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A MORPHOLOGICAL STUDY OF THE LESIONS OF AFRICAN HORSESICKNESS

S. J. NEWSHOLME, Section of Pathology, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

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Gross, histological and ultrastructural findings are described in 6 natural cases and in 2 experimental cases of African horsesickness. From the gross lesions the cases were divisible into 2 groups which represented the previously described pulmonary and mixed forms of the disease. Histologically, abundance of fibrin and inflammatory cells in oedematous lung suggests that the pulmonary lesion is an exudative pneumonia. Lymphoid depletion and necrosis in germinal centres were consistently present. Electron microscopy failed to demonstate virus particles or virus-associated structures in the tissues. Ultrastructural evidence of vascular injury was not apparent in oedematous tissues. Possible mechanisms in the development of the lung oedema are considered and a comparison is made with oedema induced by alpha-naphthyl-thiourea. Lack of structural evidence of vascular injury revealed by this study extends some hope for therapy in African horsesickness.

INTRODUCTION

From the gross lesions in natural and experimental cases of African horsesickness (AHS), Theiler (1921) distinguished 3 forms of the disease, namely, the pulmonary form, the oedematous or cardiac form, and the mixed form.

The pulmonary form was characterized by severe pulmonary oedema, with oedema of the mediastinum and of related lymph nodes. Hydrothorax, sometimes severe, was a usual feature, whereas hydropericardium was absent. This pathological form corresponded with the pulmonary clinical form, which had a peracute course and an incubation period in most cases of 4–5 days.

In the oedematous or cardiac form the striking lesions were oedema of the subcutis and the subcutaneous fat of the temporal fossa, masseteric and parotid regions, the mandibular space and the regional lymph nodes. In some cases oedema was more extensive, involving eyelids, neck, shoulders and back. In these regions, oedema also affected fascial planes and aponeuroses of the underlying musculature. Hydropericardium was almost always present and was sometimes severe. Subepicardial and subendocardial haemorrhages were usually extensive. Pulmonary oedema was slight or absent, and hydrothorax was rare. Clinically, the oedematous or cardiac form had a subacute course and a variable incubation period, which was generally longer than that of the pulmonary form.

In the mixed form, lesions occurring in the other forms were present in combination. Theiler (1921) claimed this form to be rare and stated that many such cases resulted from dual infections with various AHS viral strains. According to Erasmus (1972), the mixed form runs an acute clinical course and has an incubation period of 5–7 days. Subsequent observations indicate that rigid distinction into different forms of AHS is unjustifiable and that most cases are mixed in type (Carpano, 1931; Maurer & McCully, 1963; Erasmus, 1972).

Factors determining the development and distribution of lesions are not clear. According to Theiler (1921), the pulmonary form usually followed inoculation with virulent strains and the cardiac form was associated with more attenuated strains. Exercise during the febrile period of the disease may precipitate the pulmonary form (Howell, 1968; Erasmus, 1972). Erasmus (1972) has provided some evidence that the status of the population of infecting virus particles with regard to specific tissue tropism may be an important determinant of the disease form. Serial passage of AHS virus in horses using lung material consistently produced peracute disease with marked pulmonary involvement, whereas passage using spleen material resulted in progressively milder disease.

There are few reports on the histopathology of AHS. Maurer & McCully (1963) studied the histopathological changes in a series of natural and experimental cases of AHS in the Middle East. They described the pulmonary lesion as alveolar and interstitial oedema which they believed to be of inflammatory origin. The oedema fluid contained variable numbers of inflammatory cells and sometimes fibrin. Depletion of mature lymphocytes from lymphoid follicles was a consistent finding in lymph nodes and in spleen. In the predominantly cardiac form of AHS, myocardial oedema and haemorrhages were regarded as characteristic lesions. Myocarditis was a feature of a few cases.

Ultrastructural changes associated with replication of AHS virus have been studied in tissue cultures (Lecatsas & Erasmus, 1967; Breese, Ozawa & Dardiri, 1969; Huismans & Els, 1979). However, the ultrastructure of the lesions of AHS has not been described and the precise cellular sites of viral replication in this disease have not been established. The pathogenesis and, particularly, the mechanisms in the development of the characteristic oedematous lesions remain obscure.

This study concerns gross examinations, light microscopy and electron microscopy of the lesions and their possible mechanisms of development, and compares them with previous observations in this disease.

MATERIALS AND METHODS

Pathological changes were studied grossly and by light microscopy (LM) and electron microscopy (EM) in 6 natural cases and in 2 experimental cases of AHS. Tissues were compared to those from uninfected control horses for critical assessment where required.

Natural cases

Animals: Six dead horses, of mixed breeding, suspected as cases of AHS, were received during outbreaks of the disease in late summer, 1981. All originated from the farm, Kaalplaas, near Onderstepoort, where they had been at pasture. Cases 1–5 were foals which had been running with their dams. They had been found dead unexpectedly so that no clinical history was obtainable. Case 6 was an adult mare which had been found in lateral recumbency shortly prior to death. None of the animals had received treatment of any kind. These cases were selected from others because of the shortness of the interim period from death to examination. Age, sex and interim of these cases are recorded in Table 1.

TABLE 1 Natural and experimental cases of African horsesickness

	Case	Age	Sex	Interim
Natural cases	1	4 months	M	2–5 h*
	2	5 months	F	5–10 h*
	3	4 months	M	2 h*
	4	4 months	F	5–10 h*
	5	5 months	M	4 h*
	6	6 years	F	1 h
Experimental cases	7	1½ years	F	1 h*
	8	1 year	M	Immediate

*: estimated by gross examination
M: male
F: female

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Gross examination: A complete gross examination was conducted in each case immediately upon receipt. Tissue specimens were collected for LM, EM and virological examinations as described below.

Light microscopy: Specimens from lymph nodes, thymus, lung tissue at various sites, atria, ventricles and interventricular septum of the heart, supraorbital subcutaneous tissue, spleen, liver, kidney, brain, adrenals, stomach, large and small intestine, and skeletal muscle were collected and fixed by immersion in 10% buffered formalin.

The fixed specimens were processed routinely, embedded in paraffin wax, sections cut at 5–7 µm thickness and stained with haematoxylin and eosin (HE). The following stains were applied to selected sections: aldehyde fuchsin for elastic fibres (Gomori, 1950), a modification of Gomori's reticulum stain (Luna, 1968), Mallory's phosphotungstic acid haematoxylin (MPAH) (Luna, 1968), and periodic acid-Schiff (PAS), with and without diastase digestion (Luna, 1968).

Electron microscopy: In all cases specimens were collected from lung tissue at various sites, bronchial lymph nodes, thymus, spleen, pericardium and heart, including epicardium, endocardium and myocardium.

The specimens were diced into 1 mm cubes and fixed by immediate immersion in 4% glutaraldehyde in Millonig's phosphate buffer at pH 7,3–7,4 and 4 °C (Millonig, 1961) for periods of 24 h to 1 week. Selected blocks were rinsed with phosphate buffer and then post-fixed in 2% osmium tetroxide, also in the same buffer. Following 2 or more buffer rinses, the blocks were dehydrated in a graded ethanol series, cleared in propylene oxide and embedded in Epon 812 for 48 h at 60 °C.

Thick (1–2 µm) sections were cut with glass and diamond knives on a Reichert Om V4 ultramicrotome and stained with toluidine blue (Trump, Smuckler & Benditt, 1961) for tissue orientations. Relevant blocks were trimmed to size and gold to silver sections were obtained using the above equipment.

Thin sections were picked up on copper grids, stained for 30 min in a saturated aqueous solution of uranyl acetate (Watson, 1958) and for 3–4 min in an 0,2% lead citrate solution (Reynolds, 1963). The stained grids were viewed in a Philips 301 transmission electron microscope, operated at 80 kV.

Virology

Specimens of lung showing gross changes, and specimens of spleen were collected from each case for primary viral isolation in baby hamster kidney $(BHK)_{21}$ cells and for typing by the plaque neutralization test on African green monkey kidney (VERO) cells according to routine procedures. To measure virus concentration in the tissue specimens, 10% tissue suspensions were titrated in roller tubes of BHK_{21} cells (Erasmus, 1972).

Experimental cases

Animals: Two horses susceptible to AHS, which had been born and raised in insect-proofed buildings, were infected experimentally. During the experiment both horses were maintained in insect-proofed stables and fed hay and pellets containing cereal and lucerne. Neither horse was exercised. Morning and afternoon rectal temperatures were recorded and frequent inspections instituted. In both cases, disease was allowed to progress to death, with no therapeutic intervention. Age, sex and interim of these horses (Cases 7 and 8) are recorded in Table 1.

Infection: Case 7 was infected intravenously with a single dose of 10^6 tissue culture infective $doses_{50}$ ($TCID_{50}$) AHS virus, serotype 3, in the form of a suspension of infected BHK_{21} cell cultures. Case 8 was infected in the same manner with 10^4 $TCID_{50}$ AHS virus, serotype 7, in the form of a freeze-dried suspension of lung, obtained from natural case 5. In both cases, the virus concentration of the infective material was measured by titration in roller tubes of BHK_{21} cells (Erasmus. 1972).

Pathological and virological examinations: A full gross examination was conducted in both cases as soon as possible after death. Specimens were collected and prepared for LM, EM and virolgoical examinations as described for the natural cases.

Controls

A clinically normal, 6-year-old male horse, kept at Onderstepoort, was killed by stunning and exsanguination. Specimens from a wide range of tissues were collected at *c.* 30 min after death for LM and EM. Specimens were prepared as described for the natural cases.

Specimens of lung, lymph nodes and spleen for LM, and lung specimens for EM, were collected and prepared in the same manner from 30 horses at the municipal abattoir, Springs, Transvaal. Specimens were collected at *c.* 30 min after stunning and exsanguination. Additional lung specimens were stored at ambient temperature (20–25 °C) for intervals of 3 and 6 h before fixation in 4% buffered glutaraldehyde to investigate ultrastructural changes occurring post-mortem. Toluidine blue thick sections were prepared subsequently from this material. The youngest horses available were selected, their ages ranging from 4–9 years, as estimated by examination of the teeth. These horses had been gathered from a wide area of the country.

RESULTS

Clinical course of experimental cases

In Case 7 a febrile reaction (40,0–41,2 °C) developed on Day 5 post-infection and persisted until death on Day 8. In Case 8 a febrile reaction (40,1–40,8 °C) developed on Day 5 post-infection and persisted until Day 9. Death occurred on Day 10.

No other clinical features were recorded.

Gross pathology

Gross changes and their relative severity are summarized in Table 2.

TABLE 2 Summary of gross changes and their relative severity

Gross changes	Cases							
	1	2	3	4	5	6	7	8
Hydrothorax	4	4	4	4	4	2	0	1
Pulmonary oedema	4	4	4	4	4	3	2	2
Tracheal froth	4	4	4	4	3	0	0	0
Mediastinal oedema	3	2	2	2	2	1	1	2
Hydropericardium	0	2	0	0	1	3	3	4
Lymphadenopathy (general)	2	2	2	2	2	2	2	2
Lymphadenopathy (bronchial and mediastinal	4	4	4	4	4	2	2	2
Subendocardial haemorrhages	3	3	3	3	3	3	3	3
Oedema of atrioventricular valves	0	0	0	0	0	0	1	0
Subcutaneous oedema	0	0	3	0	0	2	3	3
Supraorbital oedema	0	0	0	0	0	0	0	3
Gastric and intestinal oedema	1	1	1	1	1	1	1	1
Mesenteric congestion	0	0	2	0	0	0	0	0

0: Lesion absent
1–4: Increasing severity (arbitrary relative units)

Natural cases 1–5

The respiratory system was most strikingly affected and showed remarkable conformity in all these cases.

Lesions in Case 6, however, resembled more closely those in Cases 7 and 8. For this reason, changes in Cases 6–8 are described collectively *(vide infra)*.

Respiratory system: Severe hydrothorax was a feature of all cases, the pleural cavity containing transparent, pale yellow, gelatinous fluid which ranged in volume from 3–6 ℓ.

The lungs in all cases showed diffuse subpleural and interlobular oedema. They failed to collapse completely when the thoracic cavity was opened, and were large and heavy. Subpleural oedema was more pronounced on the diaphragmatic surfaces and especially along the basal margins, where it was confluent with greatly thickened interlobular septa. In Cases 1 and 3, copious, focally extensive, subpleural fluid accumulations separated the pleura widely from underlying tissue, resulting in irregularly raised, pale yellow areas on the costal surfaces. The cut lung surface showed irregular, patchy congestion and copious, pale yellow fluid seeped from it on standing. Multiple, small foci of emphysema were distributed beneath the pleura, mainly in the caudal region of the costal surfaces, but varying slightly in distribution and extent from case to case. Subpleural petechiae and ecchymoses were disposed in groups over the costal and diaphragmatic surfaces. Both lung apices in Cases 1–3 were shrunken, dull-red and fleshy on palpation, suggestive of atelectasis.

The trachea in all cases was partially or totally filled by a white to pale-yellow, stiff froth. Small volumes of similar froth were evident at both nostrils in Cases 1–4. Yellow, gelatinous oedematous fluid overlaid segments of the tracheal mucosa in all cases. Oedema of the caudal part of the tracheal mucosa was discernible in Cases 2 and 4 but was particularly conspicuous in Case 2 in which it resulted in appreciable reduction in tracheal internal diameter.

The laryngeal mucosa was diffusely congested throughout in every case.

Mediastinal oedema was a consistent feature, with involvement of subpleural tissue, extending around the great vessels, oesophagus and perithymic connective tissue.

Heart: Scattered, subepicardial petechiae in the coronary grooves and left ventricular, subendocardial ecchymoses, particularly over the papillary muscles, were constant findings. A single suggillation was noted in the adventitia at the base of the pulmonary artery in Case 5. Hydropericardium was present in Cases 2 and 5 only, but was more severe in Case 2 in which the pericardial cavity contained *c.* 100 mℓ of pale fluid. The ventral region of the pericardium was thickened by oedema fluid in Case 1.

Lymph nodes and spleen: Lymph nodes consistently showed some degree of enlargement. Bronchial and mediastinal nodes were most markedly enlarged and displayed a 2–3 fold size increase compared to controls. Colourless fluid oozed from their cut surfaces.

Lymphoid follicles were conspicuous on the cut surfaces of all the lymph nodes and were particularly prominent in the more congested nodes. Interfollicular tissue showed various degrees of congestion. Apart from prominence of the white pulp the spleen was normal in size in all the cases.

Gastro-intestinal tract: Moderate, diffuse to patchy mucosal congestion involving the glandular part of the stomach was a consistent finding. This was accompanied by patchy congestion of the serosal surface of the entire length of the small intestine and a few widely scattered petechiae throughout the abdominal serosal surfaces. Mild mesenteric oedema was present in Case 3.

Musculoskeletal system: Oedema of the intermuscular fascial planes of the neck, extending from the jugular furrow dorsally to involve the ligamentum nuchae, was noted in Case 3. Fascial or subcutaneous oedema was not seen in the other cases.

A slight increase in the volume of synovial fluid was appreciated in many of the joints.

Numerous ecchymoses were distributed in the ventral surface of the tongue in Case 5.

Other organs: The only other noteworthy changes included slight hepatic swelling and moderate congestion with a faint lobular pattern, mild to moderate renal congestion, and petechial haemorrhages in the thymus of Case 3.

Experimental Cases 7 and 8, and natural Case 6

In these cases the pattern of gross changes was uniform. Lesions were similar in nature to those of Cases 1–5, but differed from these in their relative severity and distribution (Table 2).

Respiratory system: Cases 6 and 8 had a moderate hydrothorax (*c.* 200–500 mℓ fluid).

The lungs were moderately congested and collapsed normally throughout most of their area when the thoracic cavity was opened. Small, bilateral areas of the caudal pulmonary tissue, however, failed to collapse completely and remained thicker than the surrounding tissue. Features in these areas resembled closely those of the pulmonary tissue in Cases 1–5. Subpleural and interlobular oedema was conspicuous and the cut surfaces oozed yellow fluid. Multiple petechiae were present over the diaphragmatic surface of both lungs in Case 8.

The trachea and bronchi contained no fluid. The tracheal mucosa was moderately congested in Case 8. Multiple mucosal petechiation and mild peritracheal oedema were features of Cases 6 and 7.

The larynx showed congestion and petechiation in Case 7.

Mild oedema of the dorsal mediastinum was present in all cases and extended around the aorta. Subpleural oedema of the dorsal thoracic wall was marked in Case 8.

Heart: Severe hydropericardium was present in all cases. In Case 8 the pericardial sac was distended by *c.* 1ℓ of fluid.

Subepicardial petechiae in the coronary grooves, and subendocardial ecchymoses, disposed over the papillary muscles, were consistent features. Slight oedema of the right and left atrioventricular valve flaps was evident in Case 7.

Lymph nodes and spleen: All lymph nodes were moderately enlarged and lymphoid follicles were prominent. Many of the nodes showed various degrees of congestion. The marked enlargement of bronchial and mediastinal nodes, with wet cut surfaces, as noted in Cases 1–5, was not in evidence. Splenic white pulp was prominent.

Gastro-intestinal tract: The gastric mucosa was moderately congested and the small intestine showed patchy serosal congestion. Petechiae were widely distributed over the abdominal serosal surfaces. Petechiation was particularly intense throughout the *taeniae coli* in Case 7.

Musculoskeletal system: Bilateral supraorbital swelling was marked in Case 8 and oedema of the supraorbital fat was conspicuous. Subcutaneous oedema, extending to involve underlying intermuscular fascia, was distributed

over the occipital and dorsal cervical areas in Cases 7 and 8, over the lateral thorax on both sides in Case 7, and over the thoracic inlet, extending to the peritracheal connective tissue in Case 6.

Small haemorrhages were disposed in the ventral surface of the tongue in Cases 7 and 8.

The volume of synovial fluid was slightly increased in most of the joints.

Histopathology

Natural Cases 1–5.

Lung: In all these cases the microscopical features were remarkably similar, and were represented in sections from diverse sites, which suggested that their distribution was both diffuse and extensive. The changes showed more variation from one area to another in the same lung than from case to case.

Exudate was plentiful within alveolar spaces in most areas. The relative cellular and extracellular constitution of this exudate varied markedly throughout (Fig. 1–10), with fibrin (Fig. 1–3), which stained positively with MPAH and with the PAS reaction, protein-rich fluid (Fig. 4), or inflammatory cells (Fig. 5 & 6) predominating. Fibrin, however, was generally the most extensively represented constituent. The amount of fibrin ranged from a few strands to dense masses filling the alveolar space. Alveolar spaces containing most abundant fibrin frequently showed a patchy distribution, alternating with groups of empty, distended alveolar spaces (Fig. 1–3). This pattern appeared to be random and no relation to larger blood vessels or to airways could be established. Macrophages possessing abundant, eosinophilic, finely granular to vacuolated cytoplasm occupied some alveolar spaces. In toluidine blue sections a close association between some of these cells and fibrin was demonstrable (Fig. 9). Numerous neutrophils were present in some areas, and alveolar spaces were completely filled by a homogeneous, strongly eosinophilic material suggestive of protein-rich oedema fluid in other areas. Occasional extravasated erythrocytes were present.

Alveolar capillaries were congested (Fig. 6 & 7), and contained increased numbers of neutrophils, lymphocytes and monocytes. Separation of Type 1 pneumocytes from the alveolar wall was occasionally apparent in HE sections (Fig. 8). This feature was demonstrated more clearly in toluidine blue thick sections of alveoli containing abundant oedema fluid in which thin cytoplasmic segments were separated from the alveolar wall over most of the alveolar perimeter (Fig. 10).

Many arterioles and venules contained numerous mononuclear cells, which included lymphocytes and monocytes. The lumens of occasional vessels were completely filled with these cells (Fig. 11 & 12).

Perivascular changes, characterized by a striking separated appearance of the adventitial connective tissue and by a perivasculitis, characterized by adventitial inflammatory cell accumulations of varying intensity (Fig. 13, 14, 15 & 16) were in evidence. These cells comprised mainly lymphocytes, some monocytes and macrophages, and occasional neutrophils and plasma cells. The cells tended to obscure vascular structure so that the identity of the vessels involved was not always clear. However, the recognition of occasional transversely disposed smooth myocytes and the demonstration of an elastica interna by aldehyde fuchsin staining (Fig. 15) indicated that small arterioles were the principal site of involvement. The media and intima of these vessels were apparently unaffected.

The air passages were moderately dilated. Many were empty, while others contained homogeneous, eosinophilic material (Fig. 13, 17, 18, 19 & 20), fibrin coagula (Fig. 18 & 19) and neutrophils and macrophages (Fig. 18 & 19) in varying amounts and proportions. In occasional bronchioles the *lamina propria* was thickened by the presence of vacuolated eosinophilic material, which also caused epithelial separation and disruption (Fig. 20). The mucosal changes were limited to bronchioles in areas of most abundant exudation.

Scattered, bronchiole-associated lymphoid nodules (Fig. 20 & 21) were apparent in most sections in Cases 1, 3, 4 and 5. The nodules were situated within connective tissue, closely adjacent to larger bronchioles, and possessed a well-organized structure, being composed of masses of lymphocytes, interspersed with reticular macrophages. At the centre of these nodules there were occasional depletion and karyorrhexis of lymphocytes (Fig. 21). A loose framework of reticular fibres was demonstrated by Gomori's reticulum stain (Fig. 22).

In the widened interlobular and subpleural tissue noted grossly, collagen strands presented an irregular, separated appearance (Fig. 1, 23 & 24). A fine lacework of fibrin was present throughout, and was demonstrable within lymphatics (Fig. 24). Small blood vessels and lymphatics were widely dilated. Scattered groups of lymphocytes and occasional macrophages and lymphocytes were present in most areas (Fig. 23).

Lymph nodes: Bronchial and mediastinal lymph nodes were affected by oedema which was made obvious by dilatation of sinuses and by a separation of lymphoid cells, one from another, giving both medulla and cortex a loose appearance (Fig. 25). Sinuses contained scattered erythrocytes and occasional fibrin strands. Oedema of the capsule was conspicuous in bronchial nodes from Cases 1 & 3 (Fig. 26). Mild oedema was apparent in other nodes examined.

Germinal centres of most lymph nodes examined showed mild to pronounced central depletion of lymphocytes, leaving reticular macrophages prominent (Fig. 27). Karyorrhexis was often present (Fig. 28). There was little evidence of lymphopoietic activity, which was limited to a narrow border of small lymphocytes surrounding most of the germinal centres. The number and size of germinal centres and the cellularity of the medulla did not differ detectably from those of the controls.

Spleen: In all cases the germinal centres of the white pulp appeared larger and more numerous than in the controls. Lymphoid depletion was a feature of all germinal centres examined, varying from moderate, with few lymphocytes remaining, to severe, with lymphocytes absent and only the prominent reticular macrophages remaining (Fig. 29 & 30). Nuclear pyknosis and karyorrhexis of lymphoid cells were marked in the depleted centres. There was little evidence of active lymphopoiesis compared to that in controls.

Thymus: Mild thymic congestion was evident in all cases. Multiple, small foci of haemorrhage were present throughout in Case 3. Connective tissue strands of the capsule in Case 3 presented a separated appearance, consistent with perithymic oedema. Several focal accumulations of eosinophils were disposed in the medulla in Cases 1 and 3.

Heart: Subepicardial and subendocardial haemorrhages, some of which extended into underlying myocardium, were accompanied by fibrin and had dissected between myocardial fibres. Mild oedema was apparent around occasional vessels in the myocardium. Neutrophils were increased moderately in number within capillaries throughout the myocardium. Moderate epicardial

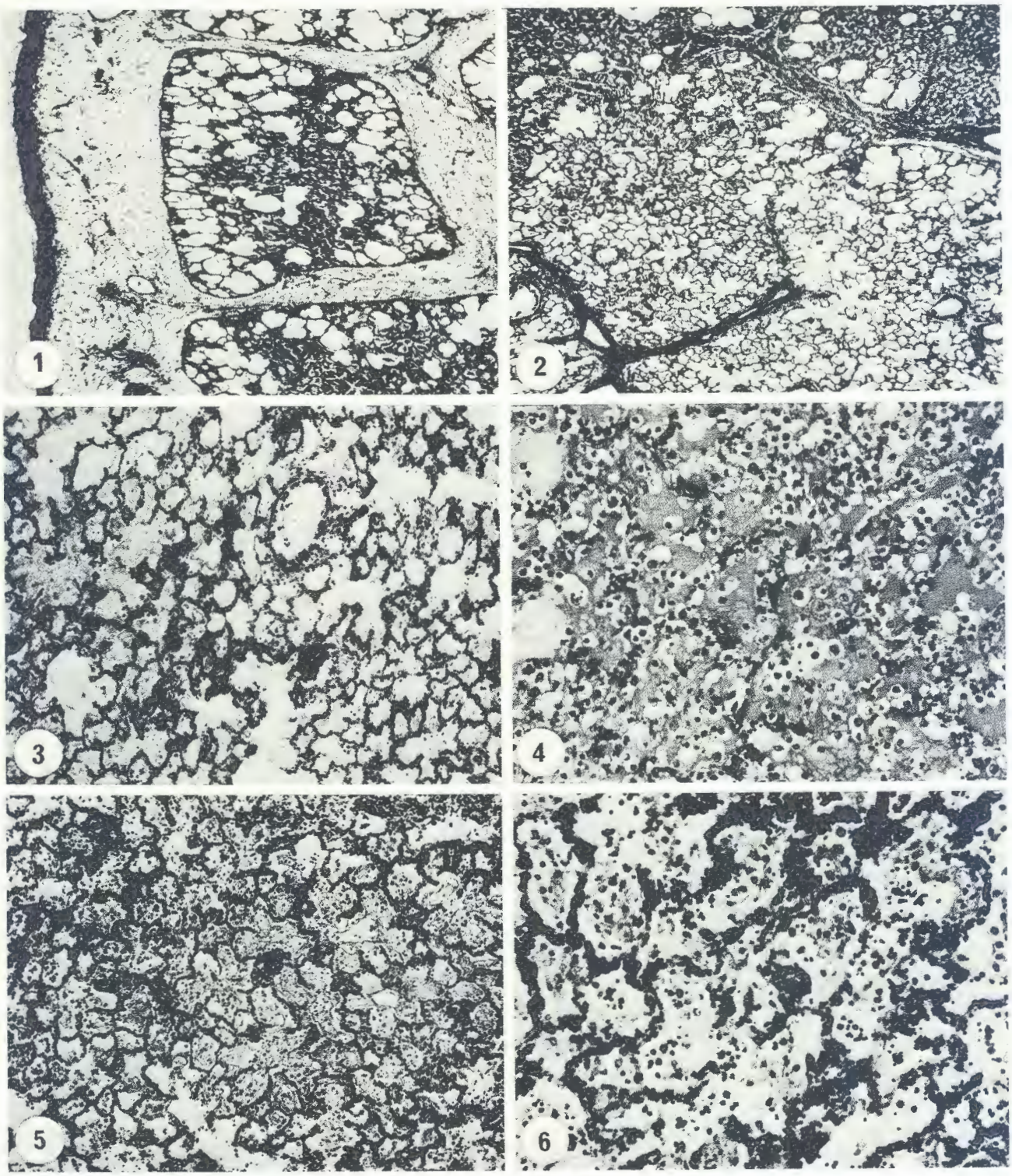


FIG. 1 Subpleural and interlobular oedema, alveolar distension and patchy distribution of fibrin within alveolar spaces: MPAH × 30
FIG. 2 Patchy distribution of intra-alveolar fibrin: MPAH × 30
FIG. 3 Patchy distribution of alveolar fibrin and mild intra-alveolar inflammatory cell infiltration: HE × 75
FIG. 4 Alveolar oedema, numerous inflammatory cells and a fine fibrin deposit: HE × 200
FIG. 5 Fibrin and numerous inflammatory cells within alveolar spaces, and alveolar capillary congestion: HE × 75
FIG. 6 Numorous intra-alveolar inflammatory cells. Capillary congestion: HE × 200

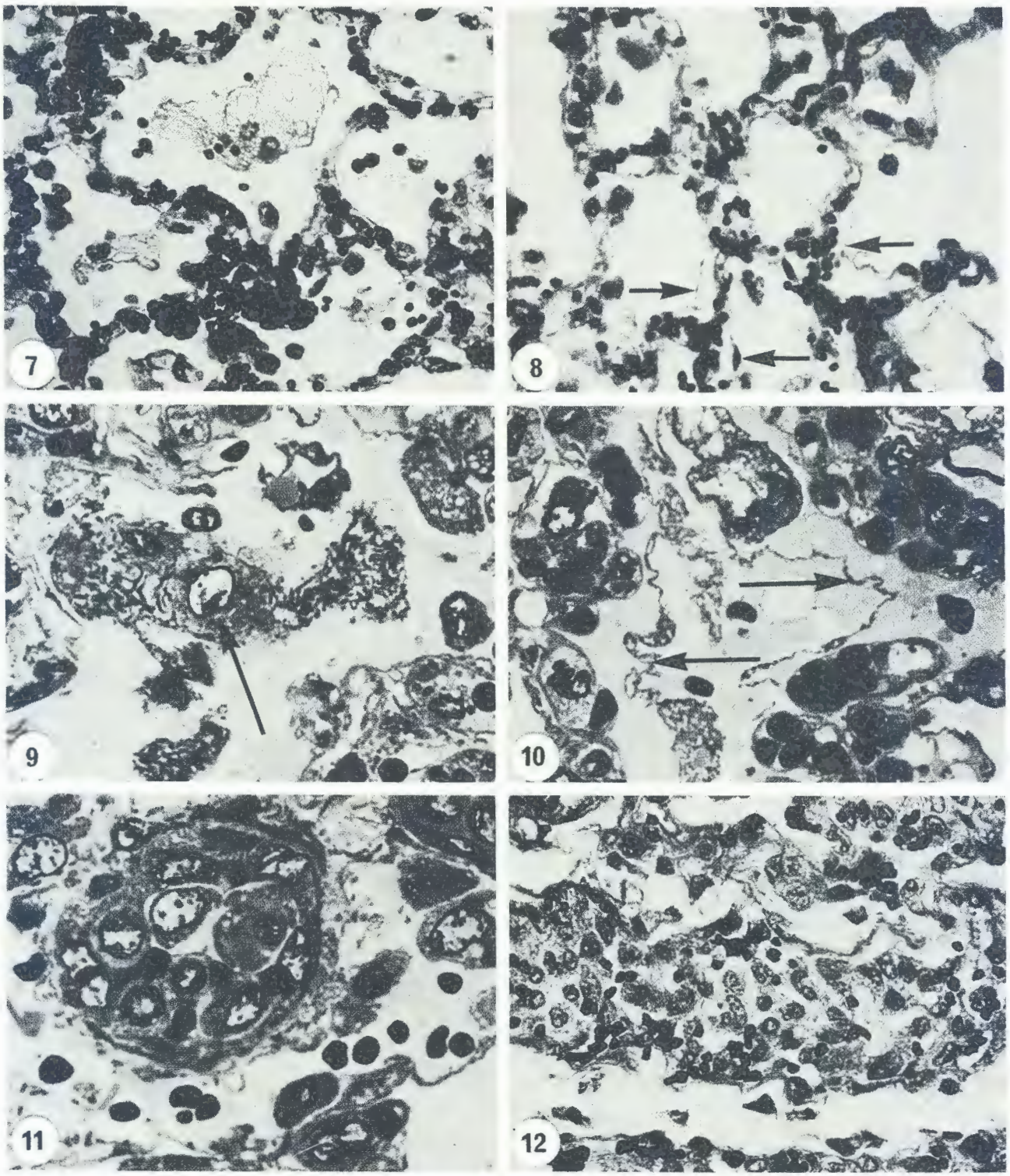


FIG. 7 Intra-alveolar fibrin and inflammatory cells. Pronounced capillary congestion: HE × 200

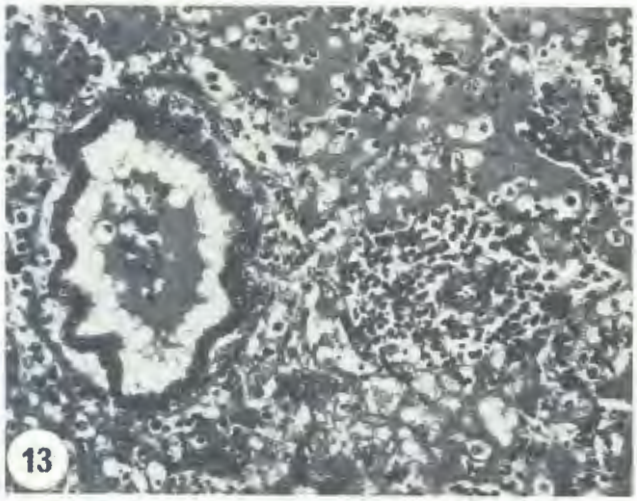
FIG. 8 Separation of pneumocytes (arrowed) from alveolar walls: HE × 200

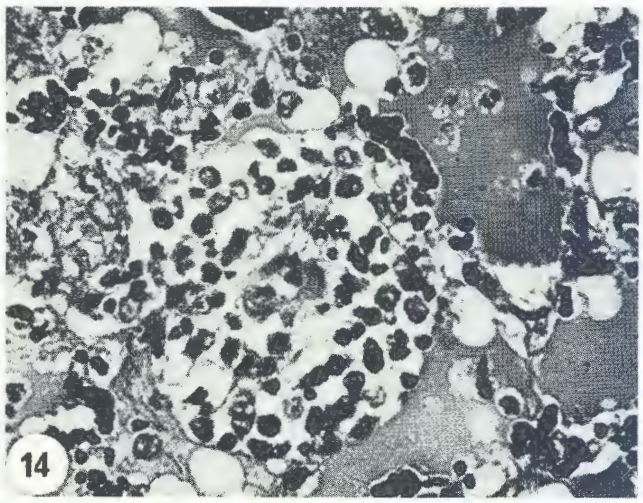
FIG. 9 Alveolar macrophage (arrow) closely associated with fibrin. Epon section, toluidine blue: × 1 200

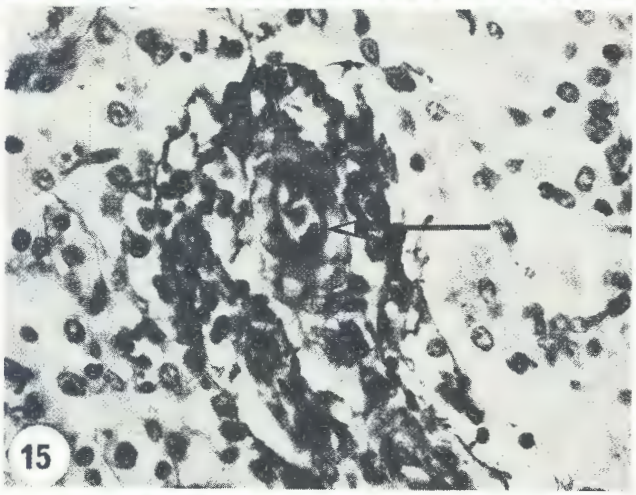
FIG. 10 Separation of pneumocyte cytoplasm (arrowed) from alveolar wall. Epon section, toluidine blue: × 1 200

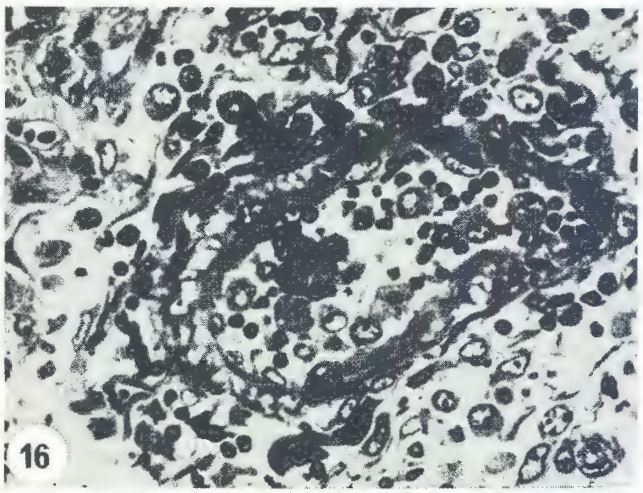
FIG. 11 Lumen of small pulmonary blood vessels filled with mononuclear cells. Scattered perivascular erythrocytes and inflammatory cells. Epon section, toluidine blue: × 1 200

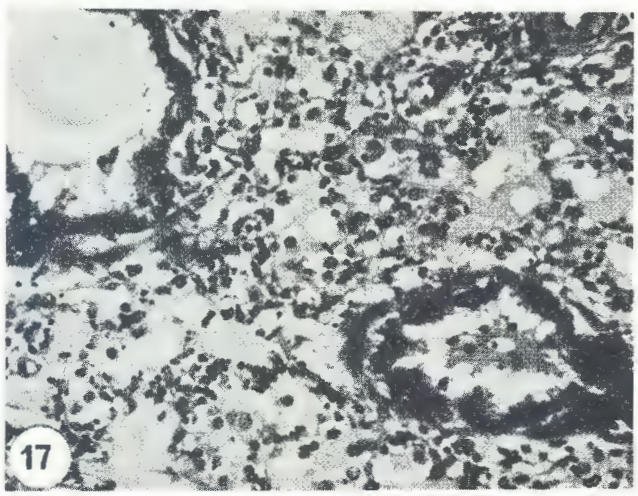
FIG. 12 Small pulmonary blood vessel packed with leucocytes: HE × 500











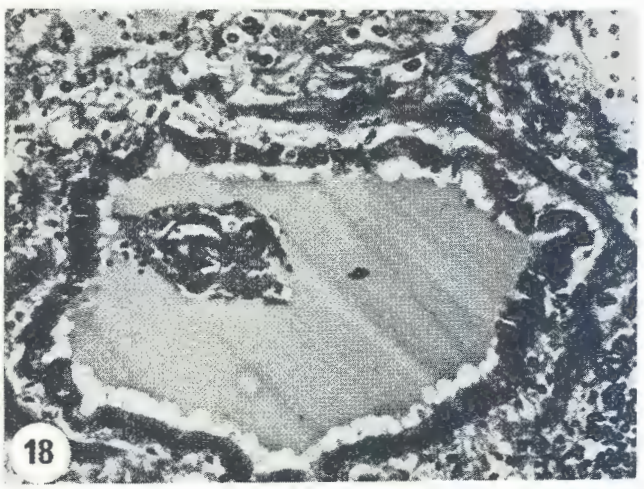


FIG. 13 Bronchiole containing homogeneous material and leucocytes. Adjacent arteriole with pronounced perivascular inflammatory cell infiltrate: HE × 200

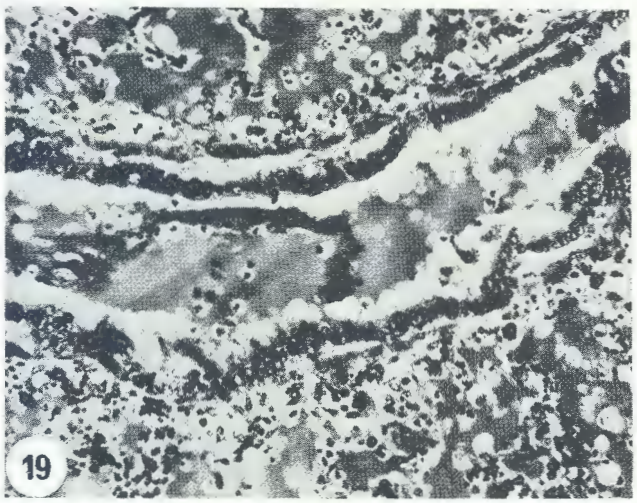
FIG. 14 Pronounced periarteriolar mononuclear cellular infiltrate: HE × 500

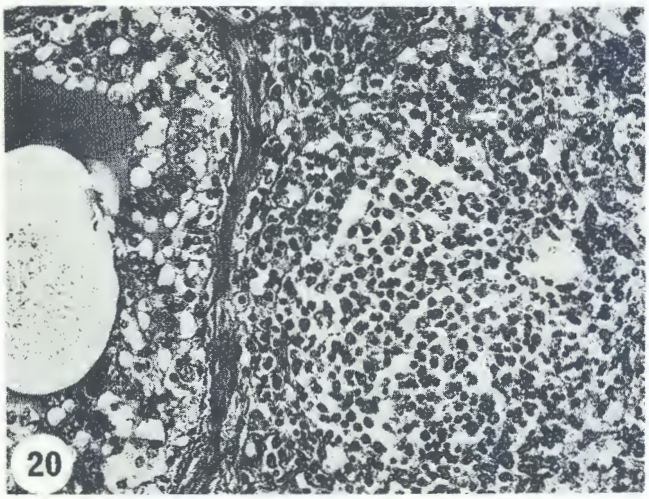
FIG. 15 Small arteriole, in lung (arrow), showing moderate perivascular cellular infiltration: Aldehyde fuchsin × 500

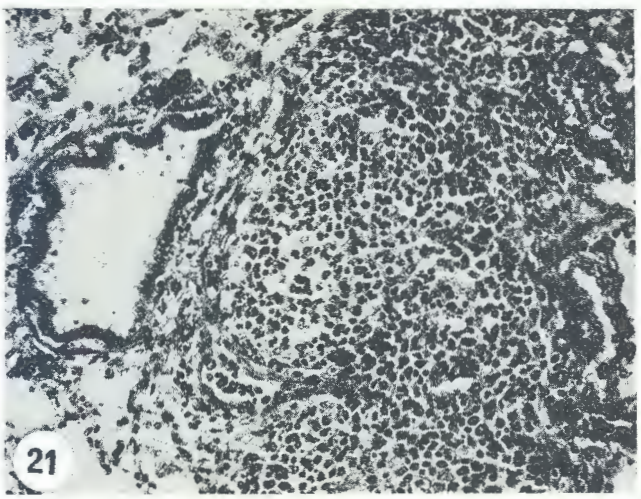
FIG. 16 Arteriole in lung, showing numerous leucocytes in the lumen, separated appearance of advential connective tissue and scattered perivascular erythrocytes and inflammatory cells: Epon section, toluidine blue × 500

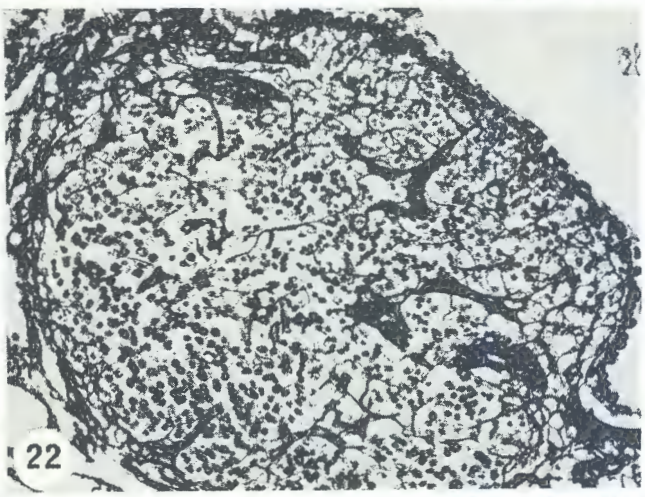
FIG. 17 Bronchioles in an oedematous area, containing homogeneous material: HE × 100

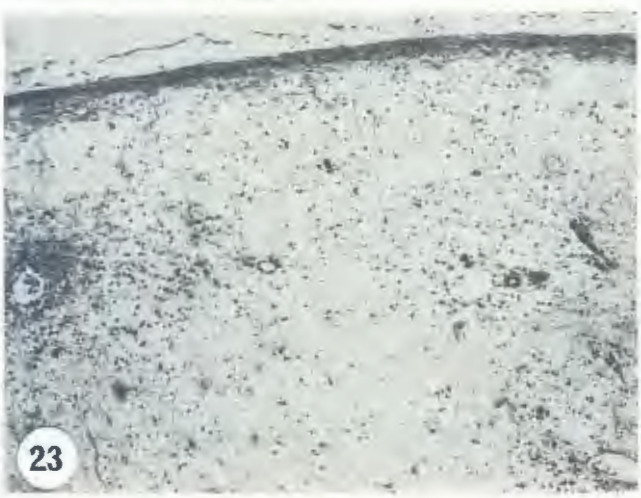
FIG. 18 Bronchiolar lumen filled with homogeneous material and a small fibrin coagulum: HE × 200











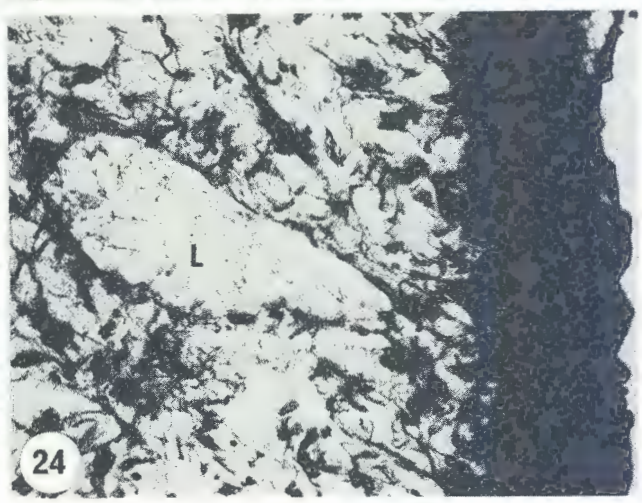
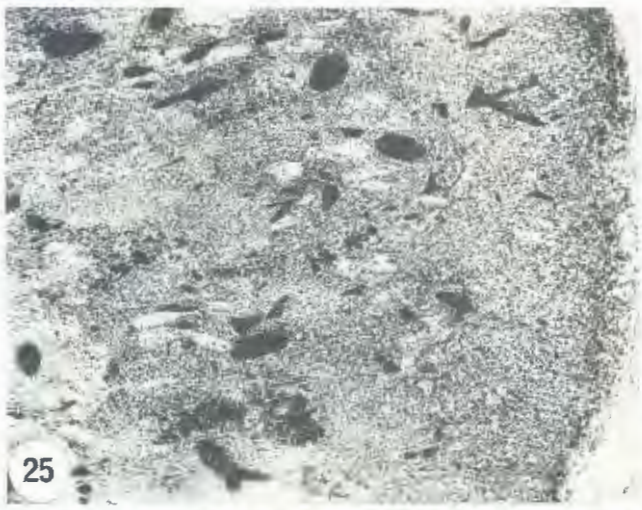
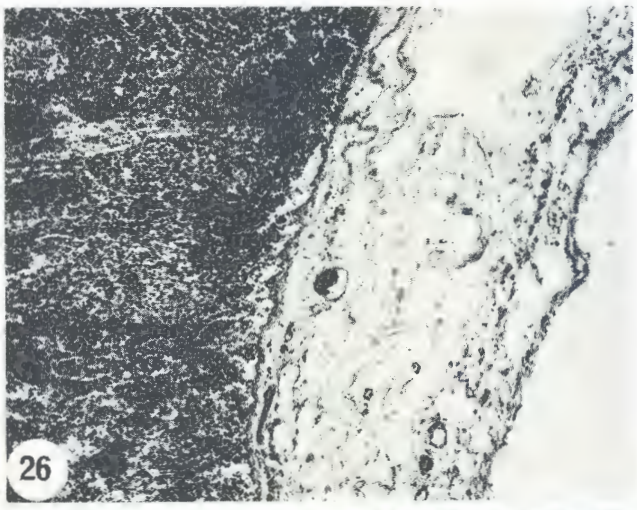
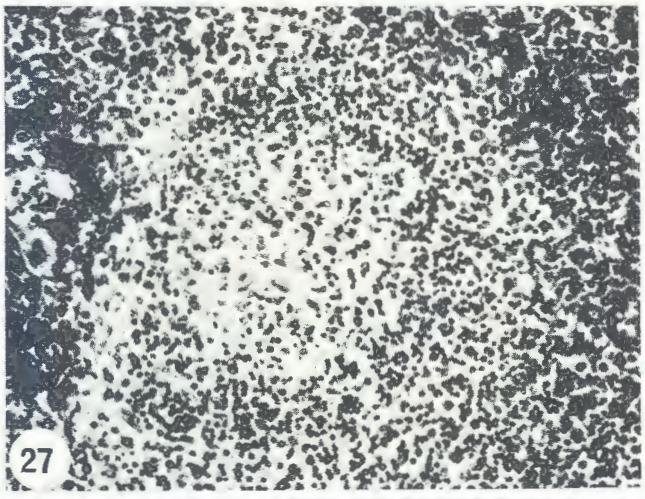
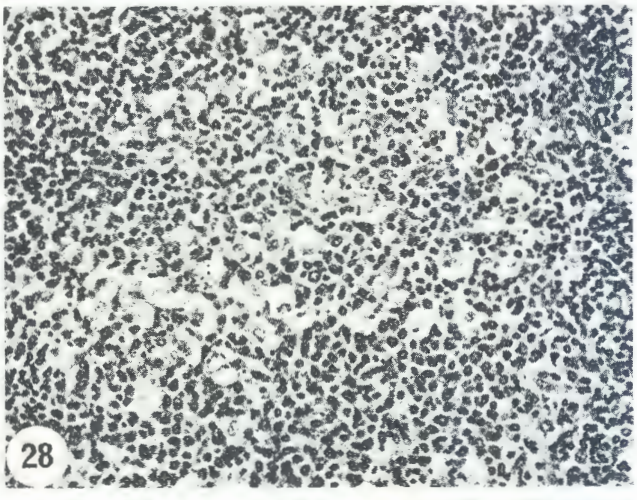


FIG. 19 Bronchiole containing homogeneous material, inflammatory cells and a fibrin coagulum: HE × 200
FIG. 20 Bronchiolar epithelial disruption associated with vacuolation. Bronchiole-associated lymphoid nodule: HE × 200
FIG. 21 Bronchiole-associated lymphoid nodule, showing central lymphoid depletion and karyorrhexis: HE × 200
FIG. 22 Bronchiole-associated lymphoid nodule, demonstrating the loose framework of reticular fibres. Gomori's reticulum method: × 200
FIG. 23 Subpleural oedema, showing dilated lymphatics and scattered inflammatory cells: HE × 100
FIG. 24 Subpleural oedema, showing separation of connective tissue, dilated lymphatic and fine, lace-like deposit of fibrin: MPAH × 500









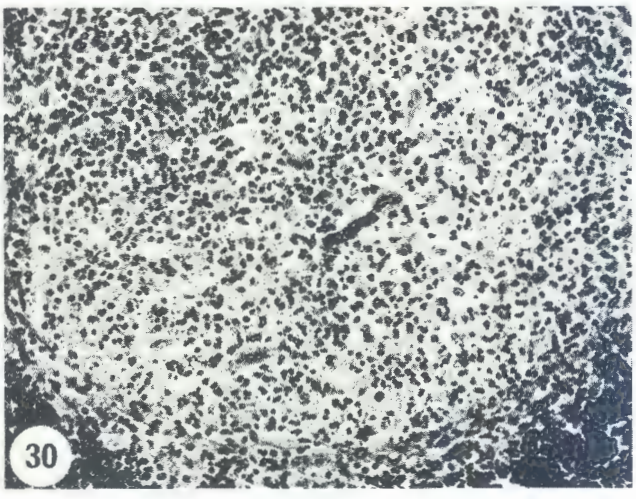
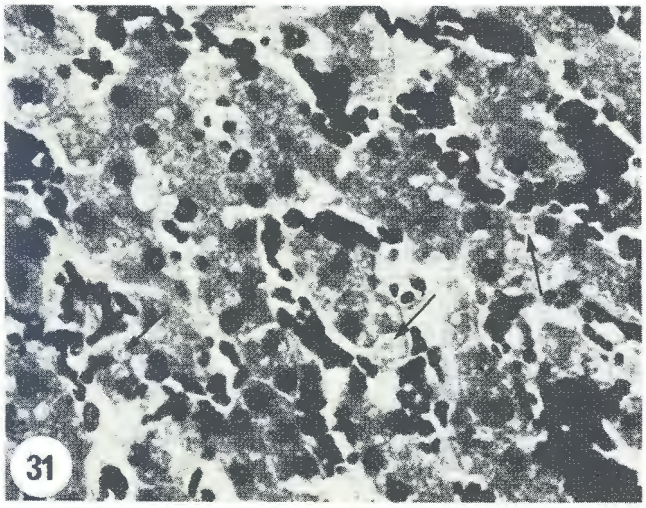
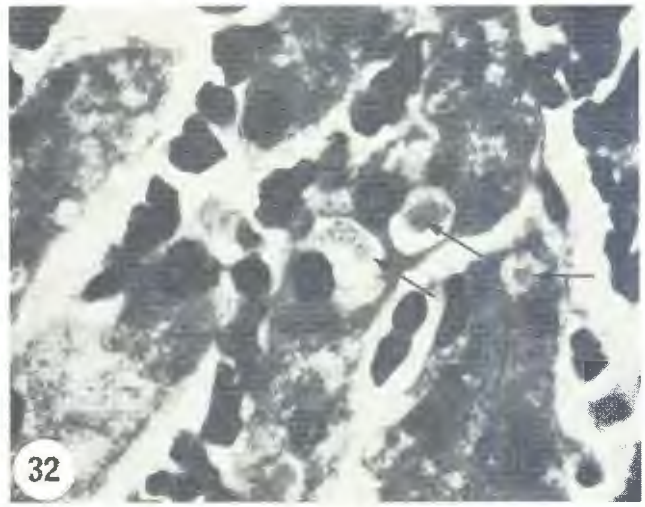
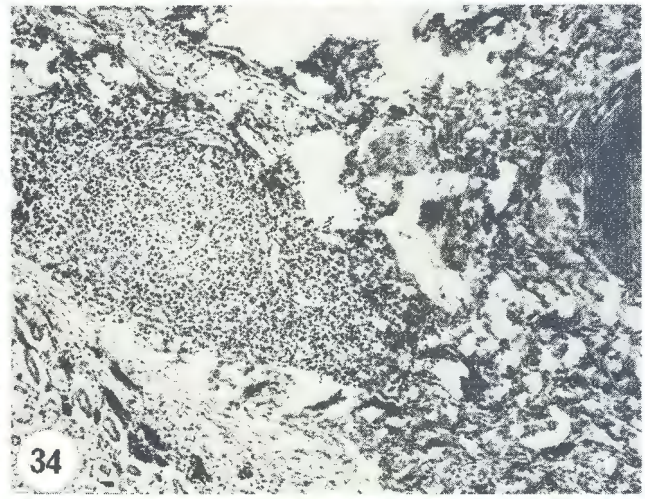


FIG. 25 Bronchial lymph node, showing congestion, sinus dilatation and separated appearance of lymphocytes: HE × 75
FIG. 26 Bronchial lymph node, showing capsular oedema: HE × 200
FIG. 27 Lymph node germinal centre, showing central lymphoid depletion: HE × 200
FIG. 28 Lymph node germinal centre, showing lymphoid depletion and karyorrhexis: HE × 200
FIG. 29 Marked lymphoid depletion in germinal centres of splenic white pulp: HE × 30
FIG. 30 Splenic germinal centre, showing lymphoid depletion and karyorrhexis: HE × 200







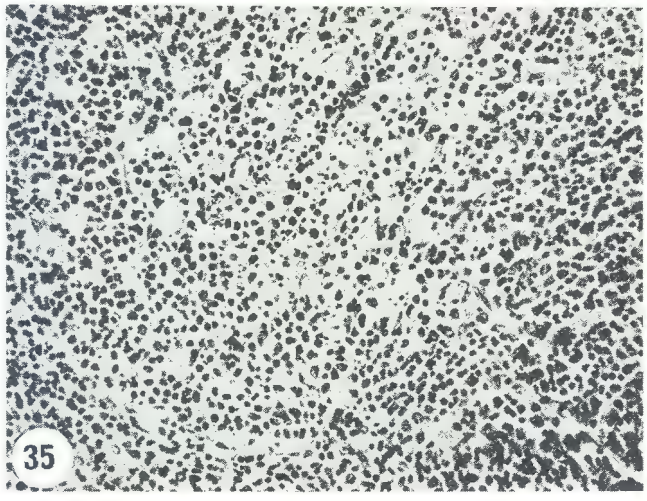
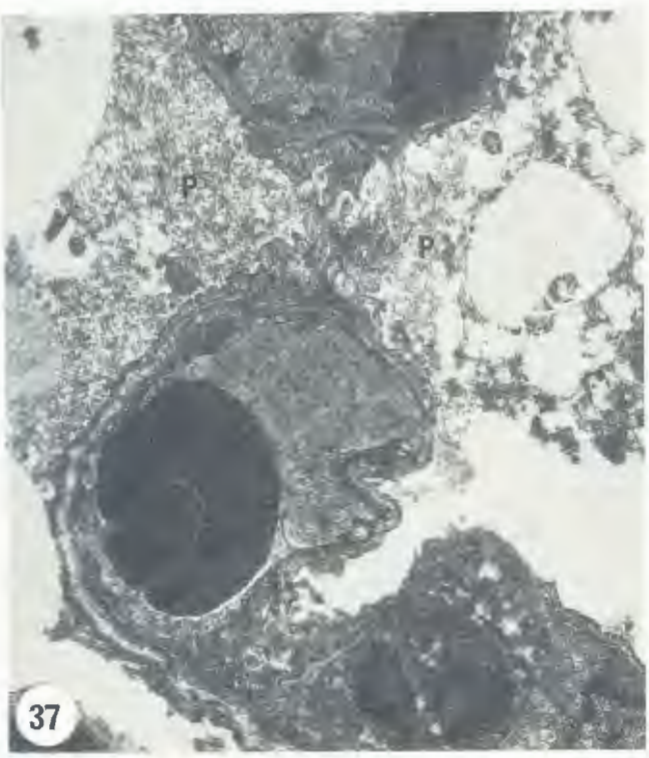
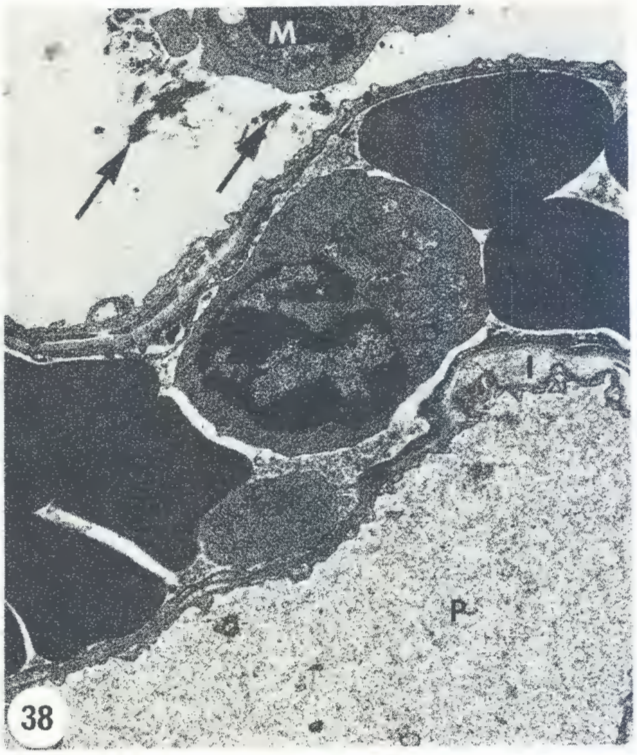
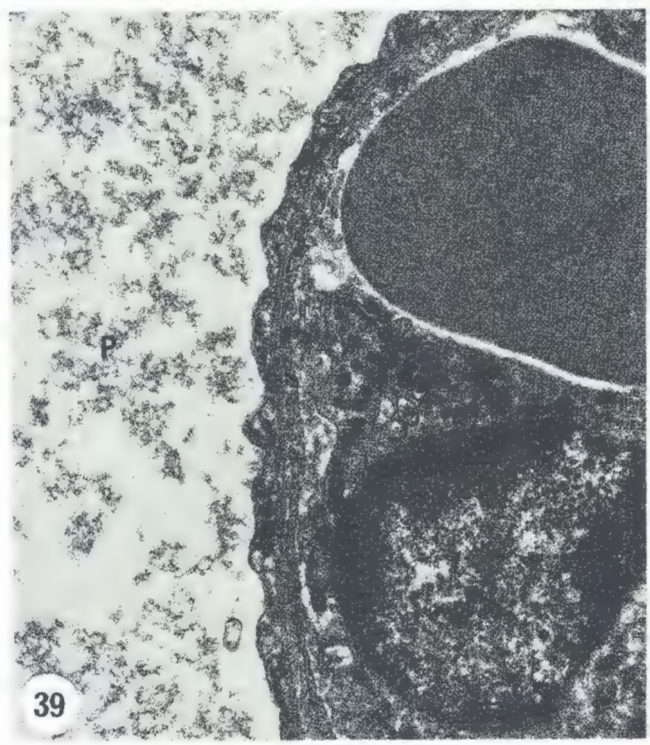


FIG. 31 Liver, showing multiple, intracytoplasmic inclusions in hepatocytes (arrowed): HE × 500
FIG. 32 Intracytoplasmic inclusions in hepatocytes (arrowed): HE × 1 200
FIG. 33 Multiple foci of extreme vasodilatation and associated interstitial oedema in renal medulla (Case 5): HE × 75
FIG. 34 Small lymphoid nodule with central lymphoid depletion, adjacement to a renal sublobular artery (Case 5): HE × 75
FIG. 35 Lymph node germinal centre. Lymphoid depletion and karyorrhexis: HE × 200
FIG. 36 Focus of necrosis in interventricular myocardium. Transverse bands are arrowed (Case 8)







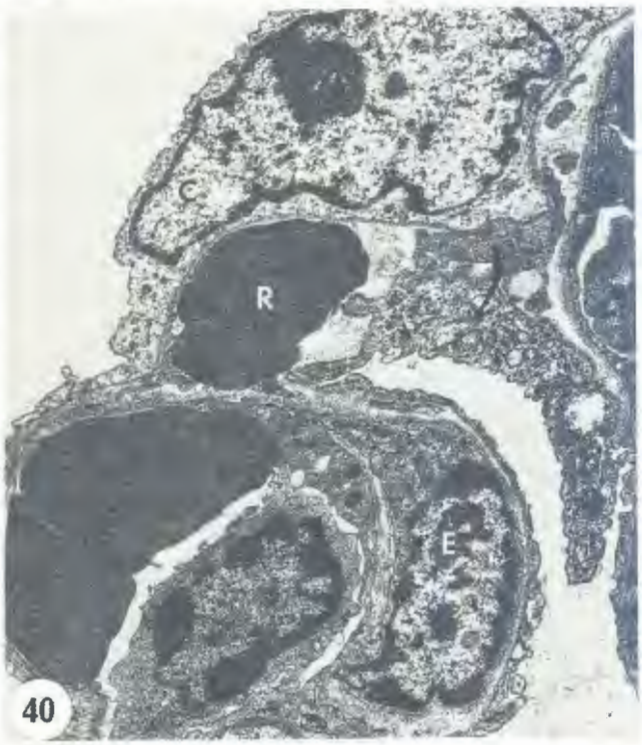
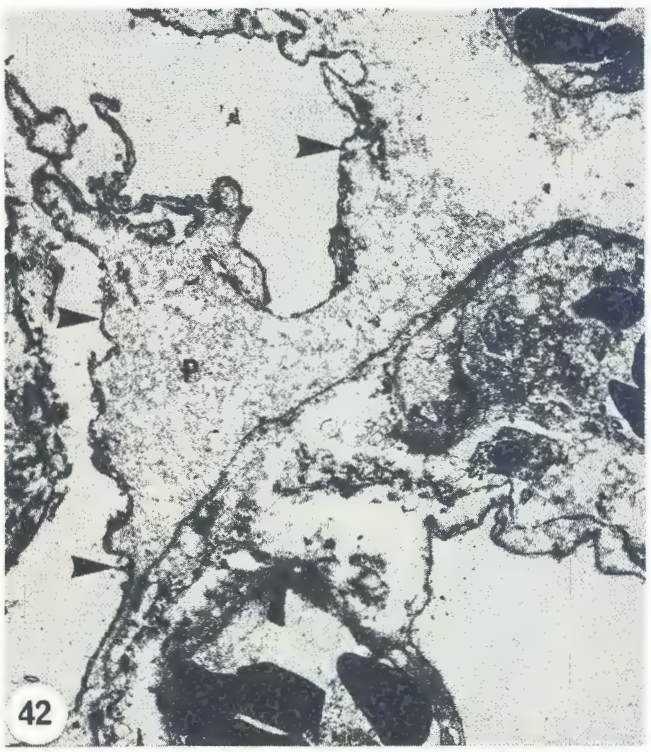


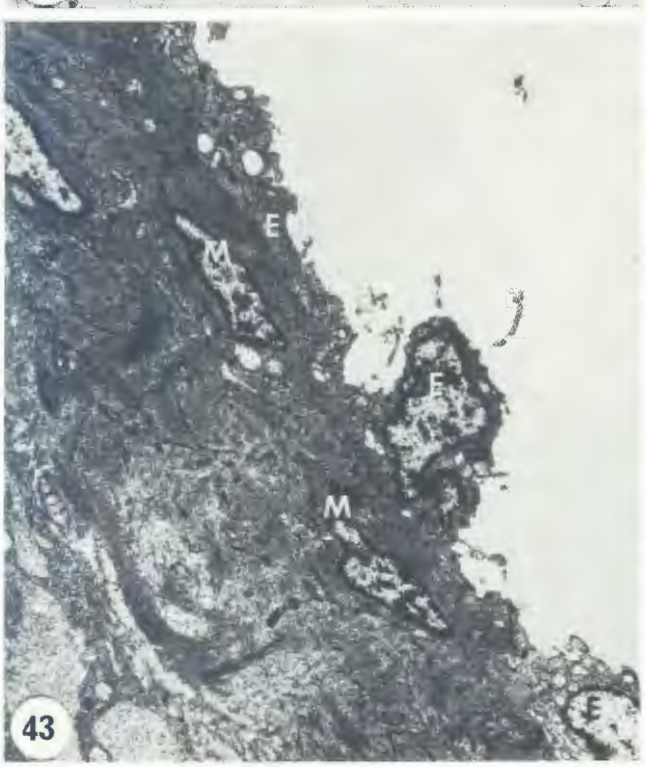
FIG. 37 Alveolar capillary with flocculent material, suggesting protein (P), in the alveolar space: × 7 000

FIG. 38 Flocculent material (P) and fibrin (arrowed) in alveolar spaces, widened interstitial space (I) and alveolar macrophage (M). A lymphocyte is present within the capillary: × 8 000

FIG. 39 Flocculent material (P) in alveolar space, and normal alveolar wall structure. A neutrophil is present within the capillary: × 11 000

FIG. 40 Alveolar wall from oedematous area showing Type I pneumocyte (C), endothelial cell (E), widened interstitial space and an erythrocyte (R) within the interstitial space. A leucocyte is present within the capillary. × 7 000





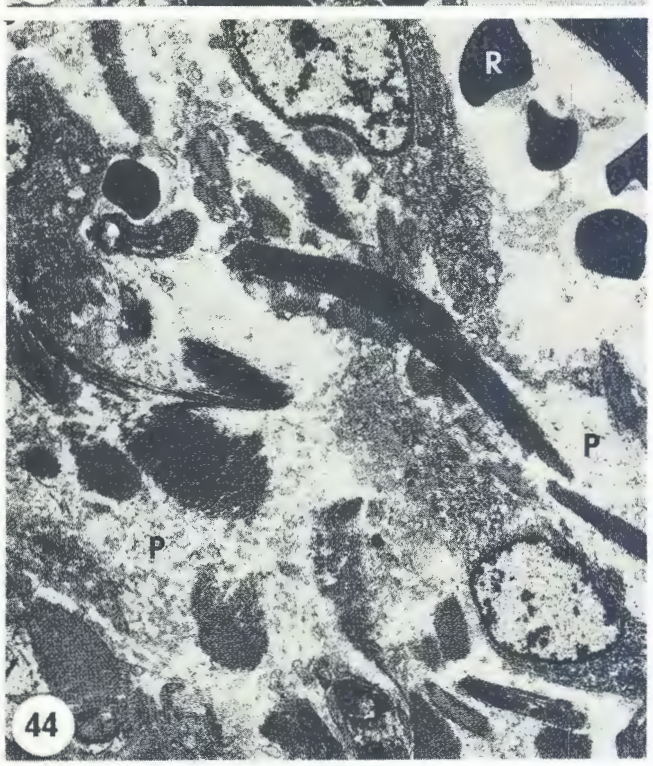


FIG. 41 Separation of Type I pneumocyte cytoplasm (arrowheads) from alveolar wall, widened interstitial space (I) and interstitial fibrin (arrowed): × 3 800

FIG. 42 Separation of Type I pneumocyte (arrowheads) from the alveolar wall. Flocculent material (P) occupies the resultant space: × 3 800

FIG. 43 Pulmonary arteriole, showing intact endothelium (E) and myocytes (M): × 3 000

FIG. 44 Adventitia of pulmonary arteriole, showing separation of fibroblasts and collagen fibres by flocculent material (P). Extravasated erythrocytes (R) are present: × 3 000

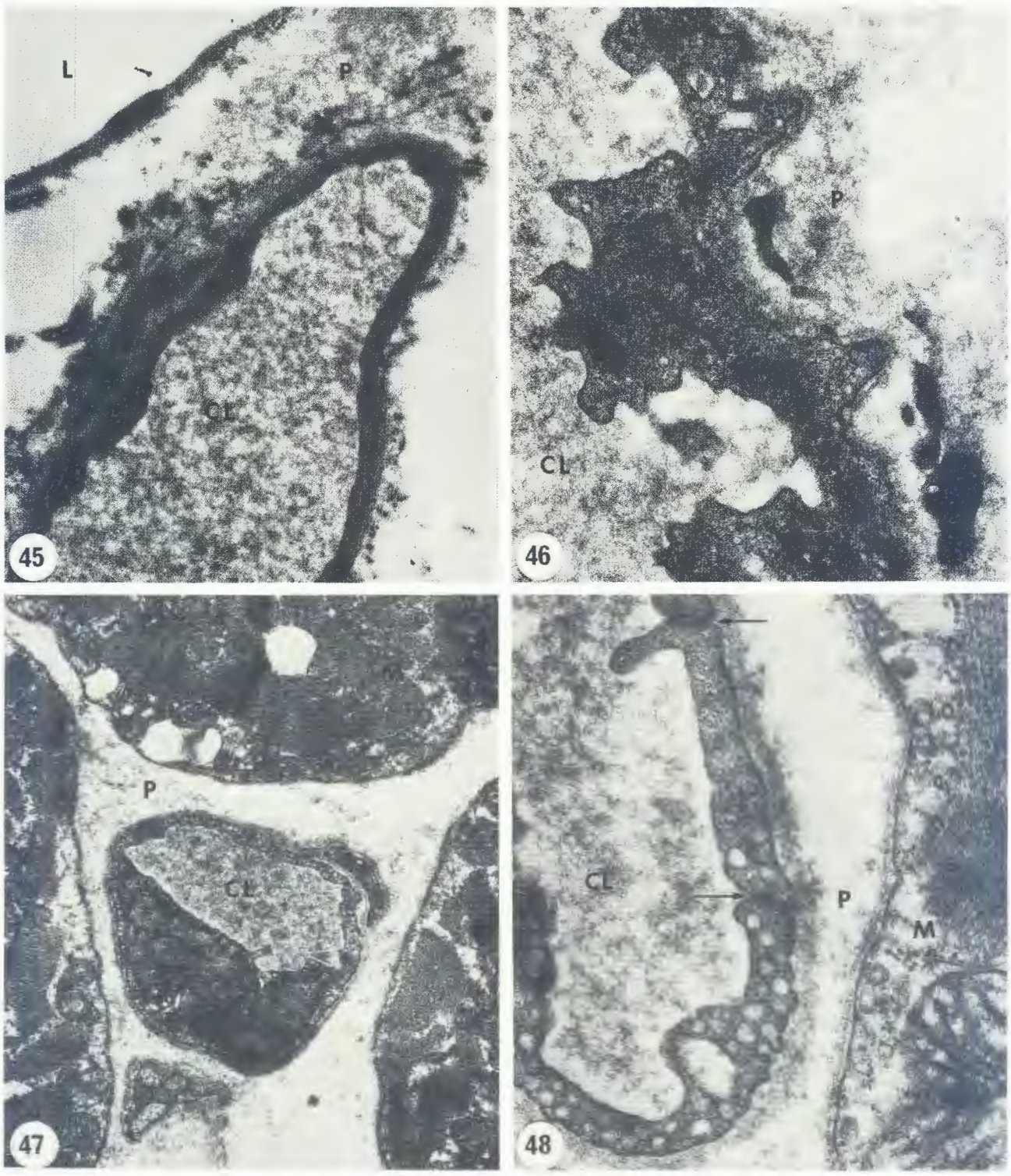


FIG. 45 Capillary in epicardial fat, showing capillary lumen (CC), endothelium, adjacent lipocyte (L), and interstitial flocculent material (P): × 12 000

FIG. 46 Capillary in epicardial fat, showing capillary lumen (CL), normal endothelial structure and extravascular flocculent material (P): × 25 000

FIG. 47 Myocardial capillary, showing capillary lumen (CL), endothelium, cardiac myocyte (M) and increased capillary space containing flocculent material (P): × 9 000

FIG. 48 Myocardial capillary, showing capillary lumen (CL), myocyte (M), increased pericapillary space, containing flocculent material (P), and normal endothelial structure with cytoplasmic vesicles and closed junctions (arrowed): × 50 000

oedema within subepicardial connective tissue and between individual adipose cells was present in Cases 2, 3 and 5.

Liver: Moderate centrilobular congestion was evident in most lobules of all cases.

Hepatocytes showed moderate cloudy swelling, with fine vacuolation in some areas, suggesting early hydropic degeneration.

Strongly eosinophilic hyaline bodies, each surrounded by a sharply defined halo, occurred in the cytoplasm of hepatocytes (Fig. 31 & 32). These bodies were strongly PAS positive with resistance to diastase digestion, indicating that they were non-glycogenic, and, including the halo, they ranged from 4–12 μm in diameter. They were distributed patchily and randomly in all cases and appeared more numerous in Case 2, where they occupied approximately 1–5% of hepatocytes. They were disposed singly and as occasional pairs or small groups within the affected hepatocytes. There was no evidence of any similar bodies outside the hepatocytes or in Kupffer cells.

The connective tissue of the portal areas contained scant infiltrates of small lymphocytes and plasma cells.

Kidney: Moderate congestion of cortex and medulla was evident in all cases. Tubular epithelium exhibited degenerative changes ranging from mild cloudy swelling to hydropic degeneration.

In the medulla of both kidneys in Case 5, there were multiple foci of intense congestion and associated interstitial oedema with scattered, extravasated erythrocytes present (Fig. 33).

A lymphoid nodule, showing moderate central lymphoid depletion with karyorrhexis, was observed adjacent to a sublobular artery at the corticomedullary boundary in Case 5 (Fig. 34).

Gastro-intestinal tract: Apart from moderate congestion of the gastric mucosa no inflammatory changes were apparent.

Mild subserosal oedema was in evidence in the jejunum of Case 5. Intestine-associated mucosal lymphoid nodules displayed central lymphoid depletion and necrosis with little evidence of activity, similar to the changes observed in lymphoid tissue elsewhere.

Adrenal: Moderate congestion of cortex and medulla, seen in all cases, was the only change detected.

Central nervous system: Moderate congestion of blood vessels in meninges, brain and spinal cord, and occasional small haemorrhages, were the only changes detected.

Experimental Cases 7 and 8, and natural Case 6

Lung: Sections from the oedematous areas noted grossly revealed changes resembling all those described for Cases 1–5. Fibrin exudation, however, was less abundant, and alveolar and perivascular inflammatory infiltration was generally less intense.

In the areas that were not recognized grossly as oedematous, mild alveolar oedema was observed. Alveolar macrophages were rarely seen. Alveolar capillaries and larger blood vessels were moderately congested and contained increased numbers of monocytes and lymphocytes. Periarteriolar changes were minimal or absent. Scant lymphocytic accumulation occurred in the adventitia of larger blood vessels to a degree which did not exceed that in most of the controls examined.

Lymph nodes: The changes in these cases were similar to those described in Cases 1–5. Oedema, however, was evident only in a bronchial node of Case 8.

Spleen: Germinal centres appeared slightly larger, but no more numerous than those of controls. They showed similar degrees of central lymphoid depletion to those in Cases 1–5.

Heart: Scattered subendocardial, subepicardial and myocardial haemorrhages, subepicardial and myocardial oedema, and a moderate increase in the number of neutrophils within myocardial capillaries were features of all the cases (as in Cases 1–5).

Several small foci of acute myocardial necrosis were observed in the interventricular septum and in papillary muscle of the left ventricle in Case 8 only. These foci were composed of groups of adjacent myocytes which were narrowed and separated with a wavy appearance. Affected myocytes showed increased sarcoplasmic eosinophilia, complete loss of transverse and longitudinal striations, and the presence of thick, irregular, eosinophilic transverse bands (Fig. 36). Their nuclei showed various degrees of pyknosis. Occasional neutrophils and small fibrin deposits were present between these myocytes.

Other organs: Changes in liver, kidney, stomach, adrenal and central nervous system closely resembled those in Cases 1–5.

Controls

In addition to points of comparative importance already recorded, bronchiole-associated lymphoid nodules were seen in only 2 of the controls, both 7 years old. These structures were compact and composed of tightly apposed lymphocytes. There was no evidence of lymphoid depletion or necrosis.

There was no evidence of separation of pneumocytes from alveolar walls in lung tissue after either immediate or delayed fixation.

Lymphoid depletion or necrosis was not apparent in lymph nodes or in spleen.

Electron microscopy

An oedematous, protein-rich fluid, represented by finely flocculent material (Fig. 37–39), and fibrin (Fig. 38) occupied many of the alveolar spaces of lung specimens that were oedematous grossly. Interstitial spaces of the alveolar walls frequently were widened (Fig. 38, 40 & 41) and contained flocculent material (Fig. 38 & 41), sometimes accompanied by fibrin (Fig. 41). Erythrocytes occupying the interstitial space were encountered occasionally (Fig. 40).

Separation of Type I pneumocytes from the alveolar wall was encountered commonly (Fig. 41 & 42). The resultant space, between pneumocyte and underlying endothelium, was occupied invariably by finely flocculent material.

Ultrastructural evidence of cellular injury or reaction was not perceived in the lung tissue examined, even in the severely oedematous areas. Features of alveolar architecture corresponded to controls and to those outlined previously for equine lung (Tyler, Gillespie & Nowell, 1971). Where pneumocyte cytoplasm had separated from the alveolar wall, the extreme thinness of this cytoplasm and its lack of organelles (Fig. 41 & 42) precluded a full assessment. However, the plasmalemma was continuous and cytoplasmic disintegration was not in evidence.

Similarly, arterioles presented intact intima and media (Fig. 43). Arteriolar adventitial oedema was represented by separation of fibroblasts and collagen fibres by finely flocculent material (Fig. 44). Immediately neighbouring capillaries of oedematous subepicardial fat in Cases 6 and 8 possessed intact endothelium with no evidence of cellular injury (Fig. 45 & 46).

Oedema of subepicardial myocardium in Case 8 was manifest as a slight separation of capillary walls from surrounding myocytes and as an increase in pericapillary flocculent material. Endothelium of these capillaries was intact. No deviation from normal size or number of cytoplasmic vesicles could be appreciated and the cellular junctions appeared to be closed (Fig. 47 & 48).

Intensive screening of pulmonary tissue, including bronchiole-associated lymphoid nodules from Case 5, bronchial, mediastinal and peripheral lymph nodes and splenic tissue, failed to demonstrate virus particles or any structures associable with replication of AHS virus.

Virology

The virus serotypes isolated from spleen and lung and the tissue virus concentrations measured are recorded in Table 3.

TABLE 3 African horsesickness virus serotypes and concentration in spleen and lung

Case	Serotype	Virus concentration ($TCID_{50}$/g)	
		Spleen	Lung
1	4	ND	ND
2	4	10^4	10^4
3	7	10^4	10^4
4	7	10^4	10^3
5	7	10^4	10^4
6	8	10^3	10^2
7	3	10^4	10^2
8	7	10^4	10^4

ND=Not done

DISCUSSION

Cases 1–5 corresponded closely to the pulmonary form of the disease, as described by Theiler (1921).

In Cases 6–8, the combination of gross lesions of both the pulmonary and the cardiac forms suggests similarity to the mixed form (Theiler, 1921). The incubation periods and disease courses in the experimental cases were also compatible with those of the mixed form.

Although clear differentiation into 2 disease forms was possible for the limited number of cases under study, individual variations occurred in the presence, severity and distribution of certain of the gross lesions. For example, hydrocardium and subcutaneous oedema, which Theiler (1921) regarded as features of the mixed and cardiac forms, also occurred among the pulmonary form cases in this series. Such variations, though minor in the present series, do give an indication that strict classification into forms of AHS on the basis of gross lesions may not always be appropriate.

Although Theiler (1921) claimed that many cases of the mixed form resulted from dual AHS virus infections, there was no evidence of dual infection in the 3 mixed form cases. Each of the experimental cases was exposed to a single virus strain only, and a single serotype only was isolated from each mixed form case. No correlation was demonstrated between the virus serotype isolated and the form of the disease.

The pulmonary form cases were all under 6 months old. This apparent correlation between age and disease form may have no significance considering the small number of cases under study. No clear relationship between age of horse and the form of AHS has been reported previously, although Theiler (1930) considered that young horses are more susceptible to AHS than are old ones. The lung lesion in the pulmonary form and, in affected areas of lung in the mixed form, appeared grossly as oedema. Microscopically, however, the widespread, abundant distribution of fibrin, and the marked accumulations of intra-alveolar and perivascular inflammatory cells, were more suggestive of an acute inflammatory process. On these grounds the pulmonary lesion in AHS should be considered more appropriately as an exudative pneumonia. Maurer & McCully (1963) considered pulmonary oedema in their cases of AHS to have an inflammatory origin. These workers also reported that, in certain areas, the inflammatory cellular component increased to the point of bronchopneumonia. They suggested that the bronchopneumonia might be the result of secondary bacterial infection and that the proteinaceous oedema fluid provided a suitable medium for bacterial growth. In the present series of cases, while bacterial involvement could not be discounted conclusively, there was no evidence of any preferential distribution of exudate within bronchi or bronchioles to suggest a bronchopneumonia, and no morphological evidence of bacterial colonization of these structures. Accumulation of neutrophils was marked in some areas. Although neutrophil accumulation is classically associated with bacterial infections, it is not restricted to them and has also been associated with viral infections. Intra-alveolar neutrophil infiltration is an early feature of feline caliciviral pneumonia (Holzinger & Kahn, 1970; Hoover & Kahn, 1973, 1975) and the role of bacteria in the development of this pneumonia was considered to be minimal (Hoover & Kahn, 1975). Furthermore, neutrophil chemotaxis *in vitro* has been shown to result from interaction of feline calicivirus with pneumocytes and alveolar macrophages (Langloss, Hoover, Kahn & Kniazeff, 1979).

The substantial increase in numbers of alveolar macrophages, their ample, eosinophilic cytoplasm, and the close association of some of these cells with fibrin suggest that an active process of engulfment and disposal of proteinaceous exudate was already in progress late in the disease. According to Green, Jakab, Low & Davis (1977), accumulated evidence suggests that most alveolar macrophages arise from blood monocytes, derived from the bone marrow. *In vitro* studies have indicated that mature alveolar macrophages are also capable of more rapid local proliferation. The time course of alveolar exudation in the pulmonary form is not known. However, the short clinical course in this form of the disease (Theiler, 1921) suggests that most of the alveolar exudation occurs shortly before death. It is likely, therefore, that the increase in alveolar macrophages seen in the pulmonary form cases was mainly attributable to local proliferation.

Staub, Nagano & Pearce (1967) have shown that during the development of oedema produced experimentally in canine lung, the loose connective tissue around larger blood vessels is one of the first sites of fluid accumulation. Similar perivascular changes were discernible in the lung in this study. The perivasculitis, involving predominantly arterioles, was not readily explicable. Changes in small arteries in association with oedema in horses have been described in equine viral arteritis (EVA) (Jones, Doll & Bryans, 1957). However, in that disease there was clear evidence of medial necrosis, and an ultrastructural study of EVA (Estes & Cheville, 1970) has demonstrated severe injury to endothelium, which appeared to be the primary site of viral insult. The arteriolar changes observed in the cases of AHS were distinct from those of EVA in being restricted to the adventitia. No arteriolar medial or intimal alterations were detected by LM or by EM in the present study. Maurer & McCully (1963) also described oedema and mild round cell infiltrates in the adventitia of small pulmonary arterioles in 2 of their cases.

The presence of bronchiole-associated lymphoid nodules with well-developed structure in 4 out of 5 of the pulmonary form cases, their apparent absence from all 3

mixed form cases, and their demonstration in only 2 out of 30 controls suggest that their occurrence is related to age of the horses rather than to AHS. The pulmonary form cases were all younger than 6 months, whereas the mixed form cases and the controls were all at least 1 year old.

Lymphoid depletion of germinal centres was a consistent feature in all cases and appeared to be generalized, involving not only lymph nodes and spleen, but also bronchiole-associated, renal and intestine-associated lymphoid tissues, where examined. The lymphoid depletion appeared to affect principally lymphocytes and was frequently associated with acute necrosis of these cells. It was not possible to ascertain whether the prominence of reticular macrophages in the lymphoid follicles was attributable to hyperplasia or to an apparent increase viewed against the background of lymphoid depletion. High virus concentrations measured in the lymphoid tissue in cases of AHS (Erasmus, 1972) raise the possibility that the lymphoid depletion resulted from direct viral injury to lymphoid cells. Alternatively, the lymphoid depletion might have arisen as a non-specific event associated with severe stress in the advanced stages of the disease, possibly related to excessive release of endogenous corticosteroids. Dougherty (1952) has reported a lymphocytolytic effect of certain adrenal cortical steroids, and a similar effect in response to stressful stimuli, mediated through endogenous adrenocorticotrophic hormone (ACTH) release in laboratory animals. Although no descriptions are apparent of the effects of ACTH or stress upon lymphoid tissues in the horse, widespread necrosis of lymphocytes and associated lymphoid depletion of germinal centres have been encountered in cases of equine colic (Elizabeth W. Howerth, 1981, personal communication). Maurer & McCully (1963) reported similar lymphoid depletion in their cases of AHS. Unlike their cases, however, little evidence of lymphopoietic activity could be found in the present study.

The severity of the hydropericardium in the mixed form cases suggests that this fluid accumulation may have contributed to death in these cases. Edington (1900) and Erasmus (1972) suggested that heart failure in the cardiac form of AHS may be attributable to hydropericardium.

Myocarditis, as described by Maurer & McCully (1963), has not been reported elsewhere and was not evident in any cases of this series. In a review of viral myocarditis in animals, Gainer (1974) has drawn attention to the profound influence which environmental factors, such as hypobaric oxygen states and conditions causing stress, can exert upon the development of viral myocarditis. Virus concentrations measured in the heart in large numbers of experimental AHS cases were very low and showed no increase over concentrations in blood, even in cases of the predominantly cardiac form, suggesting that the myocardium is not a site of virus replication in AHS (Erasmus, 1972).

The foci of myocardial necrosis in Case 8 resembled myocardial lesions, described as coagulative myocytolysis or myofibrillar degeneration, a lesion which has been recognized in a variety of circumstances in man (Reichenbach & Benditt, 1970), in showing thick, irregular, eosinophilic, transverse banding of myocytes and loss of transverse and longitudinal striations. Similar lesions have been produced by various experimental means and the development of the lesions can be prevented by beta adrenergic blockade (Reichenbach & Benditt, 1970). This has led to the unifying concept that coagulative myocytolysis results from excessive release of endogenous catecholamines. With this in view, it is unlikely that the myocardial lesions in Case 8 were directly associated with AHS virus infection, and more probable that they were associated with a state of severe stress late in the disease.

Hepatocellular, eosinophilic, intracytoplasmic inclusions, which were found consistently in this study were similar to those described in AHS by Fotheringham (1936), though generally smaller. The significance of these bodies is uncertain. Their morphological features and tinctorial properties revealed in the present study were compatible with those reported for acidophilic bodies in Bolivian haemorrhagic fever in man (Child & Ruiz, 1968). The recognition of acidophilic bodies in a variety of unrelated conditions in man, including viral and parasitic hepatitides, thermal, ischaemic and toxic hepatic insults, and malnutrition, has led to the conclusion that they arise from non-specific causes (Child & Ruiz, 1968; Klion & Schaffner, 1966).

Bodies with a striking similarity to those in the present study have been described in otherwise normal livers of healthy dogs, where their histochemical properties indicated that they were composed of glycolipoprotein complexes, probably related to lysosomes. Their occurrence in normal livers suggested that they represent only a minor form of injury (Murti & Borgmann, 1965). Since PAS positive, non-glycogenic, hepatocellular intracytoplasmic inclusion bodies are generally regarded as representing a non-specific degenerative or necrotizing process, it is likely that the hepatocellular inclusion bodies in AHS, whatever their exact nature, also conform in this regard. Little diagnostic significance, therefore, can be attached to these structures in AHS, substantiating a view expressed previously by Howell (1968).

Eosinophilic bodies were not seen in renal tubular epithelium, in contrast to previous reports (Fotheringham, 1936; Amjadi & Ahourai, 1971). Gastric mucosal congestion, considered by M'Fadyean (1900) to be a most constant feature of AHS and reported subsequently by others (Van Sacaghem, 1918; Theiler, 1921; Carpano, 1931; Maurer & McCully 1963), was also a consistent finding in the present series. More severe gastric lesions reported previously, such as haemorrhage, oedema and erosions (Theiler, 1921; Carpano, 1931) were not encountered.

Failure of thorough EM examination of tissues to reveal AHS virus particles or any structures resembling those described in association with AHS virus replication *in vitro* (Lecatsas & Erasmus, 1967; Breese *et al.*, 1969; Huismans & Els, 1979) was not completely unexpected. Tissue concentrations of virus did not exceed $10^4TCID_{50}/g$ whereas procedures employed in the *in vitro* studies resulted in much higher virus concentrations. Experience in screening by EM for other viruses in infected animal tissues indicates that virus particles are not regularly visualized *in situ* unless the tissue concentration of the virus approaches or exceeds $10^6TCID_{50}/g$. (G. Lecatsas, 1981, personal communication). Since the virus was not detected by EM in the tissues, its cellular sites of replication could not be determined. However, comparison with diseases caused by other species of the genus *Orbivirus* such as bluetongue in sheep (Stair, 1968) and epizootic haemorrhagic disease of deer (Tsai & Karstad, 1973) suggests that an expected site would be vascular endothelium.

In the development of pulmonary oedema, as pointed out by Hurley (1978), alveolar flooding occurs only as a late, and not an inevitable, stage. If the rate of fluid and protein loss from capillaries is great enough to exceed the rate of lymphatic drainage, it accumulates in the interstitual tissues, firstly, in peribronchial and perivascular connective tissue, and, subsequently, it distends

the interstitial spaces of the alveolar walls. With the accumulation of enough fluid in the interstitium, alveolar flooding occurs, individual alveolar spaces filling rapidly with fluid. The dilated pulmonary interlobular and subpleural lymphatics containing fibrin, and the oedema of bronchial and mediastinal lymph nodes associated with pulmonary oedema in the cases of AHS in this study are consistent with this general concept of the development of pulmonary oedema.

The separation of intact alveolar epithelium from the alveolar walls may have been a manifestation of an extreme stage of alveolar interstitial oedema, the epithelium being lifted away by accumulated oedema fluid. Whatever its exact significance, there is little doubt that this separation represents a true pathological change, since it was evident in Case 8, which had a very short interim, and it was absent from control material, even where fixation had been delayed by several hours.

Pulmonary oedema, in common with oedema in other tissues, may be classified into permeability, hydrostatic and lymphatic types, as outlined by Hurley (1978). Although the rate of flow and the protein content of pulmonary lymph have not been studied in AHS, the abundance of fibrin consistently observed in alveoli, pulmonary interstitial tissue and lymphatics in the pulmonary form cases of the present series suggests that the oedema fluid has a high protein content, compatible with that of a permeability oedema. This is supported by the finding that thoracic fluid, collected from several natural cases of AHS subsequent to the present study, possessed a total protein content similar to that of equine plasma (S. J. Newsholme, 1981, unpublished data). Studies of experimental cases of AHS have shown that pulmonary arterial pressures do not exceed the values obtained in clinically normal horses, even in the terminal stages of the disease. Pulmonary oedema of hydrostatic type, therefore, appears to be an unlikely feature of AHS (A. Littlejohn, 1982, personal communication). No evidence of lymphatic obstruction was apparent. Fibrin within lymphatics was limited to fine, lace-like deposits and no thrombi associable with obstruction were observed.

The absence of demonstrable ultrastructural injury to vascular endothelium in this study contrasts sharply with the EM findings in epizootic haemorrhagic disease, another disease caused by a species of the genus *Orbivirus*. Experimental infection of deer with epizootic haemorrhagic disease virus resulted in frequent enlargement and degenerative changes in some of the endothelial cells (Tsai & Karstad, 1973), accompanied by multiple haemorrhages and widespread thrombosis. In AHS, as indicated by the present study, oedema is the striking lesion, haemorrhages are much less severe and thrombi and not observed.

The evidence for increased vascular permeability as the underlying pulmonary event in AHS is not contradicted by the failure to find structural evidence of pulmonary endothelial injury. Severe pulmonary oedema of permeability type can result from minor, reversible structural changes in endothelium. For example, in rats treated with alpha-naphthyl-thiourea (ANTU), an acute pulmonary oedema develops which is of permeability type. According to Hurley (1978), despite a profuse extravasation of fluid and protein, there is evidence of only minor structural injury to pulmonary endothelium. Cunningham & Hurley (1972) have provided evidence that the pathway of fluid leakage in ANTU-induced oedema is provided by reversible gaps in vascular endothelium, and that most of these gaps are formed by separation at endothelial cell junctions.

The site and pathway of fluid escape from pulmonary vessels in AHS are not known and have not been established by the present study. Nonetheless, widespread evidence of interstitial oedema and the occurrence of interstitial fibrin, both observed at capillary level, together suggest that the capillaries are an important site of increased permeability in this desease. Since the evidence in favour of a permeability type of pulmonary oedema in AHS and the lack of pulmonary endothelial structural injury revealed by the present study are both features in common with those of ANTU-induced oedema, it is reasonable to suppose that separation of capillary endothelial intercellular junctions may also constitute the major pathway of fluid leakage in AHS. Other possible pathways that might have contributed include direct transcellular passage, diffusion through cell membranes at intercellular junctions and transport of fluid within the *c.* 50 nm diameter cytoplasmic vesicles, either by shuttling from one side of the cell to the other, in a manner resembling that proposed originally as cytopempsis (Moore & Ruska, 1957), or by transitory channels formed by the vesicles themselves (Simionescu, Simionescu & Palade, 1975). Recent evidence, as reviewed by Staub (1980), does not indicate that the cytoplasmic vesicles constitute an important pathway of fluid leakage.

It is reasonable to assume that oedema in the other tissues in AHS arises by mechanisms similar to those operative in the lung. Erasmus (1972) has argued that the common distribution of subcutaneous oedema in AHS, involving head and neck, cannot be attributed to cardiac failure in view of its dorsal distribution, and that such oedema must arise through local increase in capillary permeability.

Whatever the mechanisms involved in the development of oedema in AHS, the lack of structural evidence of vascular cellular injury revealed by the present study does extend a measure of hope for therapy.

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