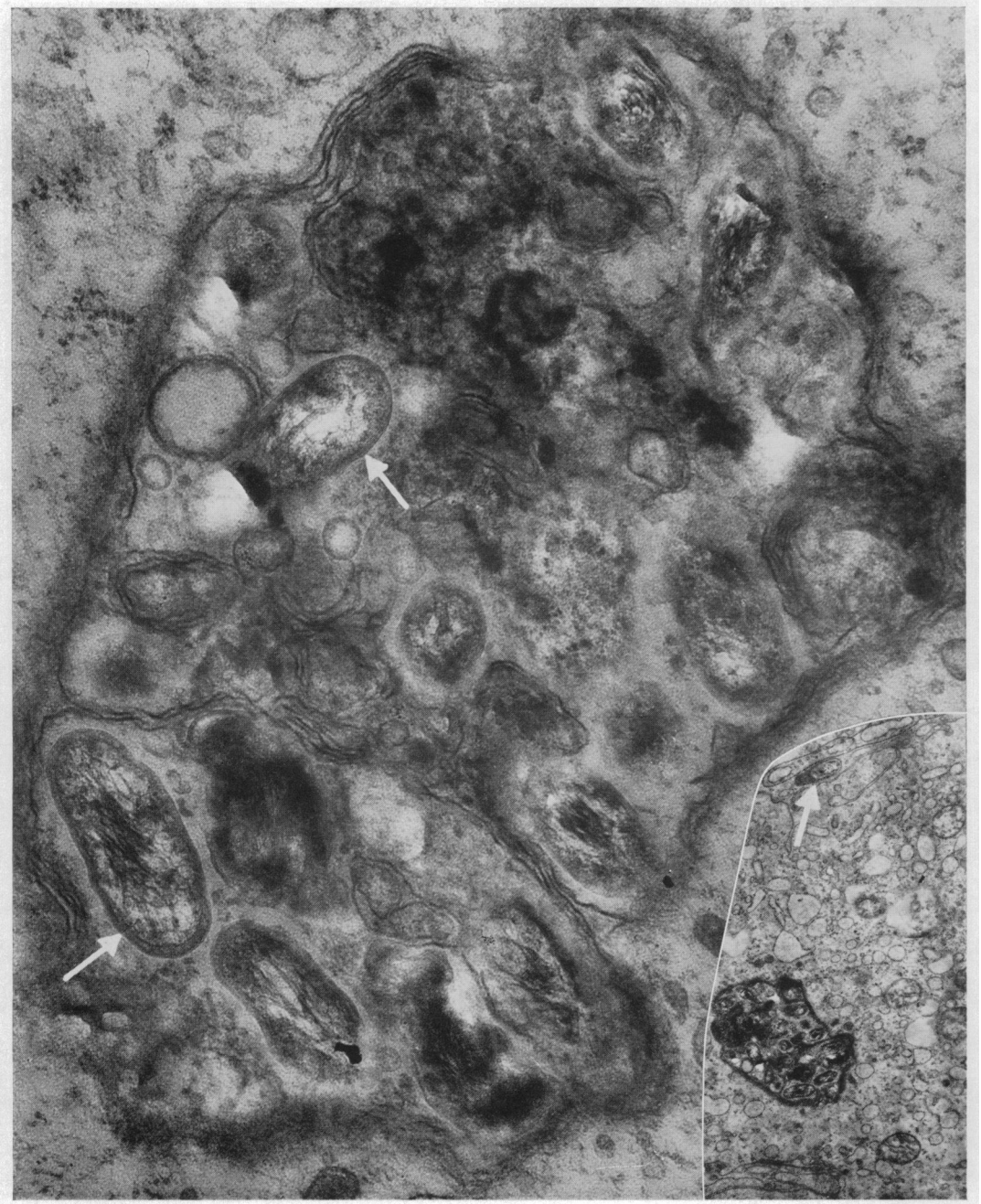
FIG. 3. *Large cytoplasmic vacuoles in the liver containing Coxiella burnetti.* × *78,000. Inset shows the same area at a lower magnification.* × *11,800. Arrow indicates a single organism also apparently enclosed within a vacuole.*

infection. It should be emphasized that the two host systems are different, i.e., one, the intact living animal, and the other, tissue cells in continuous culture. The patterns of rickettsial proliferation may well be determined by the nature of the host system. It is evident that additional electron microscopy of *C. burnetii* and its infected hosts is necessary, and should prove valuable in understanding proliferation of the parasite and its pathogenesis.