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HEARTWATER. THE MORPHOLOGY OF *COWDRIA RUMINANTIUM* AND ITS STAINING CHARACTERISTICS IN THE VERTEBRATE HOST AND *IN VITRO*

L. PROZESKY, Section of Pathology, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

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The morphology of *Cowdria ruminantium* is described and its staining characteristics in the vertebrate host and *in vitro* are summarized. Morphologically, the organisms are characterized in the cytoplasm of endothelial cells, macrophages and reticulo-endothelial cells. Based on the morphology of the internal structure of the organisms, elementary (electron-dense), intermediate and reticulate bodies are identified. Each organism is surrounded by a double membrane and a "capsule" is evident around a few organisms *in vitro*. Usually, only organisms of the same form are found within a particular vacuole, although mixed colonies are described in the *in vitro* studies.

INTRODUCTION

Although the light microscopical morphology and staining characteristics of *Cowdria ruminantium* were described by Cowdry as early as 1926 (Cowdry, 1926), the ultrastructural morphology of the Ball 3 strain of *C. ruminantium* in the choroid plexus of sheep was only reported many years later by Pienaar (1970). Basing his finding on the size of the organisms and morphology of the internal structure, Pienaar (1970) divided the organisms into small, medium, large and very large forms. According to Prozesky, Bezuidenhout & Paterson (1986), the ultrastructure of *C. ruminantium in vitro* was generally similar to that described in the vertebrate host.

STAINING CHARACTERISTICS OF *COWDRIA RUMINANTIUM*

In their description of the light and ultrastructural morphology and staining characteristics of *C. ruminantium* in domestic ruminants, Cowdry (1926) and Pienaar (1970) reported that the organisms stained negatively with the Gram's stain; a clear blue with the Giemsa method, the Löffler's methylene blue method and that of other basic aniline dyes; light red with Unna-Pappenheim's methyl-green pyronin method and red with the Fuchsin method. In autolyzed tissue the organism's affinity for basic dyes was lost before that of the nuclear chromatin (Cowdry, 1926; Burdin, 1962).

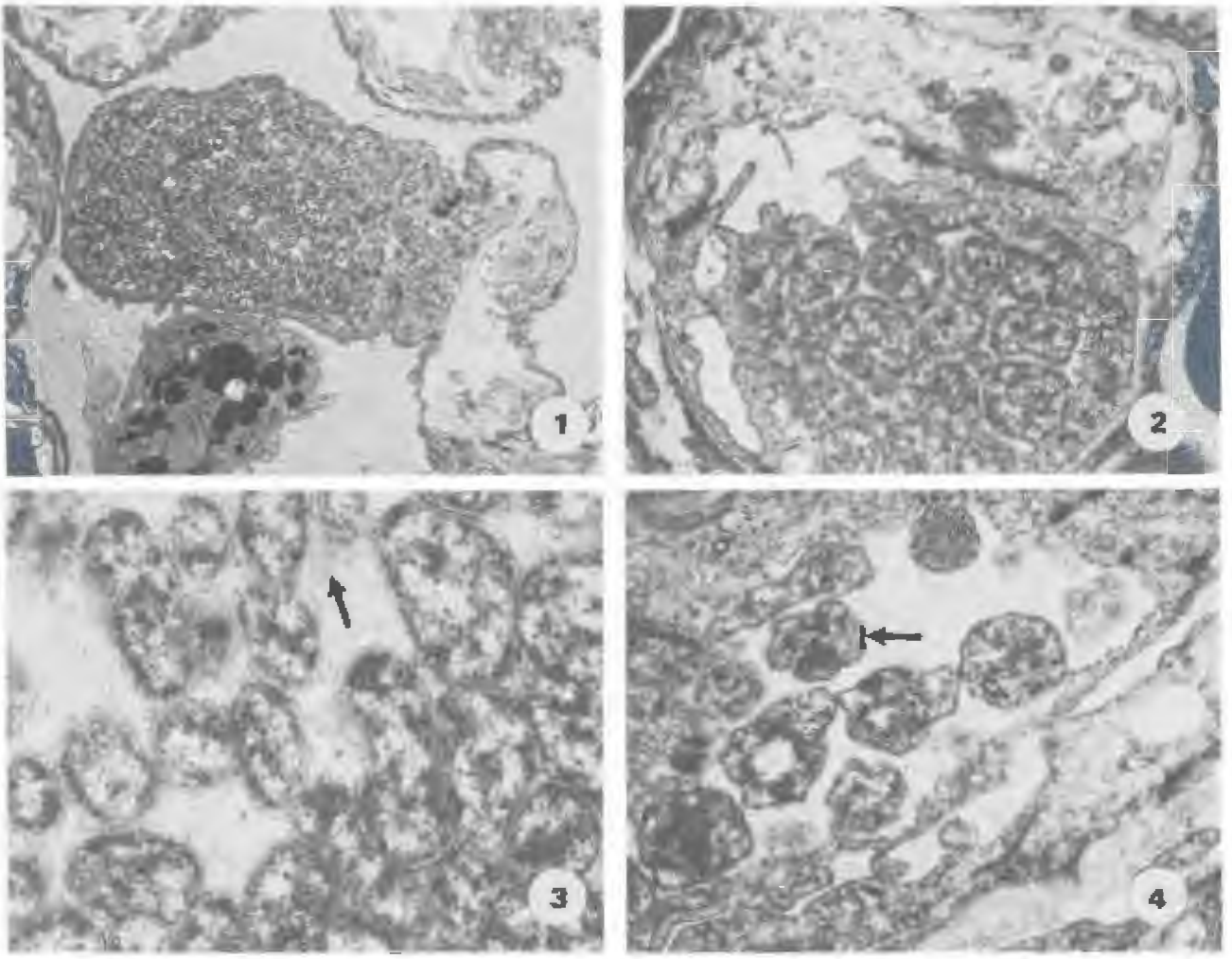

FIG. 1 & 2 A colony of organisms in an alveolar endothelial cell occluding the capillary lumen: ×5000 × 19000
FIG. 3 A fine fibrillar matrix is evident between the organisms (arrow): ×27500
FIG. 4 Each organism is surrounded by 2 membranes (arrow): ×20700

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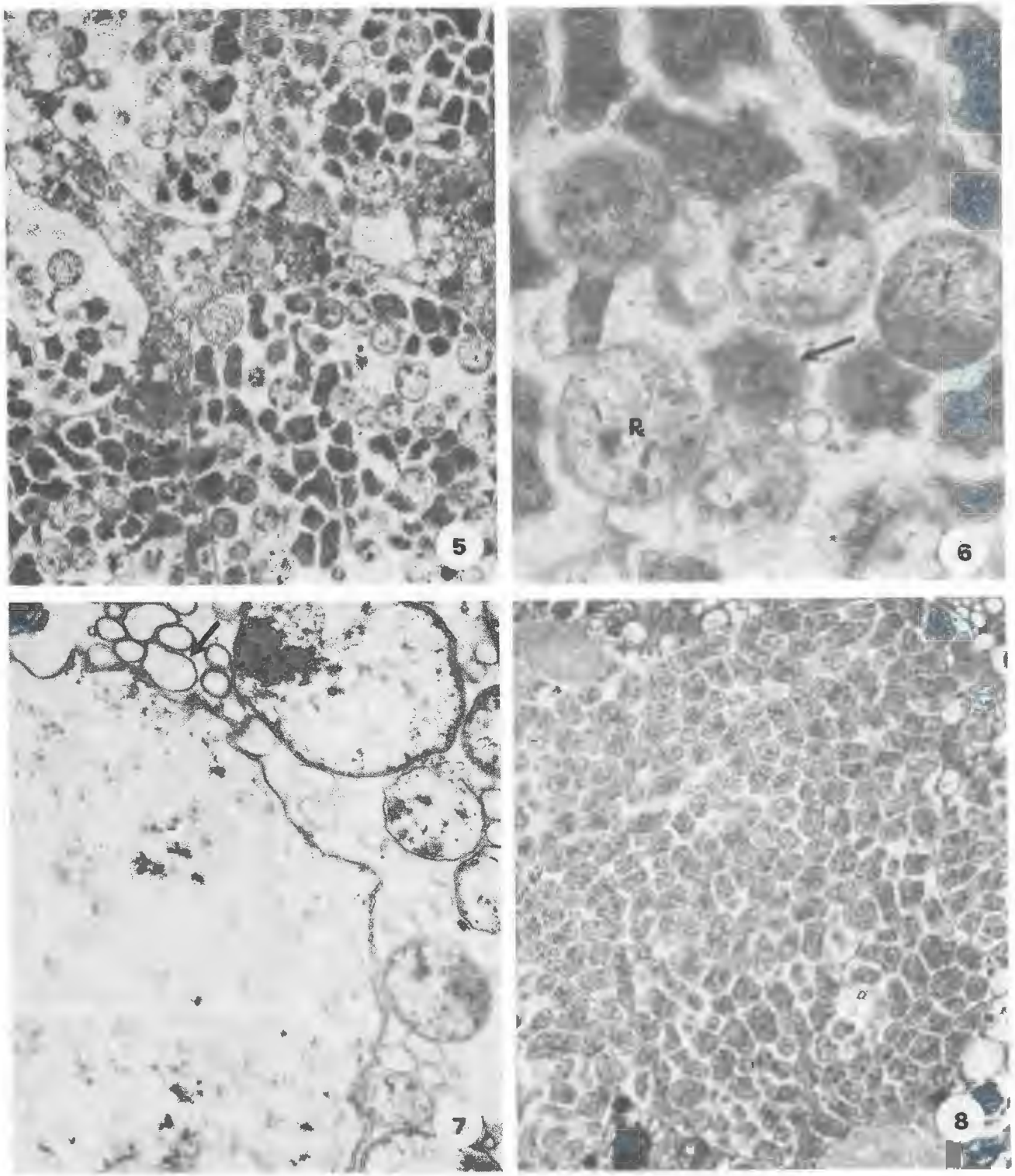

FIGS. 5 & 6 Different forms of organisms, i.e. electron-dense (arrow) and reticulated forms (R) are present within the same colony: ×9300, × 43300
FIG. 7 Vesicular structures (arrow) are visible between reticulated organisms: ×17000
FIG. 8 Small reticulated organisms: ×9000

Pienaar (1970) characterized the morphology and staining properties of heartwater organisms in brain smears prepared from sheep infected with the Ball 3 strain of heartwater and stained by the May-Grünwald-Giemsa method. The following pattern emerged; small organisms stained a reddish purple, medium-sized organisms were dark-blue to purplish-blue, large organisms had a pale blue colour and giant (very large organisms) stained pale or purplish-blue. A sharp delineation of the staining reaction between differently sized organisms was not always possible. In brain smears stained by the Gimenéz method (Gimenéz, 1964), the small organisms stained red to magenta and the larger organisms a lighter red, whilst a bluish internal structure was visible (L. Prozesky, unpublished data, 1986).

MORPHOLOGY OF *C. RUMINANTIUM* IN THE VERTEBRATE HOST

Light microscopy

The morphology of *C. ruminantium* in the endothelial cells of experimentally infected animals, originally described as coccoid, very uniform bodies 0,2–0,5 μm in

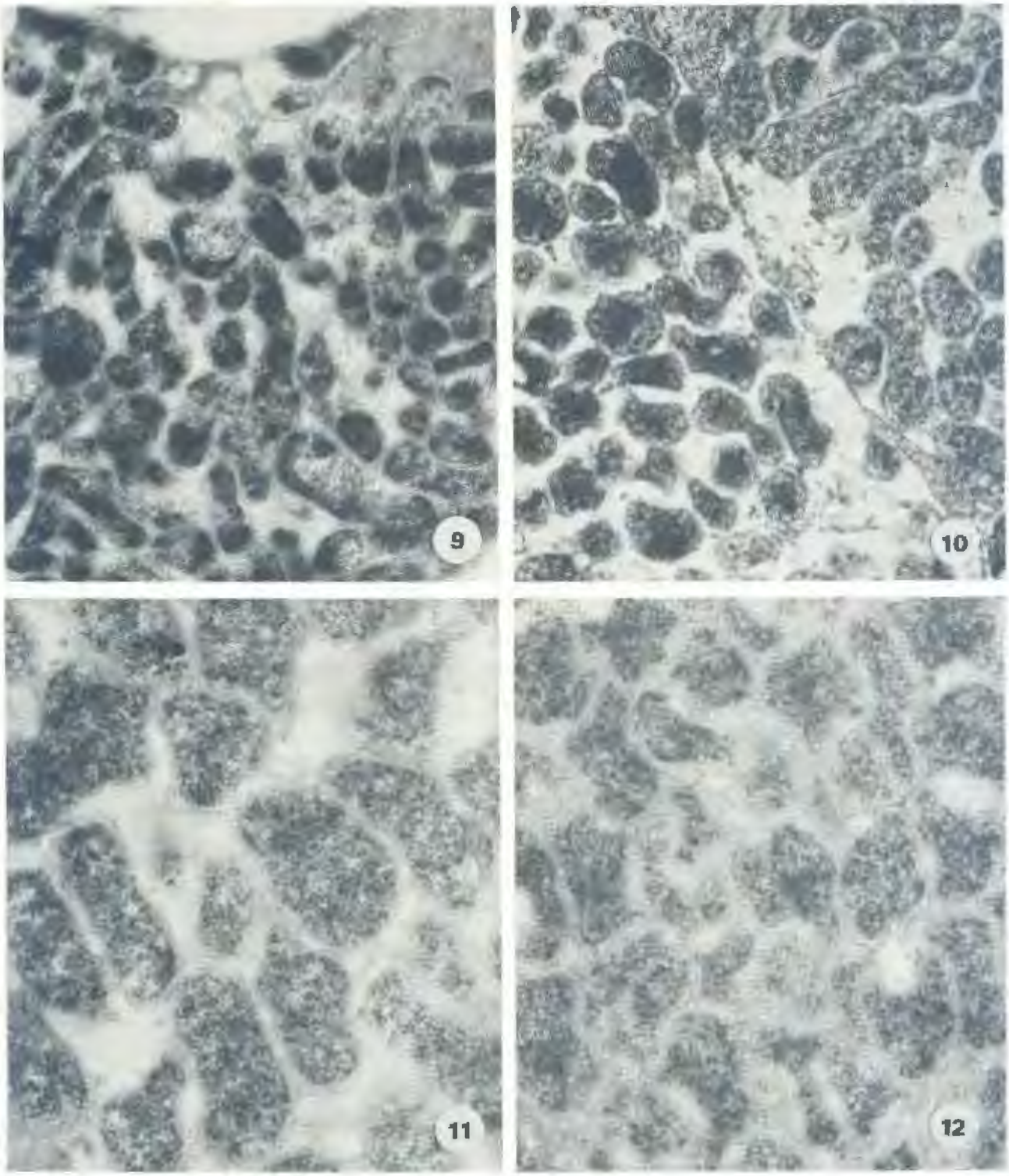

FIG. 9 Electron-dense organisms: ×27000
FIG. 10 Small reticulated and electron-dense organisms: ×18750
FIG. 11 & 12 The membranes surrounding the organisms are unclear: ×37500 × 42000

diameter (Cowdry, 1926), was later confirmed by various workers (Steck, 1928; Alexander, 1931; Henning, 1956; Pienaar, 1970). Both Cowdry (1926) and Pienaar (1970) emphasized the extreme pleomorphism of the organisms and colonies. In colonies containing mainly small organisms, a faintly staining matrix was visible between the organisms stained by the May-Grünwald-Giemsa method (Pienaar, 1970).

Developmental stages of *C. ruminantium* were described in Giemsa-stained, impression smears and in toluidine blue pyronin-stained sections of mesenteric lymph nodes in sheep infected with the Ball 3 strain, and a field isolate of heartwater (Du Plessis, 1970). Purplish-grey organisms resembling poorly outlined initial bodies were detected in cytoplasmic vacuoles in macrophages and reticulum cells. These organisms apparently divided and formed dark-purple granular bodies. Multiplication by binary fission of the granular bodies resulted in the formation of structures which were indistinguishable from the small organisms described in endothelial cells by Cowdry (1926).

Transmission electron microscopy

The ultrastructural morphology of *C. ruminantium* was studied in endothelial cells in the choroid plexus of

sheep infected with the Ball 3 strain of heartwater (Pienaar, 1970) and in the lungs of mice inoculated with the Welgevonden strain (Prozesky & Du Plessis, 1985). Morphologically the 2 strains were indistinguishable, although the very large (giant) forms observed in the sheep were not seen in the mice. The sizes of the organisms ranged from 0,49 μm–2,7 μm in diameter in sheep and *c.* 0,4–1,04 μm in mice. Exact measurements were difficult because of the pleomorphism of the organisms.

Colonies of organisms were located in vacuoles enclosed by a thin membrane in the cytoplasm of endothelial cells (Fig. 1 & 2) (Pienaar, 1970). As a rule, the small and very large organisms were widely spaced within the vacuole, whereas the large organisms were packed closer together. Usually, only organisms of the same morphological form were found within a particular vacuole, although in the sheep a few colonies were encountered with organisms of more than one form (mixed colonies). An extreme variation in size of individual organisms was often noted in colonies containing mostly very large organisms.

In colonies with small organisms, a fine fibrillar matrix in which fine granules were suspended was evident between the organisms (Fig. 3). The pleomorphic small to medium-sized organisms were coccoid, bacillary and cocco-bacillary in shape. The larger forms were either coccoid or polygonal, while the giant forms were highly pleomorphic.

Each organism was enveloped by 2 membranes and occasionally an additional double membrane was observed (Fig. 4). The inner structure of the organisms consisted of electron-dense and electron-pale areas. Electron-dense areas comprised a ground substance and many densely packed small granules, whereas an indistinct delicate fibrillar network was evident in the electron-pale areas. In small organisms, the electron-dense areas were concentrated in one area of the inner structure (centrally or eccentrically). The electron-dense areas occupied the greater part of the inner structure of the intermediate-sized organisms and stained less intensely than the small organisms. In the large organisms the electron-dense and electron-pale areas were evenly distributed throughout the inner structure. The well-defined, electron-dense areas were scattered throughout the inner structure of the very large (giant) organisms.

The ultrastructural morphology of the Kümm strain in various cells (i.e. mouse peritoneal macrophages, Kupffer's cells, myocardial capillary etdothelial cells, splenic histiocytes) of infected mice and the Ball 3 strain in lymph node histiocytes of sheep and cattle were compared (Du Plessis, 1975). He suggested that electron-dense, finely granular bodies which develop from an infective organism increase in size to form larger dense bodies. These bodies presumably undergo cleavage, giving rise to fragmented dense bodies. After further subdivision and organization, these bodies appear to form organisms with a double unit membrane, which developed further to form mature organisms.

MORPHOLOGY OF *C. RUMINATIUM IN VITRO*

Notwithstanding some morphological differences, the ultrastructure of the Ball 3 strain of heartwater in tissue culture cells concurred to a large extent with that in previous *in vivo* studies (Prozesky *et al.*, 1986).

Based on the morphology of the internal structure, elementary bodies (electron-dense organisms), reticulate bodies (medium electron-dense organisms) and a range of organisms between these 2 forms (intermediate organisms) were identified (Prozesky *et al.*, 1986). Contrary to the findings in the vertebrate host, different forms of organisms were occasionally identified within a vacuole (mixed colonies) (Fig. 5 & 6) (Prozesky *et al.*, 1986).

As was demonstrated in the *in vivo* studies (Pienaar, 1970; Prozesky & Du Plessis, 1985) most organisms in the cultured endothelial cells were surrounded by 2 membranes. A few reticulate bodies were enclosed by a "capsule" (an electron-dense layer surrounded by a well-demarcated, fine, fibrillar layer). Vesicular structures, 0,1–0,2 μm in diameter, were evident between reticulated organisms in some colonies (Fig. 7).

Preliminary *in vitro* studies with the Welgevonden strain (Prozesky & Bezuidenhout, unpublished data, 1986) revealed that small reticulated organisms predominate, although electron-dense organisms were also detected (Fig. 8–10). It was often difficult to distinguish between these 2 forms of organisms on morphological grounds alone. The 2 membranes surrounding the organisms could not always be identified, and often the membranes appeared thickened (Fig. 11 & 12).

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