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THE PATHOLOGY OF HEARTWATER. II. A STUDY OF THE LUNG LESIONS IN SHEEP AND GOATS INFECTED WITH THE BALL$_3$ STRAIN OF *COWDRIA RUMINANTIUM*

L. PROZESKY[1] and J. L. DU PLESSIS[2]

ABSTRACT

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Lung lesions in sheep and goats infected with the Ball$_3$ strain of *Cowdria ruminantium* corresponded with those reported in mice infected with the Welgevonden strain of *Cowdria ruminantium*. Ultrastructural changes in the alveolar endothelial and epithelial cells are described and the pathogenesis of the lung oedema is briefly discussed.

INTRODUCTION

Steck (1928), Alexander (1931) and Henning (1956) have described the gross and light microscopical lesions in domestic ruminants with heartwater (HW). They considered lung oedema a constant lesion in most of the animals that succumbed to the disease. Subsequent to these reports information related to the pulmonary lesions in HW infected animals was limited. Prozesky & Du Plessis (1985) have described the pulmonary lesions in mice infected with the Welgevonden strain of *Cowdria ruminantium*.

In this study the pulmonary lesions in sheep and goats infected with the Ball$_3$ strain of *C. ruminantium* are described, with emphasis on the ultrastructural changes. Possible mechanisms in the development of the lung oedema are considered, and the lesions are compared with those in mice.

MATERIALS AND METHODS

Two adult Merino sheep and 2 adult Angora goats were intravenously inoculated with 5 mℓ of blood infected with the Ball$_3$ strain of *C. ruminantium*, issued as a vaccine by the Veterinary Research Institute, Onderstepoort. The animals were stabled, observed daily, and temperatures were recorded every morning. After the peak of the temperature reaction was reached and the animals showed clinical signs of HW, they were killed, necropsied and specimens were collected as outlined below. An uninfected sheep and goat were necropsied and specimens were collected to serve as controls.

Light microscopy

Lung specimens were collected in 10 % buffered formalin, processed routinely, and tissue sections were stained with haematoxylin and eosin.

Electron microscopy

Specimens were collected from the diaphragmatic lobes at various sites. Cubes (0,5–1 mm) were cut and fixed in 2,5 % sodium cacodylate-buffered glutaraldehyde (pH 7,3–7,4) at 4 °C for 24 hours. Selected blocks were post-fixed in 2 % osmium tetroxide for 1 hour. Specimens were dehydrated in a graded ethanol series (50–100 %), passed through propylene oxide as the intermediate solvent, and embedded in Polaron* 812.

Thick (1–2 μm) sections were cut for tissue orientation and stained with toluidine blue. Thin sections from selected tissue blocks were stained for 10 minutes each in a saturated aqueous solution of uranyl acetate and Reynold's lead citrate at room temperature (Kay, 1965).

Brain smears

Brain smears were prepared from the hippocampus of each animal according to the method described by Purchase (1945), and stained with a 10 % Giemsa solution for 45 minutes.

RESULTS

Gross pathology

Respiratory tract and heart: A prominent lung oedema, characterized by dilatation of the interlobular septa and the presence of froth in the trachea and bronchi, was evident in 2 sheep and 1 goat. This was accompanied by a hydrothorax and hydropericardium, the fluid coagulating on exposure to air. Atelectasis of the ventral lung borders was attributed to the hydrothorax. Other lesions included an oedema of the mediastinum and associated lymph nodes. In the remaining goat, a mild lung oedema and hydropericardium were the only noteworthy lesions.

Other organs: A mild to moderate splenomegaly was present in all the animals, whereas a mild hepatomegaly and swelling of the kidneys were evident in the 3 animals with extensive lung oedema.

Microscopical pathology

The microscopical examination was confined to the lungs. Lesions are described for the group as a whole, although they were more pronounced in the sheep and goat with severe gross lesions.

Large areas of the lungs were consolidated, and some of the alveolar spaces contained a faintly staining eosinophilic fluid, fibrin and red blood cells, some of which were phagocytosed by alveolar macrophages. The interstitial tissue was oedematous and contained fibrin, red blood cells and scattered groups of mononuclear cells. Other changes included an intravascular increase in monocytes and neutrophils and the presence of single HW colonies in alveolar endothelial cells.

Brain smears

In all the animals HW organisms were present in the capillary endothelial cells.

Electron microscopy

Most of the alveolar endothelial cells appeared unaffected (Fig. 1), although single endothelial cells were swollen and there was a decrease in cytoplasmic electron density. In these cells, the mitochondria were pyknotic or swollen with loss of cristae. The mitochondrial matrix was decreased in density and occasionally contained electron-lucent foci or whorled membranes. The endoplasmic reticulum was often dilated, and intracytoplasmic, dark, dense bodies and membrane-bound vacuoles (*c.* 0,3–2 μm), which were either empty or contained a

[1] Section of Pathology, Veterinary Research Institute, Onderstepoort, 0110

[2] Section of Laboratory Animals and Poultry Diseases, Veterinary Research Institute, Onderstepoort, 0110

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* Polarbed, Micro Structure (Pty) Ltd

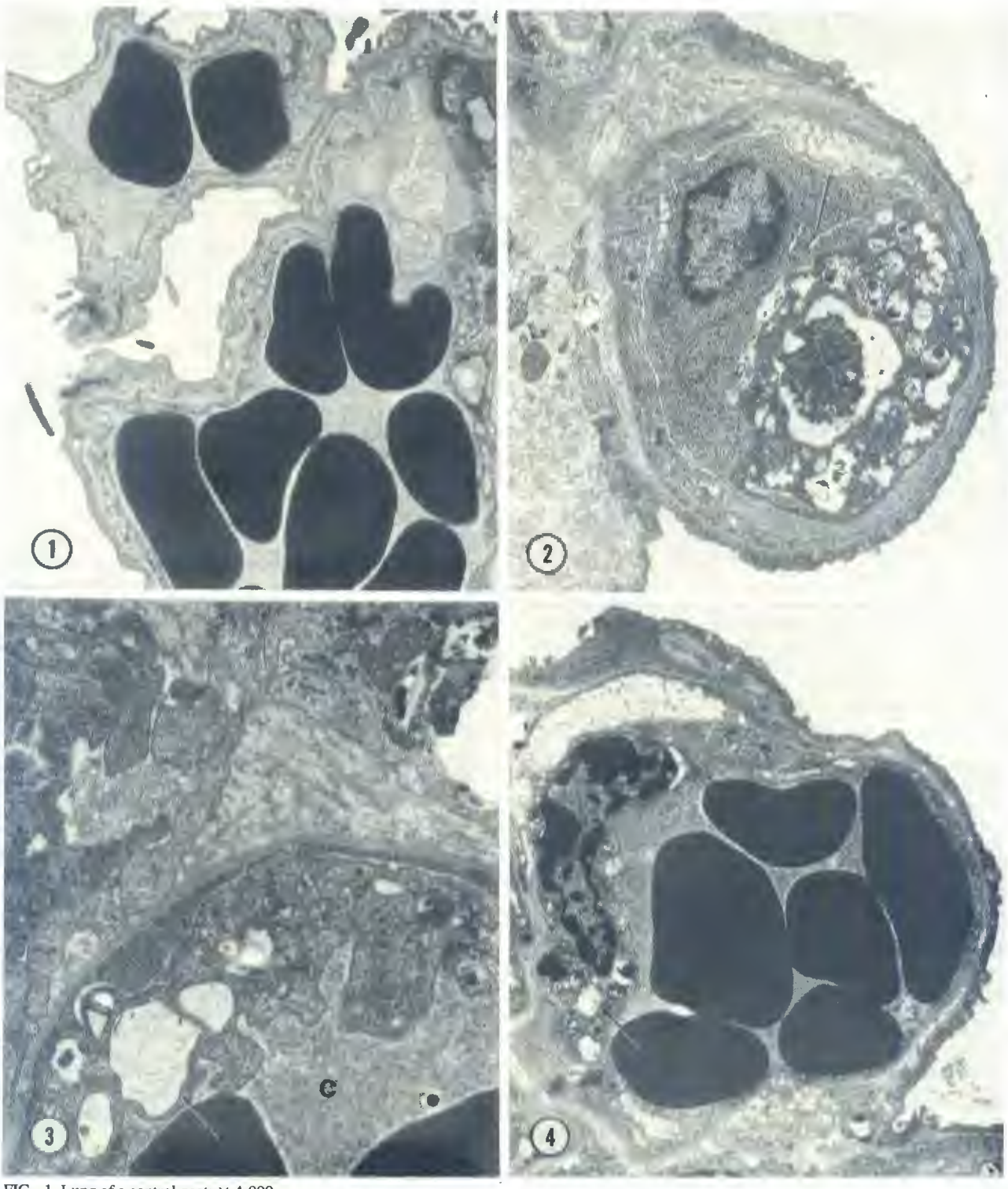

FIG. 1 Lung of a control goat: × 4 000
FIG. 2–5 Cytopathic changes in non-parasitized, endothelial cells. Note the swollen endothelial cells and the intracytoplasmic vacuoles (arrows).
s = subendothelial space c = capillary lumen:
× 4 000: × 10 000: × 4 000: × 10 000

fine flocculent or amorphous material of medium electron density, were noted (Fig. 2–5). The membranes surrounding the vacuoles were often ruptured. Necrotic endothelial cells were rarely encountered. Infrequently, gaps, which sometimes contained material of electron density similar to plasma, were seen between endothelial cells (Fig. 6). A few alveolar epithelial cells (type I pneumocytes) were swollen with cytopathic changes similar to those described in endothelial cells (Fig. 7–9).

Other lesions included an interstitial and alveolar oedema. Both the interstitial tissue and alveolar lumens contained a few fibrin strands, red blood cells and flocculent or amorphous material of medium electron density. Swollen mitochondria were present in a few interstitial cells.

A few colonies or occasionally single HW organisms were present in membrane-bound vacuoles in the cytoplasm of alveolar endothelial cells (Fig. 10–12). Single membrane-bound vacuoles, which were either empty or contained electron-lucent material, were present in some of the vacuoles containing the organisms. Apart from cytoplasmic distension due to the presence of the organisms, no other cytopathic changes were noted in parasitized cells.

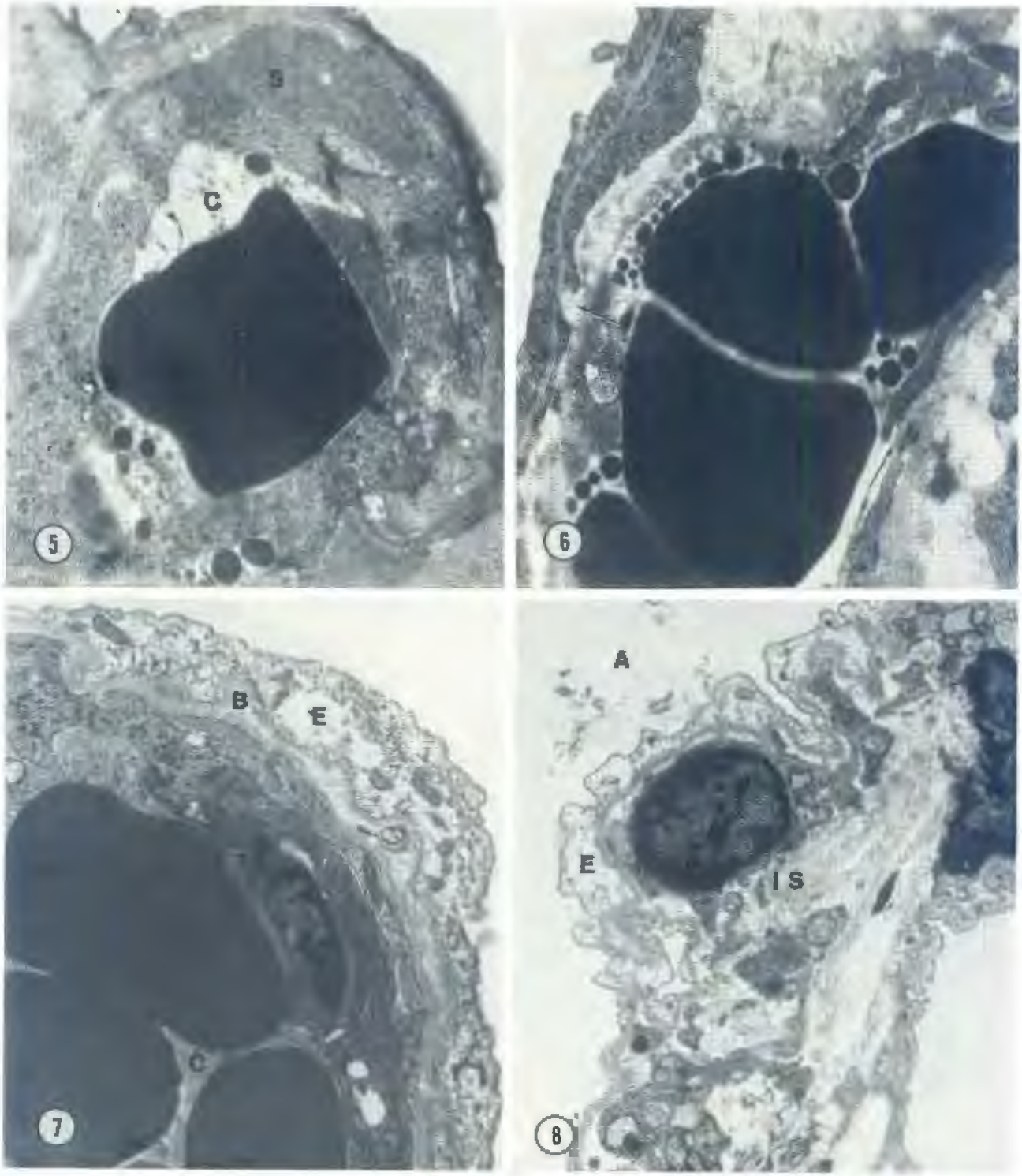

FIG. 6 Gaps (arrows) are visible between endothelial cells: × 10 000
FIG. 7–9 Swollen alveolar epithelial cells (E):
c = capillary lumen; is = interstitial space; B = basement membrane; A = alveolar space: × 10 000; × 4 000: × 17 000

Discussion

Pathological changes in the lungs of the experimental animals closely resembled those reported in mice infected with the Welgevonden strain of *C. ruminantium* (Prozesky & Du Plessis, 1985). In the mice, however, the alveolar endothelial cells were more severely affected, a higher concentration of HW organisms was present and the alveolar epithelial cells were not affected. Furthermore, the interstitial pneumonia reported in the mice was not present in the sheep or goats.

As regards the relatively mild morphological changes in the alveolar walls of the sheep and goats, it is difficult to account for the severe lung oedema seen grossly. Hurley (1978) suggested that the increased vascular permeability of slightly damaged alveolar walls is attributable to the presence of transient, reversible gaps at the endothelial junctions which are the result of direct injury to the endothelium. In this study, gaps were infrequently observed at the endothelial junctions.

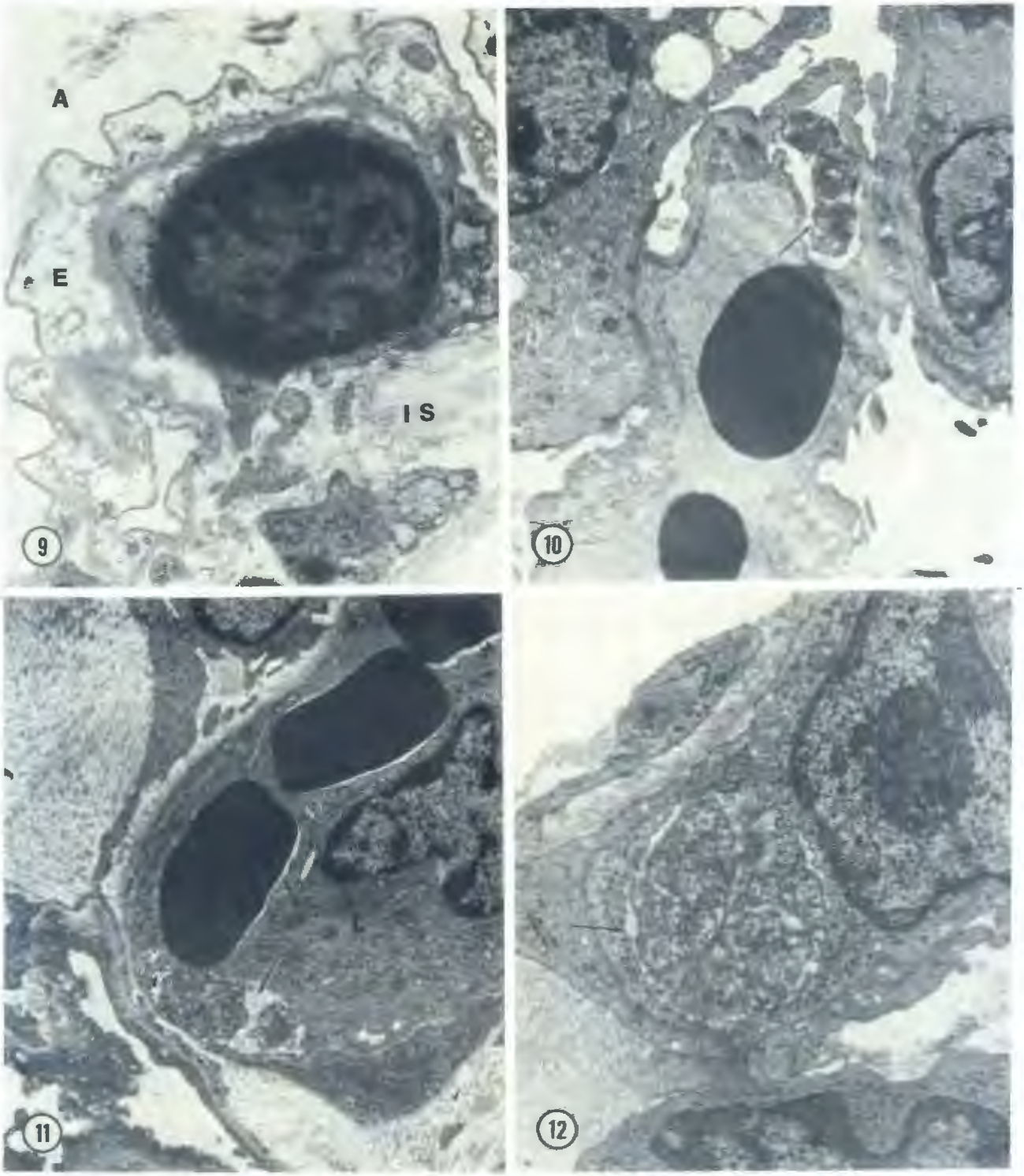

FIG. 10–12 Heartwater organisms in alveolar endothelial cells (arrows). L = leucocyte: × 4 000: × 10 000: × 10 000

The pathogenesis of HW is a controversial issue (Uilenberg, 1983). In neither ruminants nor mice is there any correlation between the severity of the lesions and the concentration of organisms (Pienaar, 1970; Prozesky & Du Plessis, 1985). Furthermore, the limited cytopathic changes in parasitized cells indicate that the organisms *per se* are not responsible for the increased vascular permeability (Pienaar, 1970; Prozesky & Du Plessis, 1985). A toxin has often been incriminated as the cause of the increased vascular permeability in animals with HW (Neitz, 1968; Pienaar, 1970). Wisseman (1968) obtained evidence of endotoxic activity in Rickettsiae and the intravenous injection of mice with lethal dose of *Rickettsia prowazeki* caused an increased vascular permeability (Parker & Neva, 1954). *C. ruminantium* stain gram-negatively, the cell wall appears morphologically very similar to that of gram-negative bacteria (Pienaar, 1970; Prozesky & Du Plessis, 1985), and the organisms can be partially concentrated by means of wheat germ lectin cellular affinity chromatography used to purify gram-negative organisms (G. J. Viljoen, unpublished data, 1984). In view of its several similarities with gram-negative bacteria *C. ruminantium* might be expected to form endotoxin.

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REFERENCES

ALEXANDER, R. A., 1931. Heartwater. Present state of our knowledge of the disease. *17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa*, 89–149.

HENNING, M. W., 1956. Animal diseases in South Africa. 3rd ed. South Africa: Central News Agency, Ltd.

HURLEY, J. V., 1978. Current views on the mechanisms of pulmonary oedema. *Journal of Pathology*, 125, 59–79.

KAY, D. H., 1965. Techniques for electron microscopy. Blackwell Scientific Publications, Oxford.

NEITZ, W. O., 1968. Heartwater. *Bulletin de l'Office International des Epizooties*, 70, 329–336.

PARKER, F. & NEVA, F. A., 1954. Studies on the toxicity of typhus rickettsiae. *American Journal of Pathology*, 30, 215–237.

PIENAAR, J. G., 1970. Electron microscopy of *Cowdria (Rickettsia) ruminantium* (Cowdry, 1926) in the endothelial cells of the vertebrate host. *Onderstepoort Journal of Veterinary Research*, 37, 67–78.

PROZESKY, L. & DU PLESSIS, J. L., 1985. The patholgoy of heartwater. I. A study in mice infected with the Welgevonden strain of *Cowdria ruminantium*. *Onderstepoort Journal of Veterinary Research*, 52, 71–79.

PURCHASE, H. S., 1945. A simple and rapid method for demonstrating *(Rickettsia) ruminantium* (Cowdry, 1926) in heartwater brains. *Veterinary Research*, 57, 413–414.

STECK, W., 1928. Pathological studies on heartwater. *Director of Veterinary Education and Research*, Reports 13 & 14. Part I. pp. 283–297.

UILENBERG, G., 1983. Heartwater (*Cowdria ruminantium*) infection: Where do we stand? *Advances in Veterinary Science and Comparative Medicine*, 27, 428–480.

WISSEMAN, C. L., 1968. Some biological proporties of Rickettsiae pathogenic for man. *Zentral Blad für Bakteriologie, Parasitenkunde Infektions krankheiten und Hygiene; Originale*, 206, 299–313.