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THE PATHOLOGY OF HEARTWATER. I. A STUDY OF MICE INFECTED WITH THE WELGEVONDEN STRAIN OF *COWDRIA RUMINANTIUM*

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ABSTRACT

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Gross and microscopical lesions in mice intravenously infected with the Welgevonden strain of *Cowdria ruminantium* closely resembled the lesions described in cattle, sheep and goats. A high concentration of organisms was present in alveolar endothelial cells. Cytopathic changes in parasitized and non-parasitized endothelial cells and the morphology of the organisms are described and compared with the $Ball_3$ strain of *C. ruminantium*. Possible mechanisms in the development of the lung oedema are considered and the role of mice as animal model is discussed.

INTRODUCTION

Most attempts to infect various laboratory animals with *Cowdria ruminantium* have been unsuccessful (Uilenberg, 1983). Mason & Alexander (1940) made 4 serial passages in ferrets after which the infection was lost. Two strains of *C. ruminantium* which are pathogenic for mice have recently been isolated from domestic ruminants. The Kumm strain (Du Plessis & Kumm, 1971) is highly pathogenic for mice, irrespective of whether it is injected intravenously or intraperitoneally. This strain is also pathogenic to sheep and goats, but almost non-pathogenic to cattle (Du Plessis, 1982). The Kwanyanga strain, on the other hand, is highly pathogenic to mice, but only if injected intravenously. It is also pathogenic to sheep and cattle (Mackenzie & Van Rooyen, 1981).

Reports on the pathology of heartwater (HW) in laboratory animals are limited (Du Plessis, 1975). The purpose of this study is to describe the lesions in mice infected with the Welgevonden strain of *C. ruminantium* and to compare them with the lesions described in cattle, sheep and goats experimentally and naturally infected with *C. ruminantium*. Emphasis is placed on lesions in the lung.

MATERIALS AND METHODS

Animals

Outbred Swiss white mice, 4–6 weeks old, were intravenously inoculated with 0,2 mℓ of tenfold serial dilutions of tissue homogenate prepared from the lungs, livers, spleens, and hearts of mice infected with the 6th passage of the Welgevonden strain of *C. ruminantium* isolated from a tick (Du Plessis, 1984). The mice were examined daily, and 11 of those that showed clinical signs 13 days or longer after infection were selected for this study. As controls, 3 non-infected mice kept under the same conditions were necropsied and specimens were collected as outlined for the experimental animals (Fig. 7–10).

Pathology

The mice were examined grossly before specimens were collected for light and electron microscopy, as outlined below.

Light microscopy

Specimens from the brain, lung, heart, spleen, kidney and liver were collected in 10 % buffered formalin, processed routinely, and stained with haematoxylin and eosin (HE). The following stains were applied to lung sections: Mallory's phosphotungstic acid haematoxylin (MPAH) and periodic acid Schiff (PAS) (Luna, 1968).

Electron microscopy

Specimens were collected from the diaphragmatic lobes in each case. 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 for tissue orientation were cut 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).

RESULTS

Clinical course

The incubation period ranged from 10–14 days. Tachypnoea, lethargy, anorexia and a ruffled coat were present in all the animals.

Gross pathology

Although there was a variation in the severity of the lesions, lung oedema, hydropericardium, hydrothorax and splenomegaly were present in all the mice. Multiple small foci of emphysema, surrounded by areas of atelectasis, were distributed throughout the lungs of 3 animals with extensive lung oedema. Scattered throughout the livers of 2 mice were a few well-circumscribed, pale areas ranging in size from *c.* 0,2–5 mm.

Microscopical pathology

Lung: In 3 mice, the alveolar spaces contained abundant protein-rich oedema fluid (Fig. 1) which stained positively with the PAS and MPAH reactions. These alveoli were patchy in distribution, alternating with areas of emphysema. Many macrophages with abundant eosinophilic, finely granular or vacuolated cytoplasm, fibrin strands and red blood cells were present within alveolar spaces (Fig. 2 & 3). The amount of intra-alveolar fibrin, which stained positively with the PAS and MPAH reactions, ranged from a few strands to dense masses which almost filled the entire alveolar space. In the remaining 8 mice, the alveolar oedema was much less pronounced, and only scattered alveoli were affected. An interstitial oedema and a mild to moderate, diffuse, mononuclear, interstitial pneumonia were present in all the animals (Fig. 4). In 2 mice, a few bronchi and bronchioles contained varying amounts of homogeneous, eosinophilic material and a few macrophages. An intravascular increase in monocytes and neutrophils was present in 5 mice.

A constant feature in all the animals was the presence of varying numbers *C. ruminantium* colonies in the alveolar capillary endothelial cells (Fig. 5). In 6 animals,

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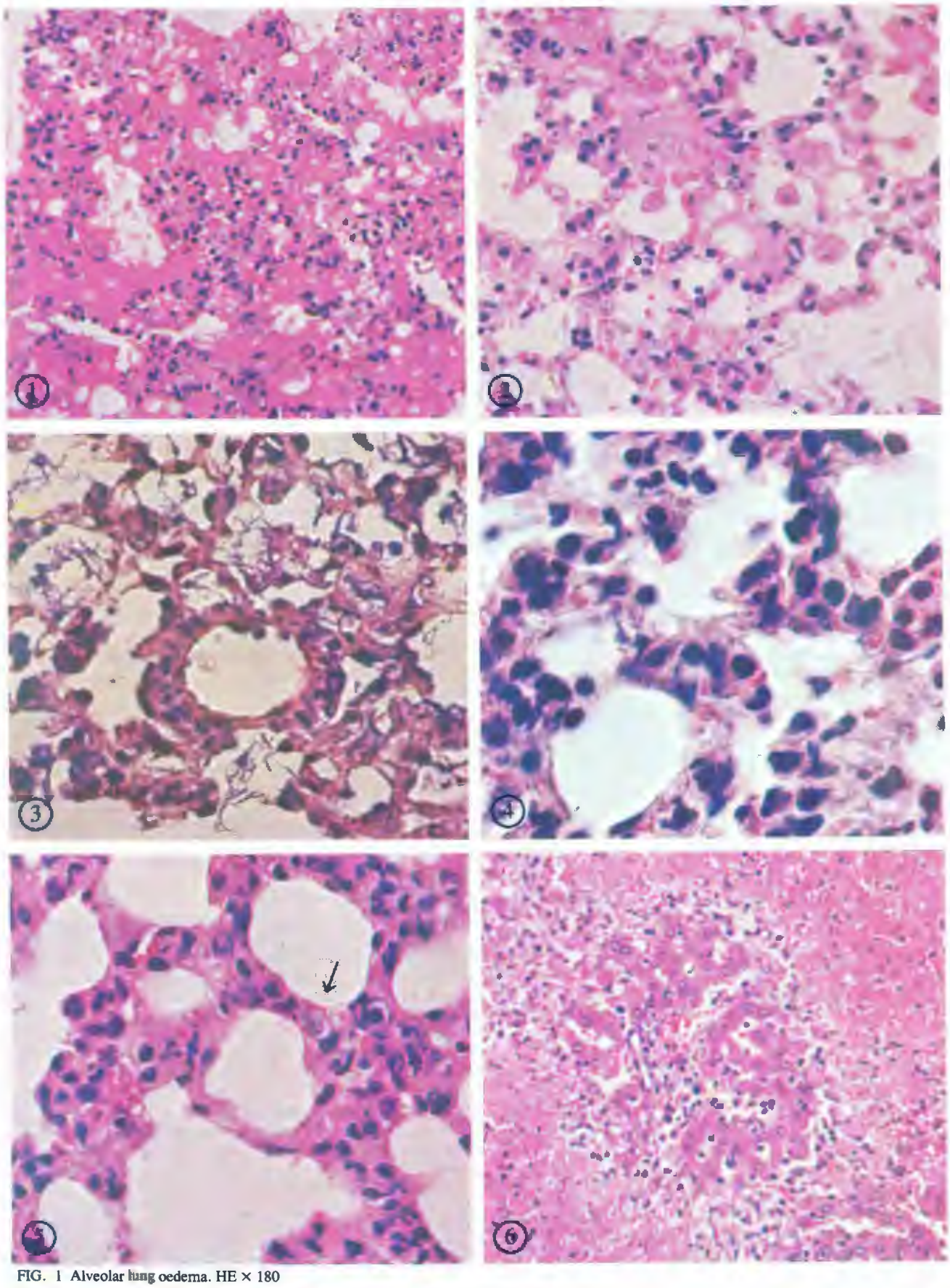


FIG. 1 Alveolar lung oedema. HE × 180

FIG. 2 Alveolar macrophages with abundant vacuolated eosinophilic cytoplasm: HE × 280

FIG. 3 Fibrin in alveolar spaces: MPAH × 450

FIG. 4 & 5 A diffuse mononuclear interstitial pneumonia. HW organisms (arrow) are visible in an alveolar endothelial cell: HE × 450 & 1000

FIG. 6 Area of hepatic necrosis. Macrophages and a few neutrophils are visible at the edge of the lesion: HE × 280

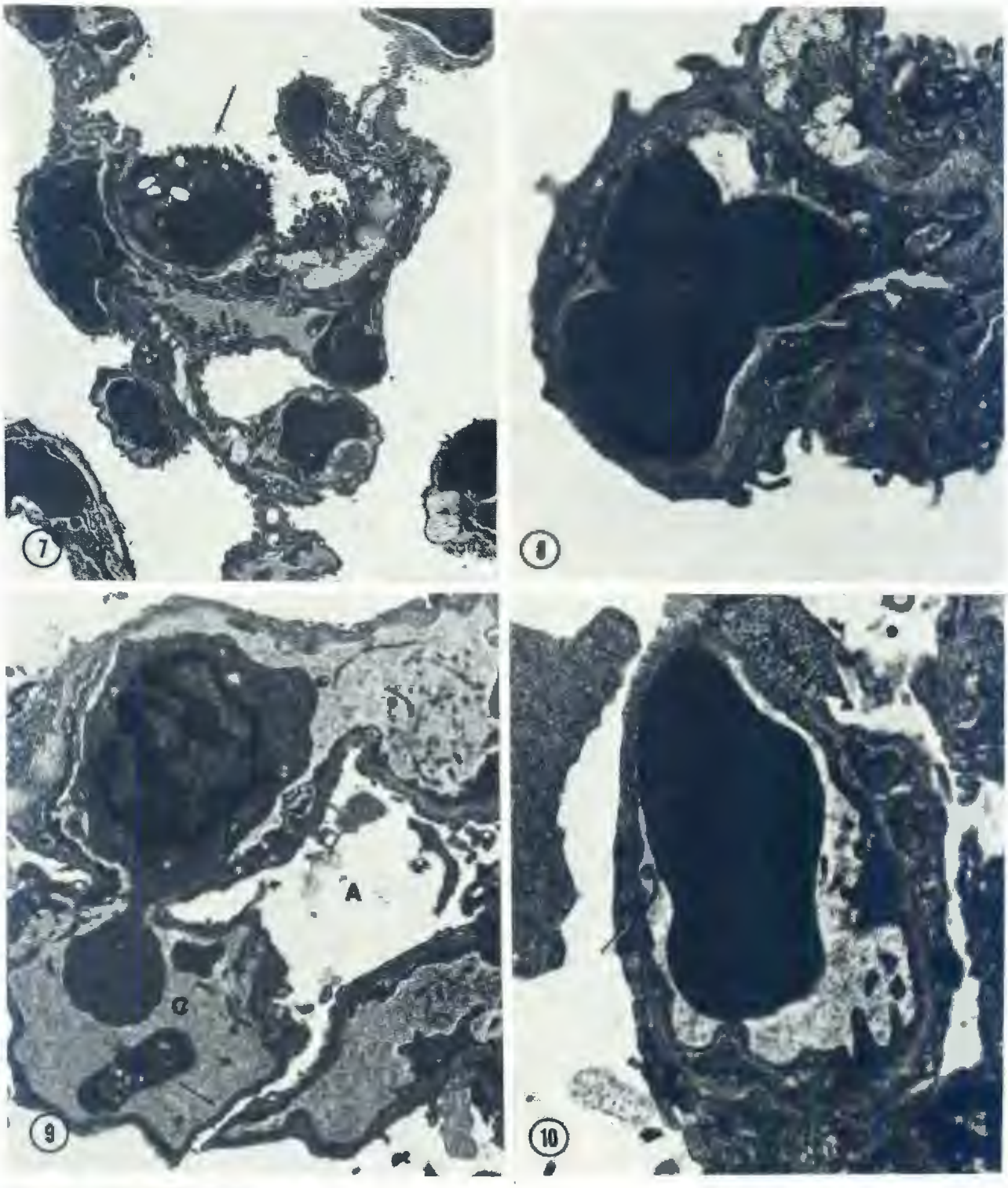


FIG. 7–10 Lung of a control mouse

FIG. 7 Type II pneumocyte (arrow) adjacent to alveolar capillaries containing red blood cells: × 3800

FIG. 8 & 9 Alveolar capillary. Note thin capillary endothelium (arrow). A = alveolar space. C = capillary lumen: × 16600 & 9300

FIG. 10 Alveolar capillary with junctions between endothelial cells (arrows): × 16600

many colonies were noted; in 2, only a few were seen, while in the remaining 3 animals it was difficult to find organisms. The colonies were often surrounded by a more lightly staining halo. The size of the colonies and individual organisms varied and corresponded with the findings of Pienaar (1970). Parasitized cells were often swollen and they occasionally almost completely obliterated the capillary lumen.

Liver: In 2 animals, multiple foci of coagulative necrosis were scattered throughout the liver with an apparent predilection for the subcapsular peranchyma (Fig. 6). Hepatocytes bordering the necrotic foci were swollen and showed a fine vacuolation of their cytoplasm. In 1 mouse, the necrotic areas were infiltrated by macrophages and neutrophils. A perivascular infiltration of mononuclear cells, which occasionally infiltrated the

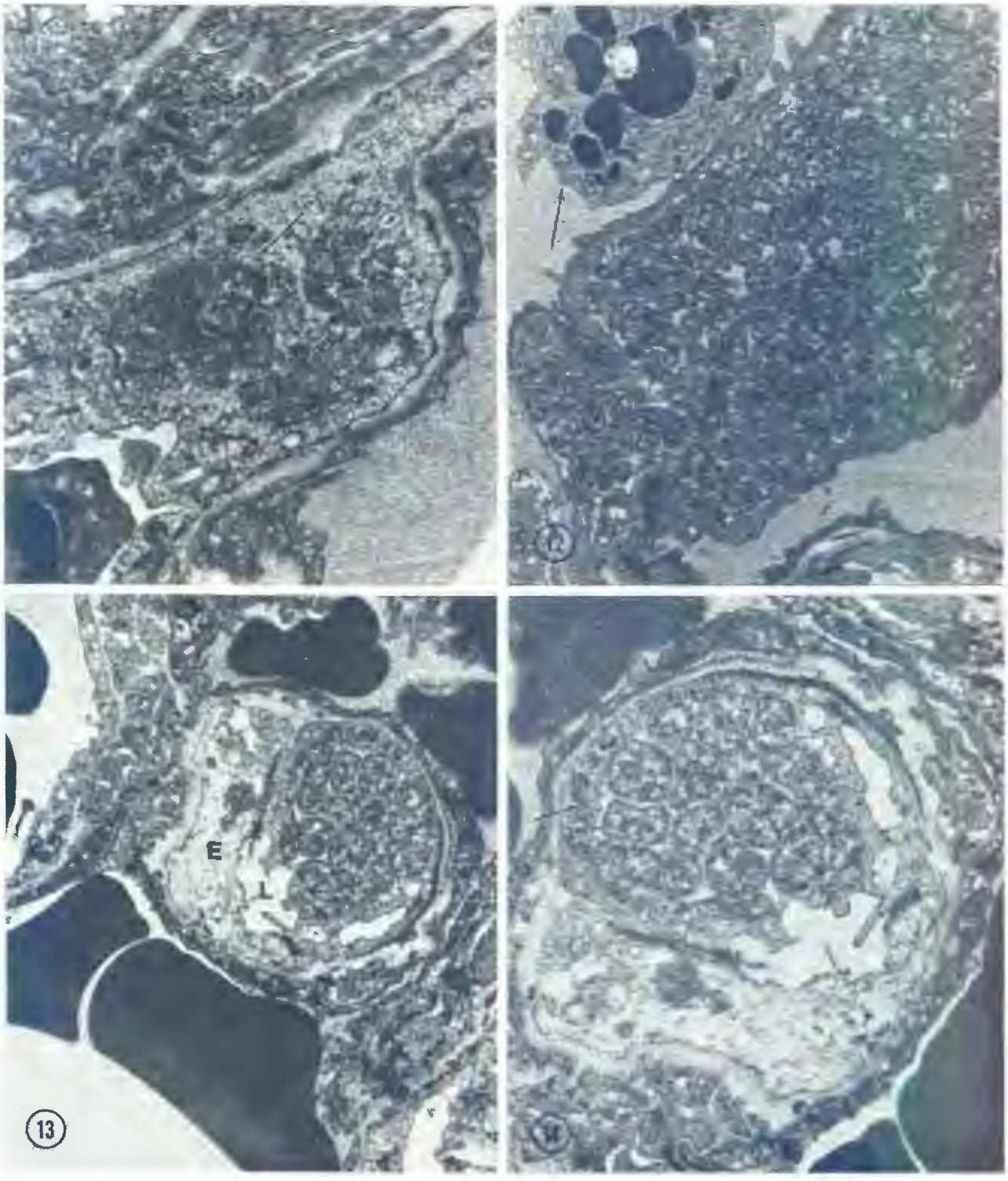


FIG. 11 Two organisms (arrow) are visible within the distended cytoplasm of an alveolar endothelial cell: × 16600

FIG. 12 The alveolar capillary lumen is occluded by a colony of organisms. An alveolar macrophage containing phagocytosed material is visible in the alveolar space (arrow): × 9300

FIG. 13 & 14 A parasitized alveolar endothelial cell with swelling of a bordering endothelial cell (E). The cytoplasm of the parasitized cell can be seen as a thin rim around the organisms (arrow). L = capillary lumen: × 9300 & 16600

vascular walls, involved the portal and interlobular vessels of both animals. Single colonies of *C. ruminantium* were present in the endothelium of larger vessels and in what appeared to be sinusoidal, endothelial cells. A sinusoidal leucostasis (macrophages and neutrophils) was present in both animals.

Other organs: In 1 mouse with hepatic lesions, a mild, diffuse, acute to subacute mononuclear interstitial myocardities was present. Zenker's hyalin degeneration and necrosis of individual or small groups of fibres was noted throughout the myocardium. Many *C. ruminantium* colonies were present in the myocardial capillary endothelial cells, and a few were seen in brain capillaries, glomerular and intertubular capillaries in the kidneys and in what appeared to be sinus endothelial cells in the sinuses of the spleen. Single HW colonies were present in the myocardial capillaries of 3 other animals.

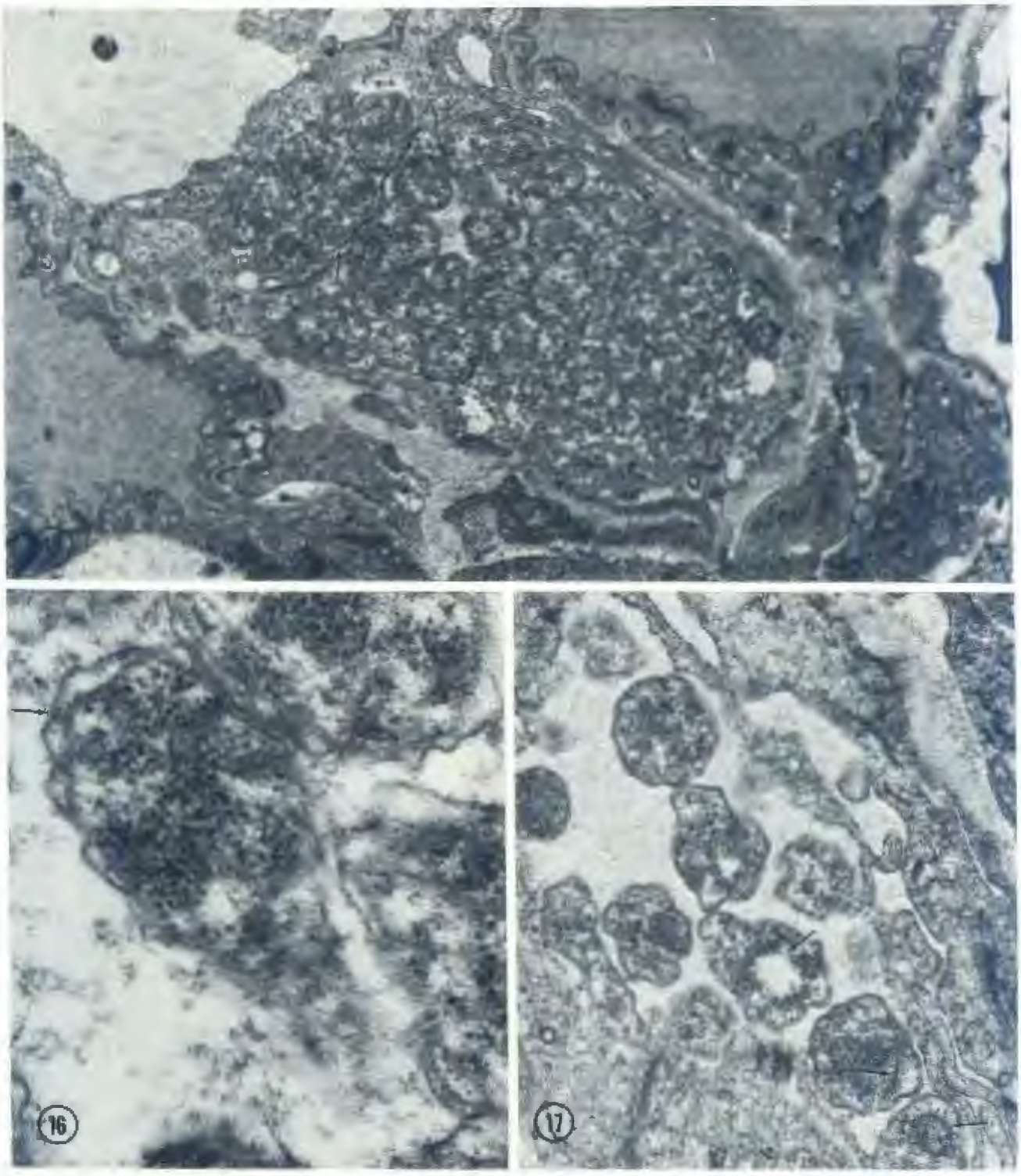


FIG. 15 Two colonies, each enclosed by a separate membrane (arrow) in an endothelial cell: × 16600

FIG. 16 Each organism is enclosed by two membranes (arrow): × 78000

FIG. 17 Vacuole in a small organism (top arrow) as well as additional membranes in 2 organisms (bottom arrows): × 25000

Electron microscopy

Lungs: HW colonies or single organisms were located in membrane-bound vacuoles in the swollen cytoplasm of alveolar endothelial cells which occasionally occluded the capillary lumens (Fig. 11–17). The cytoplasm of parasitized cells formed a rim around the vacuole containing the organisms. Occasionally, 2 colonies, each enclosed by a separate membrane, were seen in a single cell (Fig. 15).

Organisms ranged in size from *c.* 0,4–1,04 μm in diameter, although exact measurements were difficult because of the pleomorphism of the organisms. The forms most commonly seen were coccoid, bacillary and cocco-bacillary. Usually, only organisms of approximately the same size were present within a vacuole. The small organisms were less densely packed within the vacuole if compared with the larger forms. Each organism was enclosed by 2 membranes, each of which was three-layered and of typical unit membrane construction (Fig. 16). The organisms were suspended in the vacuole in a matrix of fine fibrils and granules of medium electron density. A vacuole which infrequently contained a

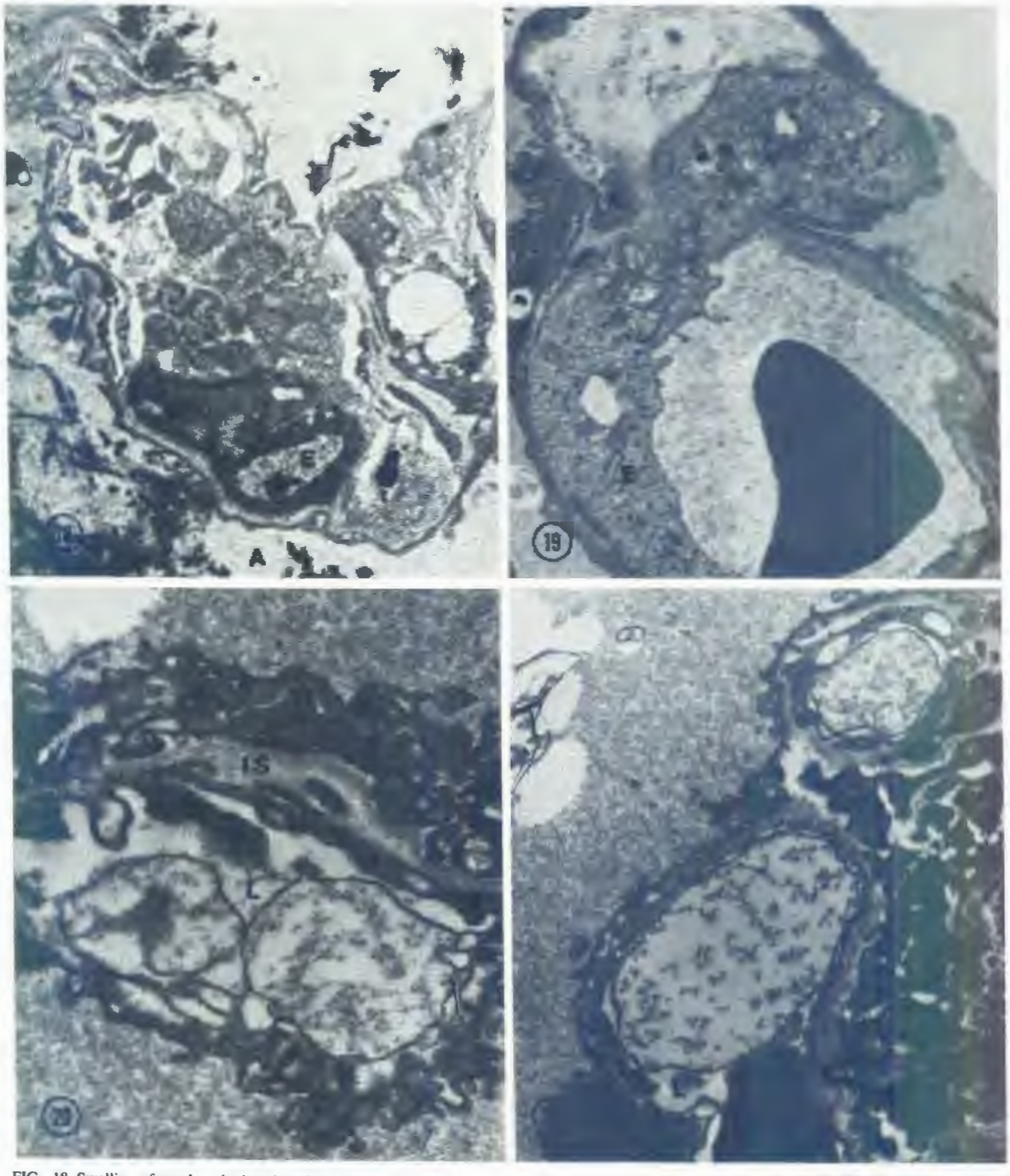


FIG. 18 Swelling of rough endoplasmic reticulum and chromatin margination in a parasitized alveolar endothelial cell (E). The alveolar spaces (A) contain fibrin strands: × 9300

FIG. 19 Swelling of some of the mitochondria in a distended alveolar endothelial cell (E): × 1600

FIG. 20 & 21 Swollen segments of capillary endothelial cells contain flocculent material. The interstitial space (IS) is dilated. L = capillary lumen: × 9300 & 9300

dark dense body was seen in a few small and intermediate size organisms (Fig. 17). Occasionally a membrane surrounded this vacuole. The internal structure of the organisms and forms of replication corresponded with the findings of Pienaar (1970).

There was a variation in the severity of the cytopathic changes in parasitized cells. In the majority of cells, no changes, or only mild swelling of mitochondria and endoplasmic reticulum were noted. Occasionally, however, severe changes such as chromatin margination, swelling of mitochondria with loss of cristae and swelling of the endoplasmic reticulum were observed (Fig. 18). More commonly, non-parasitized cells, some of which bordered parasitized cells, were swollen. In these cells the cytoplasmic density was decreased and the cytopathic changes corresponded with those in severely affected, parasitized cells (Fig. 14 & 19).

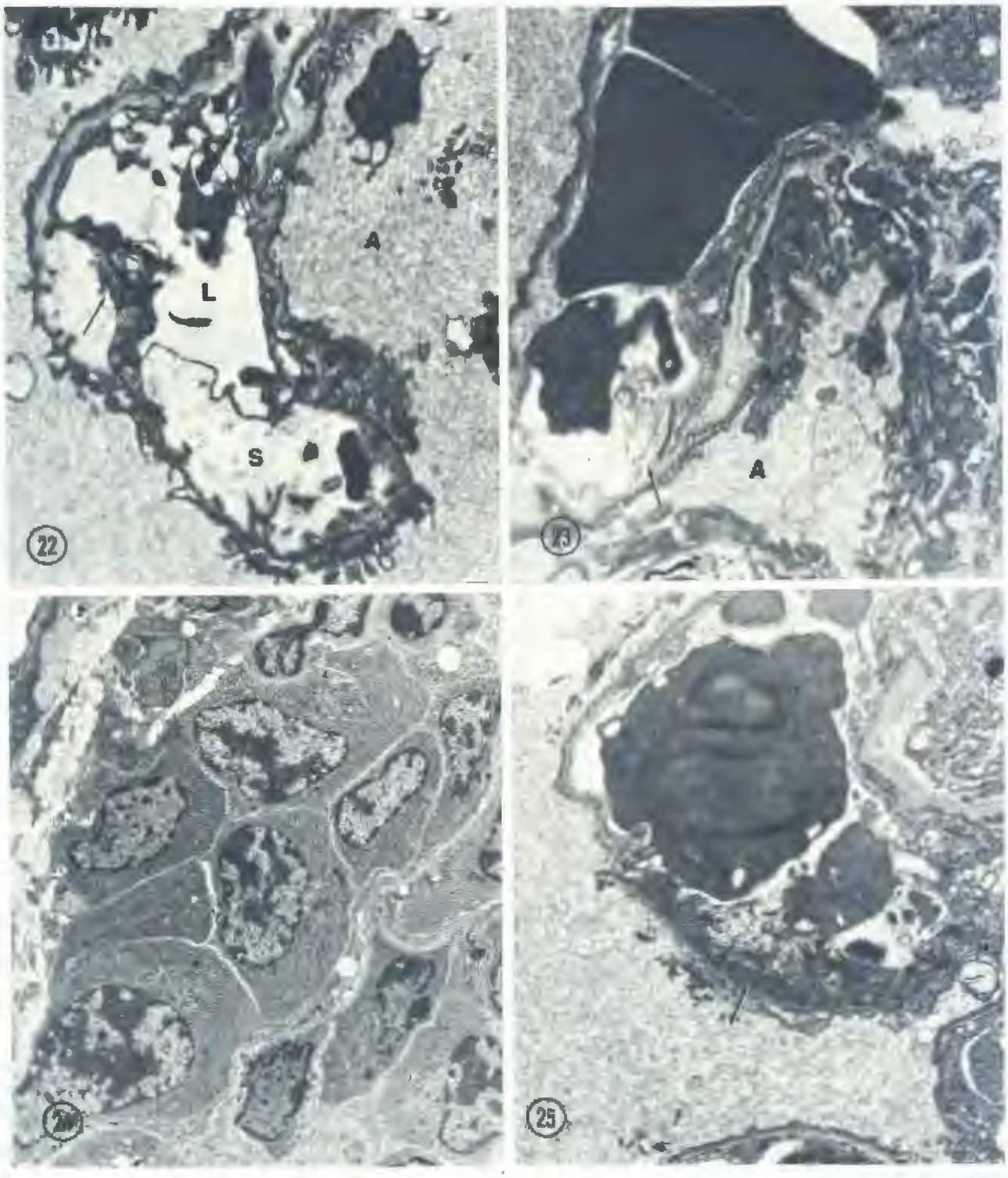


FIG. 22 Separation of the alveolar endothelium (arrow) from the basement membrane, with flocculent electron pale material in subendothelial space(s). An intracytoplasmic myelin figure is present in the detached cell. L = capillary lumen. A = alveolar space: × 9300

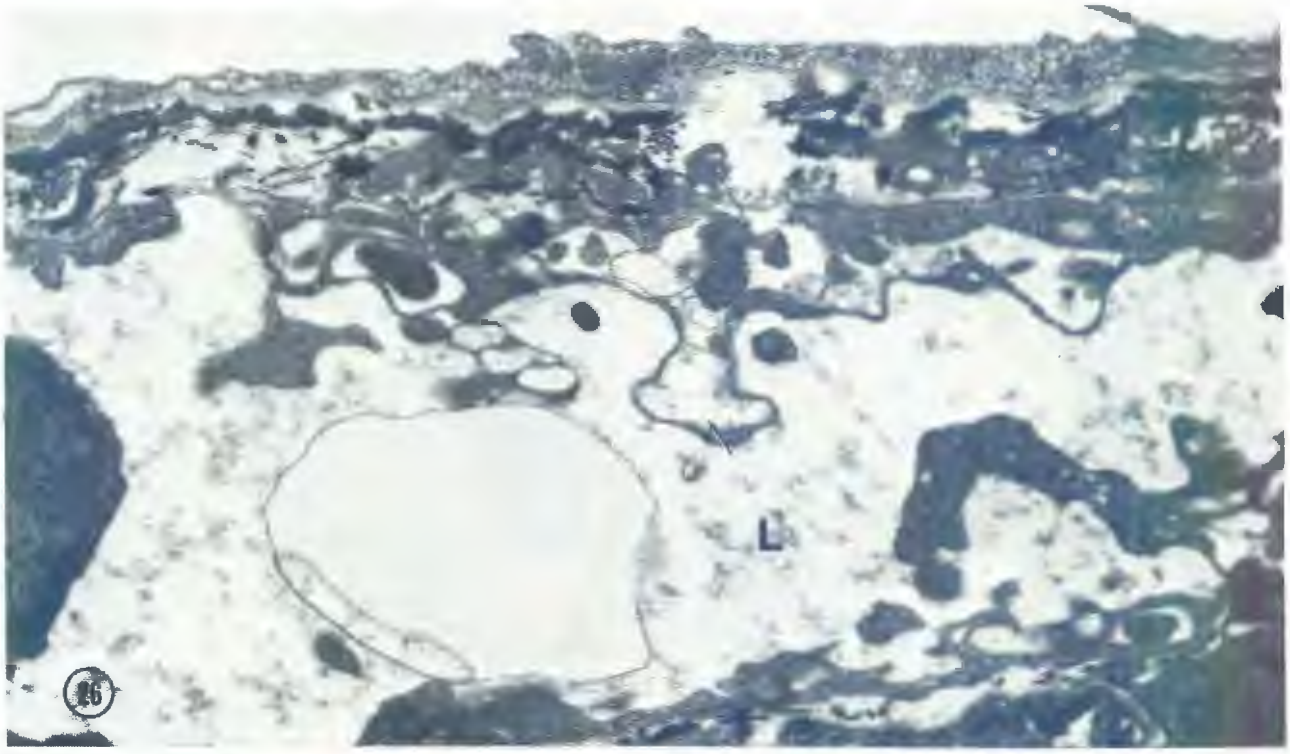
FIG. 23. Necrosis of an endothelial cell (arrow) A = alveolar lumen: × 9300

FIG. 24 Mononuclear cells with abundant rough endoplasmic reticulum in the interstitial tissue: × 9300

FIG.25 A fibrin thrombus (arrow) in an alevolar capillary: × 9300

Other changes in affected cells were the presence of intracytoplasmic membrane-bound vacuoles *c.* 0,7–2 μm in diameter, which were either empty or contained a flocculent material of medium electron density in which occasionally membranes were suspended. These cells often showed segmental distension of their cytoplasm and, depending on the plain of section, the distended segments were often lying free in the capillary lumens (Fig. 20 & 21).

Further changes included segmental separation of endothelial cells from the basement membrane which occasionally almost completely obliterated the capillary lumen (Fig. 22). The resultant space between the endo- and epithelial cells was filled with flocculent material similar to plasma. Necrosis of non-parasitized cells were rarely seen, and many normal endothelial cells were dispersed between the cells with cytophatic changes (Fig. 23).



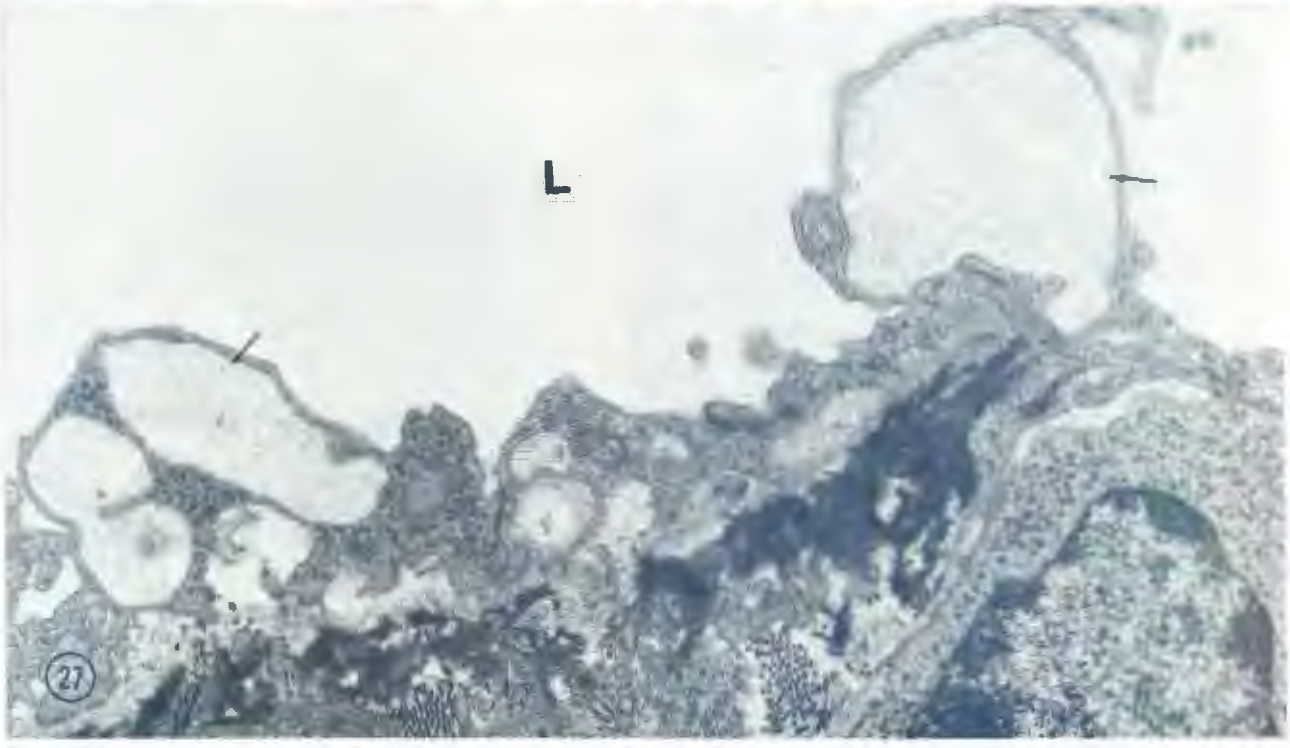


FIG. 26 Arteriole with oedema of the vascular wall. Note the dilated subendothelial space (arrow). L = vascular lumen: × 9300

FIG. 27 Vacuoles (arrows) in a swollen endothelial cell of an arteriole. L = vascular lumen: × 600

The interstitial tissue was dilated and contained flocculent material of medium electron density, fibrin and many mononuclear cells which often had abundant rough endoplasmic reticulum (Fig. 24). Lesions less frequently encountered included fibrin thrombi in capillaries and segmental detachment of the alveolar epithelium from the basement membrane (Fig. 25). Only a limited number of larger blood vessels was examined. Endothelial changes corresponded with the lesions in the alveolar capillaries, while oedema of the vascular walls was evident (Fig. 26 & 27).

Fine flocculent material, fibrin, macrophages and multilaminar structures, very similar to multilaminar structures present in Type II pneumocytes, were present in the alveolar spaces of mice with extensive lung lesions.

Discussion

Gross and light microscopical lesions in mice infected with the Welgevonden strain of *C. ruminantium* closely resemble the lesions in cattle, sheep and goats artificially or naturally infected with *C. ruminantium* (Steck, 1928; Alexander, 1931; Prozesky & Du Plessis, 1985). According to Pienaar, Basson & Van der Merwe (1966), brain oedema associated with a vasculitis is a constant lesion in cattle, sheep and goats with HW. Although not

all the brains of the mice under study were examined, brain oedema was not evident in those available.

The interstitial pneumonia observed in the mice has also been reported in mice and Angora goats with HW (Du Plessis, 1975; Prozesky & Du Plessis, 1985). However, Steck (1928) does not consider it as a constant lesion in HW infected domestic ruminants.

The lungs contained the highest concentration of organisms, followed by the heart. Only rarely were organisms seen in brain capillaries. According to Cowdry (1926), HW organisms are easily detectable in the capillary endothelial cells of the renal glomeruli and superficial grey matter of the cerebral cortex, and are rare or absent in the liver and lungs of cattle, sheep and goats. The reason for the significant variation in the concentration of organisms in the individual experimental animals and the preferred location of this strain for the lungs is not clear.

Morphologically the Welgevonden strain is indistinguishable from the organisms described in the choroid plexus of sheep infected with the $Ball_3$ strain *C. ruminantium*. However, the very large organisms described by Pienaar (1970) were not seen.

According to Cowdry (1926) and Pienaar (1970), intracytoplasmic growth of *C. ruminantium* has little deleterious effect upon the host cell. These results were confirmed in the lungs, although occasionally marked cytopathic changes were noted in non-parasitized and, to a lesser extent, in parasitized cells. These changes included swelling of cells with cytoplasmic vesiculation and/or detachment from the basement membrane. Necrosis of both non-parasitized and parasitized endothelial cells was rarely encountered and it was not always possible to correlate the concentration of organisms with the extent of the lesions, particularly in the lungs.

Lifting of the endothelium from the underlying basement membrane has been observed in many types of lung injuries (Hurley, 1978). In animals injected with ferritin, marker particles were present in the resulting subendothelial space in large numbers. Hurley (1978) suggests that the endothelium is lifted off the basement membrane by oedema fluid and protrudes into the capillary lumen because of the low pressure within pulmonary vessels.

Pulmonary lesions observed in this study closely resemble those observed in rats with ANTU poisoning, although the formation of interendothelial gaps in the latter (Cunningham & Hurley, 1972) was not seen in the mice with HW. Cunningham & Hurley (1972) suggested that the increased vascular permeability in the ANTU-intoxicated rats was attributable to the presence of reversible gaps at endothelial junctions. In the HW-infected mice there was no evidence of morphological changes at the alveolar capillary junctions. However, Hurley (1978) concluded that focal and often reversible lesions in small pulmonary vessels may cause a sufficient increase in the vascular permeability to cause a severe acute pulmonary oedema. Because of the limited number of larger vessels examined, the significance of the endothelial lesions and oedema of the walls of larger vessels is not clear at this point, but it does indicate that the vascular lesions are not limited to capillaries.

The pathogenesis of HW is a controversial issue and is dealt with in another report (Prozesky & Du Plessis, 1984). We realize the dangers of interspecies comparisons and that the pathogenesis in mice may be different from that in ruminants. The resemblance of the gross, light and ultrastructural lesions in the lungs of mice and domestic ruminants infected with 2 different strains is, however, striking (Prozesky & Du Plessis, 1984). It is therefore reasonable to suggest that the Welgevonden strain may serve a useful purpose in the study of the pathogenesis of HW.

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