

HEARTWATER, THE DEVELOPMENT AND LIFE CYCLE OF *COWDRIA RUMINANTIIUM* IN THE VERTEBRATE HOST, TICKS AND CULTURED ENDOTHELIAL CELLS

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ABSTRACT

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Various aspects of the development and life cycle of *Cowdria ruminantium* are discussed. *C. ruminantium* is transmitted transstadially by certain *Amblyomma* species. Apparently organisms initially develop in the gut epithelial cells of ticks and subsequent stages of *C. ruminantium* invade and develop in the salivary gland acini cells of the vector. Stages at which transmission to the final host are attained appear to be coordinated with the feeding cycle of the ticks and the vertebrate host is infected via salivary glands of the tick.

In the vertebrate host, ticks and cultured endothelial cells, different morphological forms of *C. ruminantium* (electron-dense and reticulated forms) are found. Organisms enter cells through a process resembling phagocytosis and reticulated forms of the organisms appear to be the main vegetative stage. In the vertebrate host, organisms proliferate in vascular endothelial cells, neutrophils, macrophages and reticulo-endothelial cells.

INTRODUCTION

Different morphological forms of *Cowdria ruminantium* were identified in endothelial cells of blood vessels (Pienaar, 1970; Prozesky & Du Plessis, 1985), cultured endothelial cells (Prozesky, Bezuidenhout & Paterson, 1986), and in midgut epithelial cells and salivary gland acini cells of *Amblyomma hebraeum* nymphae (Kocan, Bezuidenhout & Hart, 1987).

In *A. hebraeum* nymphae organisms develop initially in the midgut epithelial cells and subsequent stages of the organisms invade and develop in salivary gland acini cells (Kocan *et al.*, 1987).

Du Plessis (1970) demonstrated heartwater organisms in lymph nodes of sheep *c.* 3 days before parasites could be detected in brain capillary endothelial cells of capillaries in the brain. He suggested that *C. ruminantium* initially replicate in the lymph nodes after which the organisms are released into the blood stream and endothelial cells are parasitized.

Information regarding the development and life cycle of *C. ruminantium* in both the vertebrate and invertebrate host is limited. The purpose of this paper is to summarize the available information and to identify aspects on the development and life cycle of *C. ruminantium* that should be further investigated.

Infection of cells by C. ruminantium

Adsorption of *C. ruminantium* to cultured endothelial cells was studied electron microscopically (L. Prozesky, unpublished data, 1986). Organisms in contact with the cell membranes were taken up through a process resembling phagocytosis. The organisms were enclosed in a vacuole surrounded by a membrane derived from the cell membrane (Fig. 1–3). Occasionally small vesicles were present in the vacuole containing the organisms. Sometimes single or small groups of organisms were surrounded by an electron-dense membrane *c.* 40 nm in diameter. The origin of the electron-dense membrane remains obscure.

A preliminary *in vitro* electron microscopical study indicates that intracellular spread of organisms takes place. In the process organisms are released from a colony into the surrounding cytoplasm of the host cell. The released organisms are surrounded by a membrane which presumably develops from the membrane enclosing the

original colony of organisms (L. Prozesky, unpublished data, 1986).

Different forms of C. ruminantium

Small, medium-sized and large forms of *C. ruminantium* were identified in the vertebrate host (Pienaar, 1970; Prozesky & Du Plessis, 1985) and electron-dense and reticulated forms of heartwater were detected in ticks (Kocan *et al.*, 1987) and in cultured endothelial cells (Prozesky *et al.*, 1986). Although organisms within a particular vacuole were usually of a specific form, mixed colonies were identified in both cultured endothelial cells infected with Ball 3 or the Welgevonden strain of heartwater (Prozesky, 1987). The factors responsible for the formation of the mixed colonies are not known. They may be a consequence of unfavourable intracellular conditions or the lack of an immune response in cultured endothelial cells. Mixed colonies were not detected in the vertebrate host. Occasionally in cultured endothelial cells infected with either the Ball 3 or Welgevonden strains pleomorphic, and, occasionally, very large (giant) organisms up to *c.* 4 μm in diameter were observed (Prozesky *et al.*, 1986; Prozesky, 1987).

The role of either the electron-dense or reticulated forms in the life cycle of *C. ruminantium* is unclear mainly because of the inability to separate the 2 forms.

Multiplication of heartwater organisms

In the vertebrate host (Pienaar, 1970; Prozesky & Du Plessis, 1985), the tick (Kocan *et al.*, 1987), and in cultured endothelial cells (Prozesky *et al.*, 1986), organisms replicate mainly by binary fission and it appears that the reticulated forms are the predominant vegetative form (Fig. 4). Organisms can also replicate by endospore formation (Pienaar, 1970). Vesicular structures which apparently bud from large reticulate organisms were seen in cultured endothelial cells (Fig. 5 & 6) and may represent another method of replication (Prozesky *et al.*, 1986; Prozesky, 1987). The origin and infectivity of these structures should be further investigated.

Binary fission of organisms was not detected in many heartwater colonies in endothelial cells of the vertebrate host nor in cultured endothelial cells (Prozesky, unpublished data, 1986). A possible explanation is that organisms proliferate very rapidly during the early stages of colony formation and they later divide at a much slower rate or may even stop dividing.

Du Plessis (1975) postulated that the developmental forms of *C. ruminantium* in reticulo-endothelial cells were electron-dense structures (initial bodies) which, after further subdivision and organization, gave rise to colonies of organisms. This primary phase of replication

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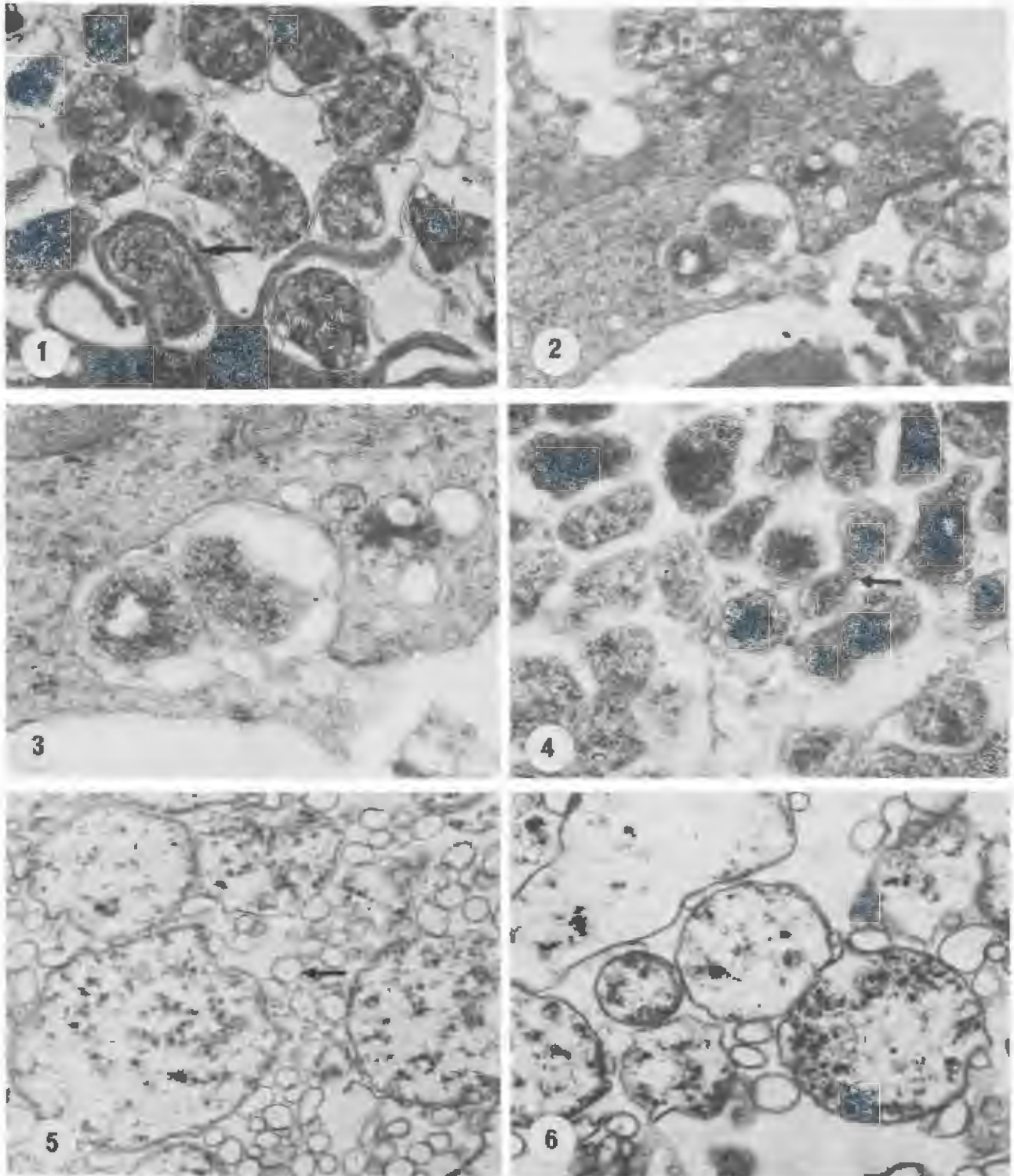


FIG. 1-3 Organisms are engulfed by cultured endothelial cells through a process resembling phagocytosis and enclosed by a membrane derived from the cell membrane; cell process (arrow): $\times 22\ 400$; $\times 22\ 000$; $\times 40\ 500$

FIG. 4 An organism apparently undergoing binary fission (arrow): $\times 28\ 000$

FIG. 5 & 6 Vesicular structures (arrow) which apparently bud from reticulated organisms are evident between the organisms: $\times 15\ 000$; $\times 15\ 000$

(organization of initial bodies) was compatible with the developmental cycle described for the Küm m strain of heartwater in the peritoneal macrophages of mice.

Release of heartwater organisms from the host cell

In vitro and *in vivo* studies revealed that heartwater organisms are released following rupture of parasitized endothelial cells (Fig. 7 & 8) (Pienaar, 1970; L. Prozesky, unpublished data, 1986).

C. ruminantium in blood fractions and lymph nodes

The presence of heartwater organisms in various blood fractions have been studied by various workers with conflicting results (Neitz, Viljoen, Bezuidenhout, Oberem, Visser & Vermeulen, 1986). Single free organisms were detected electron microscopically in the blood of heartwater-infected animals (Pienaar, 1970; Stewart & Howell, 1981). By means of an enzyme-linked immunosorbent assay method Neitz *et al.* (1986) demonstrated

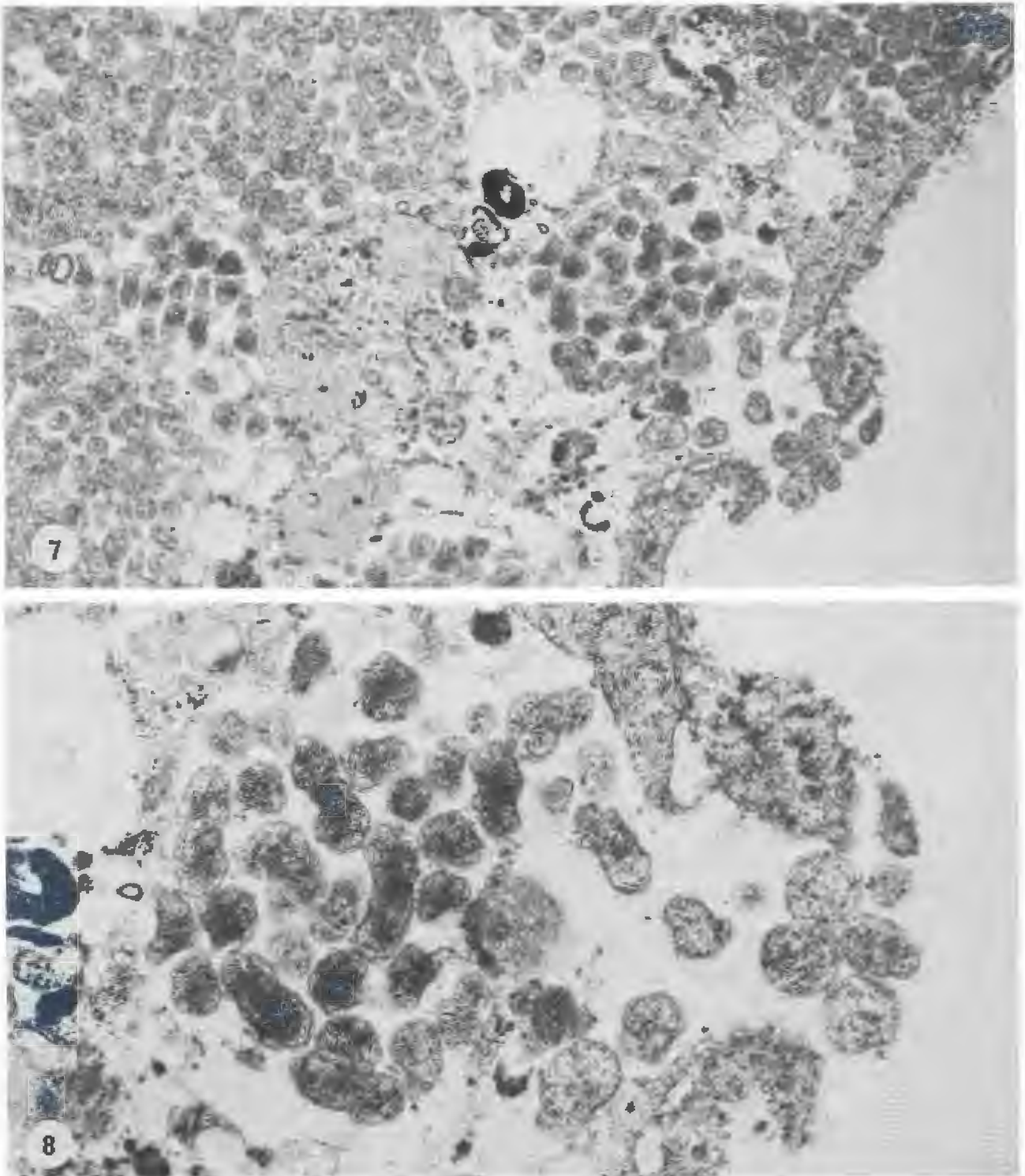


FIG. 7 & 8 Organisms are released from a ruptured endothelial cell: $\times 11\ 200$; $\times 24\ 000$

that *C. ruminantium* antigen was associated with the plasma, serum, red blood cells and leucocyte fractions of blood from heartwater-infected sheep. They deduced from these results that after heartwater organisms have entered the blood stream they are initially detectable in the plasma and serum. The red blood cells are then invaded, followed by the leucocytes. Invasion of the red blood cells and leucocytes co-incided with a decrease of organisms in the serum and plasma.

Jackson & Neitz (1932) observed heartwater colonies in leucocytes of heartwater-infected animals and Du

Plessis (1975) described the morphology of the Kümmer strain of *C. ruminantium* in mice peritoneal macrophages. Heartwater organisms were found in leucocytes of infected goats and 4 strains of *C. ruminantium* were cultured in neutrophils (Logan, Quintero, Whyard & Mebus, 1985).

C. ruminantium was detected in lymph nodes of sheep c. 1–3 days prior to their appearance in endothelial cells of capillaries in the brain (Du Plessis, 1970). He suggested that the organisms initially replicate in reticulo-endothelial cells in lymph nodes and are then released

into the efferent lymph stream and eventually into the blood stream where endothelial cells are parasitized.

C. ruminantium in ticks

Both electron-dense and reticulated forms of *C. ruminantium* were identified in midgut epithelial cells and salivary gland acini cells of *A. hebraeum* nymphae infected as larvae (Kocan *et al.*, 1987). These workers suggested that organisms initially developed in the gut epithelial cells and subsequent stages invade and develop in the salivary glands. This is followed by transsalivary transmission to the vertebrate host. It was also proposed that the development of the transmitting stages of the organisms (the stages released from the gut epithelial cells which subsequently invade the salivary gland acini cells) were consonant with the feeding cycle of the tick (Kocan *et al.*, 1987).

Infective tick material is not confined to the salivary glands, but also includes homogenates of hypodermal tissue, haemocytes and rectal ampules of prefed adult *A. hebraeum* (Du Plessis, 1985; J. D. Bezuidenhout, personal communication, 1986). The role that organisms in these tissues may play in the life cycle of *Cowdria* is unknown.

Proposed life cycle

Considering the information as outlined in this paper it is clear that in many respects adequate information is not available to finalize the life cycle of *C. ruminantium*. Nonetheless, the following preliminary life cycle can be suggested:

C. ruminantium is transmitted transstadially by certain *Amblyomma* species and transovarially only occurs infrequently (Bezuidenhout & Jacobsz, 1986). It would appear that organisms initially develop in the gut epithelial cells and subsequent stages invade and develop in the salivary gland acini cells of the vector. The development of the transmitting stages of the organisms seems to be coordinated with the feeding cycle of the ticks. The vertebrate host is infected transsalivary (Kocan *et al.*, 1987).

In the vertebrate host the spread of organisms from the site of infection to the rest of the body is poorly understood. Du Plessis (1970; 1975) proposed that following infection, initial development of organisms appears to be mainly, but not exclusively, confined to reticulo-endothelial cells. Apparently the parasitized reticulo-endothelial cells eventually rupture and the organisms are released into the general circulation to invade endothelial cells (Du Plessis, 1975). Depending on the host, organisms appear to have a predilection for endothelial cells in certain organs. In ruminants the highest concentration of organisms are found in the brain, followed by the kidneys, whereas in mice infected with the Welgevonden strain the highest concentration of organisms is in the lungs (Prozesky & Du Plessis, 1985).

The role that leucocytes play in the life cycle of *C. ruminantium* is poorly understood. It is suggested that in naturally infected animals infective organisms are drained from the site of infection by the afferent lymphatics (Du Plessis, 1975) or phagocytosed by leucocytes. Parasitized leucocytes are either drained by the

afferent lymphatics to the regional lymph node or directly into the general circulation. After multiplication in the leucocytes, organisms are released into the general circulation and parasitize endothelial cells.

Infected animals serve as a source of infection for ticks. Alexander (1931) reported that blood from sheep was infective 24 h before the onset of the febrile response and remained infective for up to 60 days. According to Oberem & Bezuidenhout (1987) certain animals such as the crowned guinea fowl (*Numida meleagris*) and leopard tortoise (*Geochelone pardalis*) can serve as subclinical carriers of *C. ruminantium* and act as a source of organisms for ticks.

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