Histopathological lesions in spontaneous dictyocaulotic pneumonia of the reindeer (*Rangifer tarandus tarandus* L.)

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Summary: The histopathology of pneumonitic lesions in natural lungworm infection has not been previously described in reindeer. In the present study, light microscopical studies of dictyocaulotic lung tissues were performed on 12 slaughtered reindeer specimens obtained from the eastern part of Finnish Lapland in order to reveal the histopathological lesions characteristic of spontaneous *Dictyocaulus eckerti* infection. The lungs of 8 healthy reindeer carrying no lung worms were investigated to elucidate normal microscopic structure.

Parasites in different stages of development and their eggs were detected not only in inflamed sections of small airways, but also wandered freely in the alveolar lumina. The eggs were rich in carbohydrates for reserve energy and were surrounded by a superficial layer of acidic mucins.

The pleural serosa over acutely inflamed areas was swollen and in chronically altered sections, fibrotic thickenings of pleural and subpleural tissues were evident. The lung tissues were either condensed by an inflammatory exudate rich in migrating cells or emphysematically altered. Mucus secretions were abnormally profuse and apparently less acidic in histochemical compositon. Interstitial tissues were also inflamed and contained separated foreign body and eosinophilic granulomes. Furthermore, a vasculitis with endothelial vacuolations and muscular hypertrophy was noticeable in some tissue sections.

Keywords: histopathology, lungs, *Dictyocaulus eckerti,* lungworm disease, dictyocaulotic pneumonia

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Introduction

Many wild and domesticated animals are exposed to parasitic infections, resulting in a potential inflammatory response by the host. A consequential phenomenon may be a disturbance in the function of the tissues and organs harbouring the parasites. Thus, the symptoms of different parasitic diseases in animals are produced by the disordered function of the organs directly injured by the parasites, or especially, by the destructive inflammatory response of the animal. Similar changes occur also in the reindeer, which may be affected with severe clinical illness due to lungworm infections and pneumonitic lesions (Holmström *et al.,* 1989; Korhonen *et al.,* 1989).

Parasitological studies of fecal samples have shown that even clinically healthy reindeer in Finland are carriers of subclinical infections due to *Dictyocaulus sp.* and *Elaphostrongylus rangiferi* (Korhonen *et al.,* 1989). Closer analyses of the parasitological samples have revealed these lungworms to be identical to *D. eckerti* as described by Boev in 1934 (Saari *et al.,* 1991). Since the

pathoanatomy of spontaneous dictyocaulosis in the reindeer is inadequately documented in the literature, we considered it important to perform light and electron microscopical investigations on the lung lesions produced by natural *D. eckerti* infection in reindeer. The purpose of this paper is to describe certain histopathological findings characteristic of natural *Dictyocaulus*-infection specimens in the lungs of slaughtered reindeer.

Materials and methods

The material consisted of tissue specimens from spontaneously dictyocaulotic lungs of 12 slaughtered reindeer originating from the eastern part of Finnish Lapland. The lungs of 8 healthy animals with parasite-free pulmonary tissues were studied to elucidate normal microscopic structure. These animals appeared to be clinically healthy male reindeer showing no lower respiratory tract abnormalities at post mortem or histopathological examinations. Five infected animals were about 8 months of age, and the others were fullgrown older animals that had been treated with ivermectin (Ivomec, Lääkefarmos, Finland) during previous autumns and slaughtered in January 1989. Dictyocaulosis was verified on the basis of macroscopical and microscopical examinations of the specimens. The parasites in different stages of development encountered in the present material were identified as *D. eckerti* described by Boev in 1934.

For the histopathology, 2 or 3 lung tissue specimens were fixed in diluted neutral formalin (25% formaldehyde), embedded in paraffin, sectioned at 4 microns and routinely stained with haematoxylin and eosin (HE) and toluidine blue at pH 4.0.

For closer light microscopical observations additional sections from 5 reindeer were stained employing histochemical methods according to the Manual of the AFIP (1968) and Pearse (1968).

Herovic's staining and Masson's trichrom-staining according to Ladewigs modification were applied for the studies of mesenchymal elements.

Iron was determined by Hueck's modification using the Turnbull blue method and calcium by von Kossa's silver nitrate staining method. Identification of haemoglobin and globule leukocytes was attempted with amidoblack staining. Lipidic materials resistant to fat solvents were visualized with oil red O applied to paraffin sections.

The following procedures were applied for the analyses of mucinous and other carbohydrate-rich substances and granular cells such as mast cells:

For the determination of neutral glycoproteins and glycogen, the sections were stained with periodic acid-Schiff without (PAS) or with diastase pretreatment (d-PAS). For acidic mucins the sections were stained with alcian blue at pH 2.5 and pH 1.0 (AB 2.5; AB 1.0), with nuclear fast red counterstain and with aldehyde fuchsin at pH 1.7 (AF). Identification of sialomucins was attempted with AB 2.5 staining of sections pretreated with a sialidase digestion for 1 hour at pH 8.9 and at 40°C (Neuraminidase, Sigma); the control slides were treated with an enzyme-free buffer. Acidic (blue or purple) mucins were differentiated from neutral (red) mucosubstances in the same sections by the sequential staining of the sections with AB 2.5 followed by PAS (AB 2.5-PAS). Correspondingly, sulphomucins (blue or purple) were differentiated from neutral mucins (red) with the sequence AB 1.0-PAS and with the sequence AF-AB 2.5 which stains sulphomucins purple and nonsulphated acidic mucins blue. Furthermore, toluidine blue at pH 4.0 and 0.5 (TB 4.0; TB 0.5) was used to identify mast cells.

Results

Normal structure

The histology observed in the healthy reindeer specimens appeared normal. Thus the small airways were free of parasites, containing scant amounts of secretions and cells. The interstitial tissues showed some inflammatory cells and separated lymphatic follicles. Mucus secretions were scanty and sialidaselabile sialomucins could not be visualized.

Parasitological observations

Light microscopy (Figures 1 to 8) revealed separated eggs and sections of parasites in different stages of development, surrounded by an inflammatory response. Some eggs, rich in the PAS-positive materials characteristic of lungworms, were located in alveoli, compressing the alveolar septa. In addition, parasites lacking a

peripheral cellular reaction were observed to be wandering freely in the airy pulmonary sections.

Histopathological findings

Pleural thickenings consisting of hypertrophied serosal cells with underlying fibrotic scars were seen in some animals. In addition, inflammatory cells were present in the subpleural sections.

Bronchial and alveolar lumina were either empty or contained oedematic and mucotic fluids with inflammatory cells and parasites. Large sections of pulmonary tissues were sometimes condensed and inflamed; in the other sections emphysematic alterations were visible. The epithelial cells in different parts of the airways were hypertrophied and actively secreting mucus with a histochemical composition somewhat different from that in normal areas. Acidic mucins tended to occur in lower amounts in the inflamed areas than in the unaffected areas. Moreover, muscular hypertrophy of the bronchial walls and sections with regenerating epithelium were observed, presenting epithelial cells with strongly basophilic cytoplasm. Globule leukocytes were not detectable in the epithelium.

Interstitial tissues were thickened, due to oedematic fluids, inflammatory cells and fibrosis. The thickenings occurred not only as alterations typical of alveolitis, but also in the peribronchial and perivascular sections, where both acute and chronic inflammatory changes were seen. The inflammatory response appeared occasionally in the form of nodules of cells of the lymphocytic series and as foreign body granulomes with parasite residues and tissue components. In some sections, eosinophilic granulomes were identified. Lipid containing macrophages and foreign pigmented material showing a positive reaction for iron were noted, too. Mast cells were numerous and stainable with TB 4.0 and 0.5 stainings. Using the AB 1.0-PAS and AB 2.5-PAS staining methods, the granules of mast cells appeared turquoise in colour, but purple with the TB-methods.

Blood vessels of small caliber contained plasma with typical of microthrombosis. A normal finding was local vasculitis showing inflammatory cells in different layers of the blood vessel walls with muscular hypertrophy. Vacuolisation could also be noticed in the cytoplasm of endothelial cells of blood vessels.

Discussion

There are a variety of parasites which cause infections of the lower respiratory tract. For example, the lungworm *Dictyocaulus viviparus* is pathogenic in cattle and deer although the immature stages of *Elaphostrongylus cervi*, too, can damage the lungs (Munro and Hunter, 1983; Corrigall *et al.*, 1988). In the present material, only different stages of *D. eckerti* were identified in naturally infected reindeer. However, in this kind of an investigation it is not possible to exclude the simultaneous occurrence of other causal agents of pneumonia. Nevertheless it is considered that the results of the present study will form a realistic basis for histopathological diagnostics of naturally occurring lungworm disease in reindeer in Finnish Lapland.

There are many comprehensive studies on the pathology in experimental and spontaneous lungworm infections in various animal species such as cattle and sheep (Jarrett *et al.*, 1957; Simpson *et al.*, 1957; Armour, 1987; Corrigal *et al.*, 1988), deer (Munro and Hunter, 1983), pigs (Subramaniam *et al.*, 1967; Jindrak and Alicata, 1968; Armour, 1987), in carnivores (Hamilton, 1966, Dubey *et al.*, 1968; Jindrak and Alicata, 1970; Stockdale, 1970; Hirth and Hottendorf, 1973; Suzuki *et al.*, 1981; Poli *et al.*, 1991) and in small laboratory animals (Beresford-Jones, 1960). Since the descriptions of the pathoanatomy of the lungs in these reports seem to be fairly uniform in several animal species, it is therefore not surprising that lungworm disease in reindeer described in the present paper does not essentially differ from that in other animal species.

Hellesnes (1935) reported on the clinical findings in reindeer suffering from dictyocaulosis. The animals had coughs and showed mild catarrhal bronchitis at post mortem examination. Correspondingly, the histopathological alterations described in the present material are considered to be characteristic for bronchitis or bronchopneumonia, too. However, changes indicating an alveolitis and interstitial pneumonia were also observed by the present authors. Probably the microscopic studies mentioned by Hellesnes (1935) dealt only with the parasite-containing mucotic material filling the bronchial lumina. Therefore the histopathology was obviously not conducted to elucidate pathological changes of the interstitial tissues.

Fibrotic areas of subpleural tissues with thickened serosa were observed in some sections of the reindeer lungspecimens. In the dog, Hirth and Hottendorf (1973), showed that such subpleural scars are the result of lungworm migration although no parasites were identified in the fibrotic areas. In the present material all of the parasites observed under the microscope were located in different sections of the airways. Thus, the subpleural inflammation might be a reaction against the parasites without traumatic lesions.

On the basis of the present and previous studies (e.g. Urquhart *et al.,* 1987), the pathogenesis of dictyocaulosis appears to be very complicated. The first phase of dictyocaulotic pneumonia is associated with the appearance of larvae within the alveolar region, causing alveolitis. The larvae move up towards the bronchi, causing bronchiolitis and bronchitis, respectively. Then cellular infiltrates consisting of neutrophils, eosinophils and macrophages plug the lumina of the distal airways, causing the collapse of distal alveoli. During this phase the immature lungworms can readily be seen in the mucus containing airways with low power microscopy.

During the patent phase of *Dictyocaulus* spp., numerous adult worms surrounded by mucus are present in the lumina of the bronchi. The hyperplasia of the bronchial epithelium and inflammatory cells, containing eosinophils in particular, are the prominent features of this phase. In the alveoli, aspirated eggs and L_1 stages act as foreign bodies and cause pneumonitic lesions with atelectasia, oedema, emphysema and the cellular reaction rich in macrophages and multinucleated giant cells.

During the recovery phase of dictyocaulotic pneumonia adult lungworms can no longer be detected in the bronchi. Alveolar epithelialisation caused by the proliferation of type 2 pneumocytes, accompanied by emphysema and pulmonary oedema, are the typical histopathological features of this phase.

When the animals that have acquired immunity from previous lungworm infection are challenged by a few infection, the lesions that are considered typical of the so-called reinfection syndrome may occur. These lesions are associated with the destruction of the reinfecting larvae in the bronchioles by the host's immune response. Histologically, the proliferation of the lymphoreticular cells is the most prominent feature. These cells form nodules composed of a central core of eosinophilic or calcified parasitic debris surrounded by macrophages, giant cells and hyperplastic bronchial epithelial cells. Immediately external to this is a ring of eosinophils, macrophages, plasma cells, lymphocytes and the giant cells. A thick zone of lymphocytes and plasma cells surrounds the lesion and begins to form mature lympho-reticular tissue with germinal centers (Armour, 1987). The cell population of these lymphoreticular cell aggregates is considered to be responsible for the hosts immune response to future Dictyocaulus infections.

All of these lesions were detected in the present study. The prominence of the cellular reactions by the lympho-reticular cells shows that most of the reindeer, although quite young, became reinfected with *Dictyocaulus* larvae.

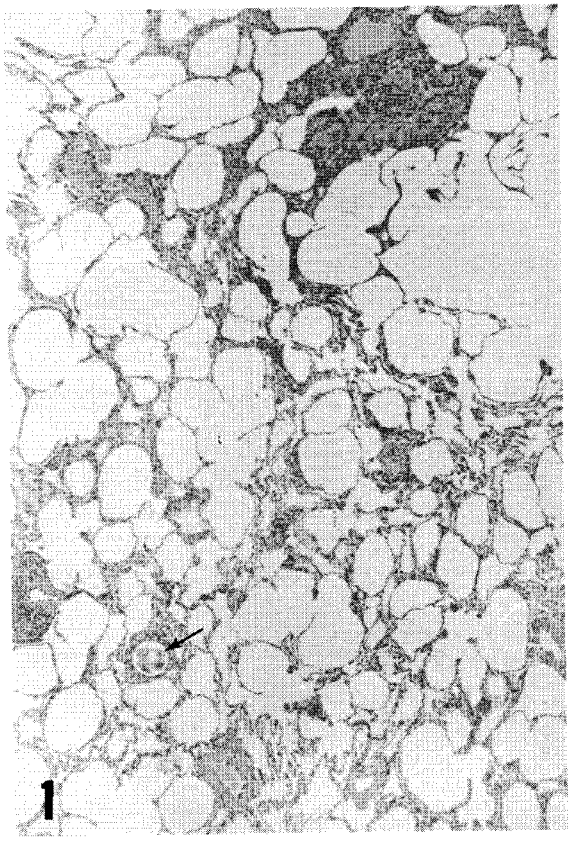
Globule leukocytes were not identified in the present material, but stainable mast cells were seen. Previously an abundant appearance of globule leukocytes in lungworm disease of cattle, sheep and pigs (Armour, 1987) and of wild Japanese Serows (Suzuki *et al.,* 1981) has been reported. The failure of the present authors to identify globule leukocytes in the bronchial epithelium of reindeer is puzzling. A recent study has shown that an abundance of globule leukocytes is present in the bile-duct epithelium of slaughtered reindeer (Rahko and Nikander, 1990).

Fig. 1. A low-power field showing a Herovic-stained section of lungs infected with *Dictyocaulus eckerti.* Emphysematous structure is dominant. The parasite is marked by an arrow. x 65.

Fig. 2. An egg of *D. eckerti* located freely in an alveol. Note the black alcianophilic layer of acidic mucins covering the egg (marked by arrowheads). AB 2.5. x 400.

Fig. 3. Regenerating epithelium of alveolar walls (arrowheads) deformed during the reparative processes. Herovic, x 260.

Fig. 4. An oblique section of a lung worm (marked by arrow). A nodule of inflammatory cells around a blood vessel (marked by arrowheads). Herovic, x 160.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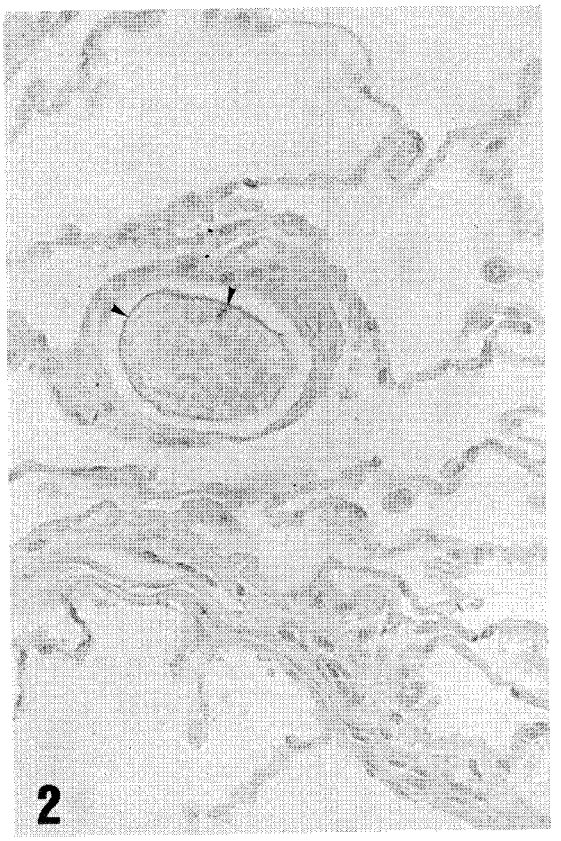
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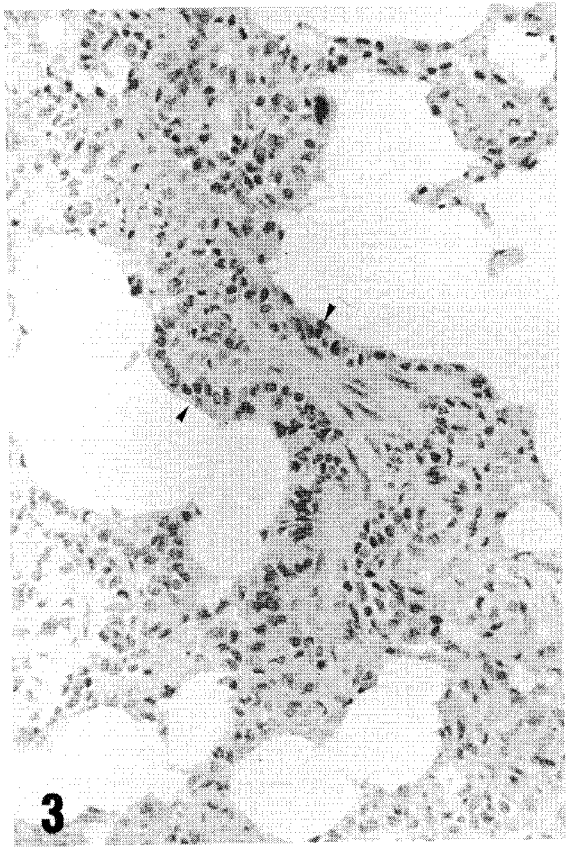
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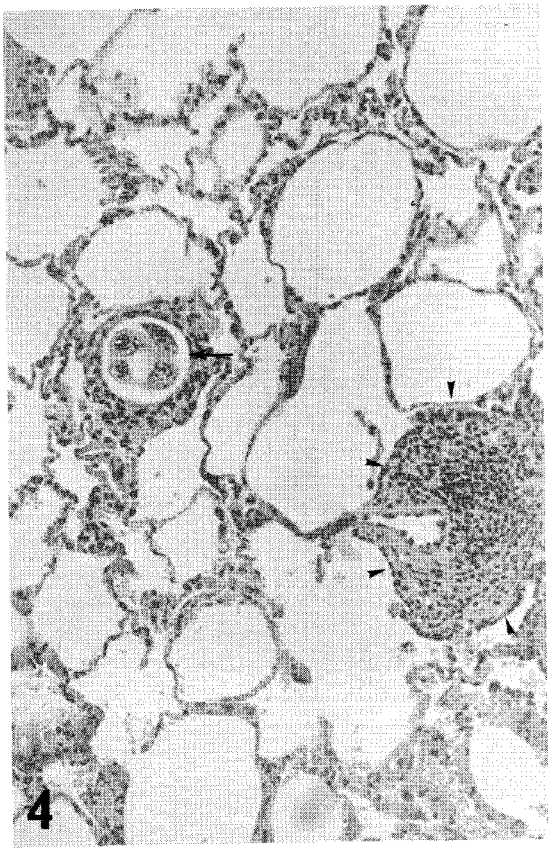
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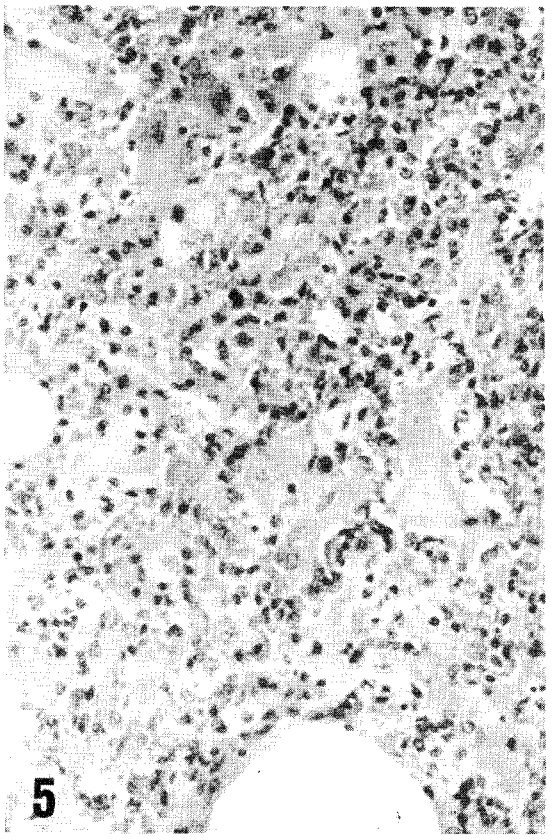

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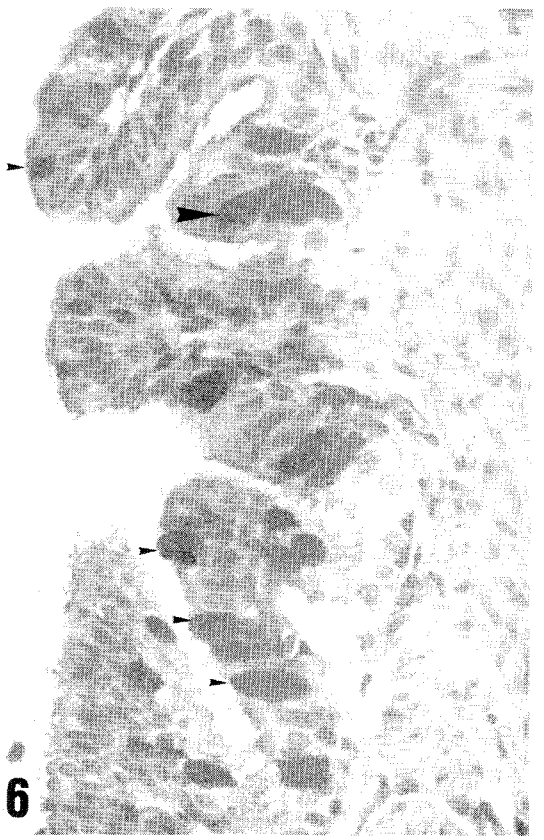

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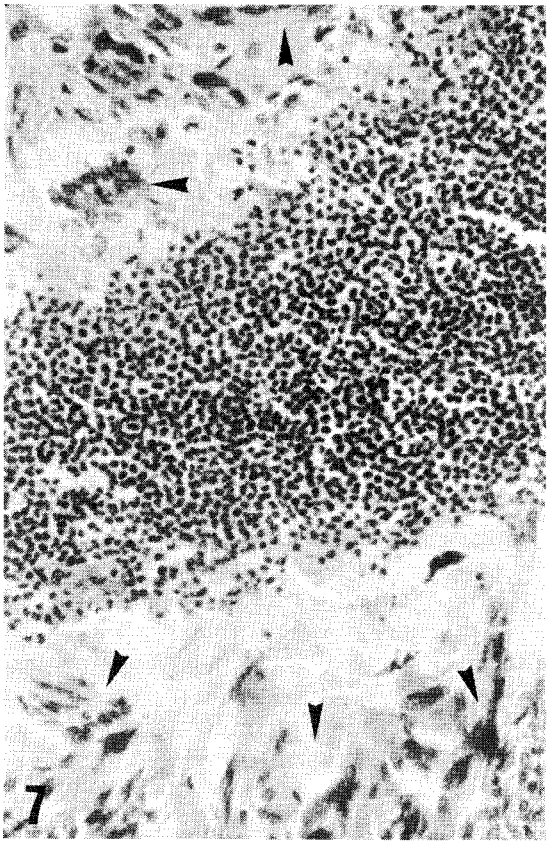

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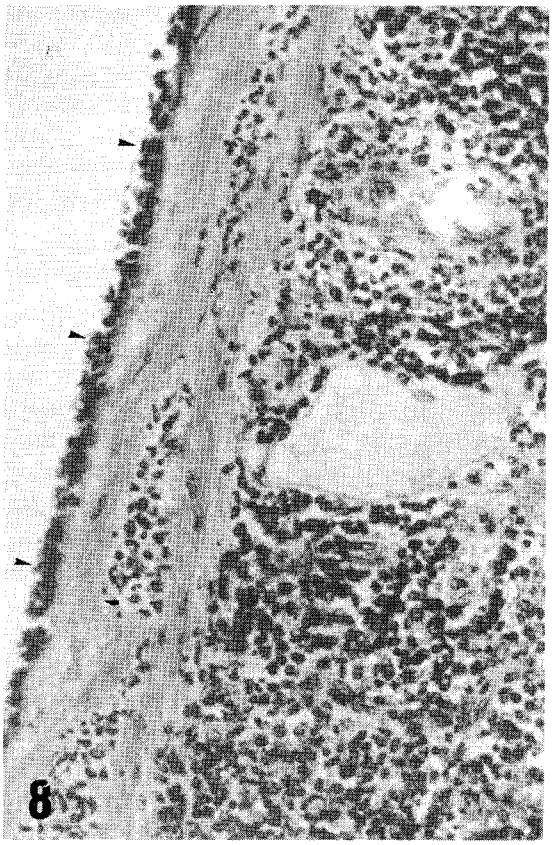

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Fig. 5. A condensed section of lungs containing inflammatory cells and exudate in alveolar lumina. Herovic, x 260.

Fig. 6. A cross-section of a bronchial wall containing numerous goblet cells with neutral mucopolysaccharides in their cytoplasms (marked by arrowheads). PAS, x 400.

Fig. 7. An eosinophilic granulome containing a peripheral layer of multinucleated giant cells (marked by arrowheads) around the central core of the eosinophilic granulocytes. HE, x 260.

Fig. 8. A swollen serosal layer of the pleura (marked by arrowheads) covering lungs infiltrated by numerous inflammatory cells, Herovic, x 260.

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