A TRANSMISSION ELECTRON MICROSCOPIC STUDY OF SOME BRONCHIOLAR-ALVEOLAR REACTIONS IN SHEEP AND CATTLE

by

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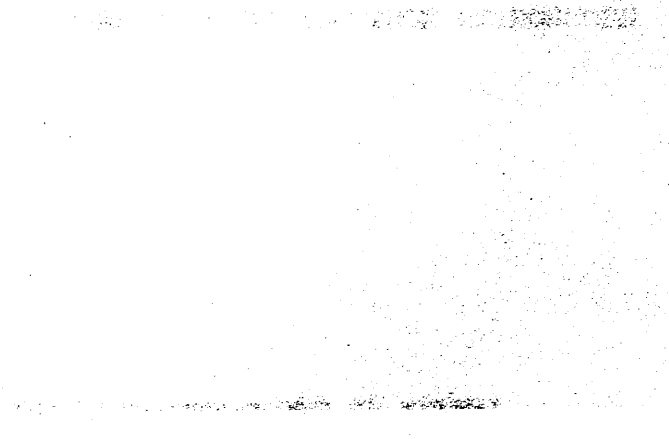
DECLARATION

I hereby declare that the work presented in this thesis has been carried out by me under the supervision of Professor H.M. Pirie. I also certify that no part of this thesis has been submitted previously in any form to any University.



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SUMMARY

The two main objectives of this thesis were firstly to study the morphological features of the alveolar macrophages (AM's) in normal sheep and in normal cattle and secondly to study AM's in sheep pulmonary adenomatosis (SPA) and in calves infected with the lungworm, Dictyocaulus viviparus, in order to compare their morphological characteristics in the two diseases.

The history and development of the transmission electron microscope (TEM) are outlined in Chapter 1. The use of the TEM in diagnostic pathology and research into animal and human diseases is also discussed with some examples relevant to studies in animals.

A review of the ultrastructure of the cell types in the normal bronchiolar and alveolar epithelium in sheep and cattle is presented in Chapter 2. The AM in normal lungs in introduced and considered in this chapter.

The structure of the lungs of normal sheep were studied to provide comparisons for subsequent examinations of SPA lungs at both light microscopic and electron microscopic levels. This work is reported in Chapter 4. Ciliated cells and Clara cells were identified in the bronchiolar epithelium while type I and type II pneumonocytes were

recognised in the alveolar epithelium. The morphology of these different cells were described. Ewes with SPA were studied by light microscopy (LM) and TEM. The results confirmed that the proliferating tumour cells were due to the transformation of the type II pneumonocytes. No viral particles were identified in the cases examined.

The AM's were consistently found in the alveolar lumen and their morphological characteristics revealed that they were activated. These activated AM's could be classified into two groups, based on their morphological features. The possible roles of the AM's in the pathogenesis of SPA is discussed.

The histologic and ultrastructural appearance of cattle lungs were studied and compared with the lungs of calves that had been infected with lungworms; this is reported in Chapter 5. Ciliated cells and Clara cells were found in the bronchiolar epithelium while type I and type II pneumonocytes were noted in the alveolar epithelium. Alterations were observed both in the cell types of the bronchiolar epithelium and in the alveolar epithelium in calves with lungworms. In the bronchiolar epithelium, moderate to severe degenerative changes were recognised in the Clara cells and there was a loss of cilia from the ciliated cells. There was severe degeneration and type II pneumonocyte proliferation with hydropic swelling of the

cytoplasm of the type I pneumonocytes. Inflammatory cells were a frequent finding in the bronchiolar and alveolar lumen. Interalveolar septa were markedly thickened in many cases. Alveolar macrophages were commonly found in the inflammatory exudate. They were similarly activated as the AM's in SPA. Morphologically, only one group of AM's could be identified.

Some aspects of the cell kinetics of the lung, in particular the AM's, are discussed in Chapter 6. The morphological differences of the AM's in the two diseases are presented and discussed at the end of this chapter.

ABBREVIATIONS

AM	Alveolar macrophage
LM	Light microscopy
PIM	Pulmonary intravascular macrophage
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy

GENERAL INTRODUCTION

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The use of the transmission electron microscope (TEM) in many fields of biomedical research and disease diagnosis has reached a stage in which the instrument is providing one of the most powerful means of investigating the complex organization of tissues at the cellular and molecular level. This indispensable tool has contributed enormously to the understanding of the structural intricacies of normal and disordered cells and tissues. The TEM has played a significant role in the understanding of neoplastic processes by providing information on the ultrastructure of tumours and neoplastic cells. The diverse ways in which the TEM can be used to study structural organisation is illustrated by the fact that the TEM has been employed not only to study the ultrastructural detail of cells but also the microstructure of crystals, particularly those of metal.

Respiratory diseases, primarily those affecting the lower respiratory tract, still remain important as they cause significant losses in all age groups of farm animals. In North America alone, $2-4 billion is spent annually on prevention and treatment of respiratory diseases in cattle (Barker and others, 1986). In Britain, the annual cost of respiratory disease in beef cattle has been estimated as being in the order of five million pounds (Anonymous, 1979).

Alveolar macrophages play an important role in many respiratory tract infections as they are a component of the pulmonary defense mechanisms operating at the alveolar level (Rossman and Douglas, 1988). Alveolar macrophages are known to play a prominent part in sheep pulmonary adenomatosis (Hod and others, 1977) and in bovine lungworm infections with Dictyocaulus viviparus (Jarrett and others, 1954). It was therefore decided that an ultrastructural study of alveolar macrophages in sheep pulmonary adenomatosis (SPA) and bovine lungworms could yield useful information on the morphological features of these cells during the course of an active response to two different types of disease.

CHAPTER 1

THE USE OF THE TRANSMISSION ELECTRON MICROSCOPE IN PATHOLOGY

THE USE OF THE TRANSMISSION ELECTRON MICROSCOPE IN PATHOLOGY

The first electron microscope was built in 1931 by Knoll and Ruska in Germany (Ham and Cormack, 1979). However, it was not until 1939 that the transmission electron microscope (TEM) was first used successfully to study a biological specimen (tubercle bacilli) at the University of Toronto in Canada (Mandal and Wenzl, 1979). Two years later, in 1941, the first commercially available TEM in North America was produced.

The kidney was one of the earliest tissue studied with the TEM when it was used for a detailed investigation of the glomerular capillary, renal corpuscles and the renal tubules (Pearce and Baker, 1950). Since then a stream of information has flowed from electron microscopic studies of cells and has opened up a new world of knowledge about the detailed structures of cells and tissues. In biological investigations, despite being limited to a small area of the whole specimen, TEM studies have helped in the better understanding of the fine microanatomical structures of many organs in the mammalian body. They have also helped to resolve cellular constituents such as the mitochondria, Golgi complexes, endoplasmic reticulum and as well as membrane structures like the cellular membranes, nuclear membranes and plasma membrane infoldings.

The pioneering studies of Low (1953) clearly demonstrated that the alveoli were lined by a continuous layer of epithelium. The identification of specific cell types in cattle became possible when TEM studies resolved the morphology and identity of the type I and type II pneumonocytes in the alveolar lining cells of the blood-air barrier (Epling, 1964).

The pathogenesis and cytopathological changes in many viral diseases affecting the respiratory system has been investigated. The marked tropism of viruses for cell types, structures involved in damage subsequent to infection and the different exudative and regenerative phases following injury have been studied for acute viral pneumonia produced by feline calicivirus (Langloss and others, 1978) and bovine parainfluenza 3-virus (Bryson and others, 1983).

Enteric infections with resulting diarrhoea is an important problem especially to young farm animals. There are many causes of diarrhoea and many investigations including TEM studies have been conducted. The location and distribution of Campylobacter in calves and lambs have been described (Terzolo and others, 1987). Transmission electron microscopic studies in calves with spontaneous Cryptosporidium infection indicated a marked predilection of the protozoa for the follicular associated epithelium over

the ileal Peyer's patches (Landsverk, 1987). The cellular events associated with the establishment and proliferation of trypanosomes in the skin have been reported using TEM studies and T congolense were observed between interstitial cells, bundles of interstitial fibres and collagen fibres where the skin might be a privileged site for the parasite (Dwinger and others, 1988).

In the study of neoplasms, classification and histogenesis of the tumour are of paramount importance. Although most pathological diagnosis of tumours can be reasonably confirmed with light microscopy (LM), about 1-8% do not conform to the general classification (Henderson and others, 1986). In such circumstances, TEM can often contribute to the diagnosis by demonstrating the ultrastructure beyond the resolution of LM. Many studies on tumours have been directed towards the histogenesis and changes during the growth and development of the tumour. Imai and others (1988) in their investigations on bronchogenic squamous cell carcinomas, have classified the tumour cells in the basal layer into three types according to the electron density of the cytoplasm and the size of their intercellular space. In the identification of cell types in adenomatoid tumours, a tumour affecting the fallopian tubes, uterus and epididymis, many theories have been proposed as regard to their histogenesis. These included the endothelial, epithelial, mesonephric and

mesothelial origin. With a combination of ultrastructural, mucin histochemical and immunohistochemical studies, the mesothelial theory of histogenesis for adenomatoid tumours has been strongly accepted (Stephenson and Mills, 1988).

Transmission electron microscopic studies have also been useful in the identification of viral inclusions and viral replication in cells. Baskerville (1972) studied the ultrastructural changes in the pulmonary airways of pigs infected with Aujeszky's disease virus and noted that the characteristic herpesvirus inclusions were located in the nucleus of bronchial epithelial cells, nucleus of bronchiolar smooth muscle cells and in macrophages.

Vaccination is a common method by which animals may be protected against pathogens. In bacterial vaccine production, for example, it is often important to identify and localise the protective antigens on the cell walls. In this aspect, the TEM has been used extensively to study details of many bacterial cell wall structures, composition and characteristics. Besides studying cell walls, it is also possible to determine the capsular materials associated with the bacterial cells by TEM (Pyliotis and Mukkur, 1981).

Studies with TEM on infectious diseases not only involve examining tissues from animals but also investigations on infected tissue culture preparations. Recently, by using

such a system, Belanger and others (1988) have identified well defined bridges between the viral particles of bovine respiratory syncytial virus, an important agent affecting the respiratory system in cattle. Although these bridges had been identified for the first time, their significance has not yet been established.

Although used mainly in research, the TEM may be used helpfully for routine diagnosis of viral infections by demonstrating viruses in clinical material by negative staining electron microscopy e.g. contagious ecthyma poxvirus in sheep. The use of the TEM to assist in the rapid diagnosis of viral diseases of veterinary importance has been reviewed (Gibbs and others, 1980). They concluded that a system of priority should be established for handling specimens as it would be unrealistic to examine all clinical samples by TEM. It was also considered essential in some situations where a rapid laboratory diagnosis was required, as with exotic diseases, where enforcement and control measures have to be instituted immediately and no other quick methods of diagnosis were available. Although the TEM is expensive to install and operate, the TEM may provide the only evidence for diagnosis in clinical samples containing viruses, that are difficult to isolate, for example rotaviruses (Fenner and others, 1987), mixed virus populations or viruses that do not grow in the generally available culture systems or do not produce obvious

cytopathic effects. England and Reed (1980) showed that the negative contrast electron microscopy (NCEM) can be used as a primary tool for the diagnosis of viral infections of veterinary significance, especially those viruses associated with enteric, respiratory and skin infections. Although it was suggested that the diagnosis should be confirmed wherever possible by viral isolation, immunofluorescence or by serological procedures, their experience indicated that NCEM had provided reliable results.

In conclusion, there is no doubt that the TEM has come to occupy a well established role in diagnostic pathology and in research into animal and human diseases. The growth in the number of TEM studies, especially in research, has been phenomenal in recent years and seems likely to continue in the future as new marker systems for cell and tissue structures are developed.

CHAPTER 2

A REVIEW OF THE ULTRASTRUCTURE OF NORMAL BRONCHIOLES AND ALVEOLI IN SHEEP AND CATTLE

A REVIEW OF THE ULTRASTRUCTURE OF NORMAL BRONCHIOLES AND ALVEOLI IN SHEEP AND CATTLE

In an extensive and detailed review, Breeze and Wheeldon (1977) described the cells lining the lower respiratory tract of mammals and the avian species. They mentioned at least thirteen cell types, eleven epithelial cells and two mesenchymal cells (Table 1). The general structure of these cell types and the cell types which are found in animals are well known. The five epithelial cells in the bronchioles were earlier described by Breeze and others (1976). It is, therefore, intended to consider in general the basic features of cells in the bronchioles and alveoli as stated in standard textbooks (Leeson and others, 1985; Spencer, 1985) and include additional specific information about sheep or cattle where appropriate.

ULTRASTRUCTURE OF THE BRONCHIOLE

There have been no specific studies of the bronchiolar ultrastructure in sheep and cattle except for the study in cattle by Bryson (1980). The bronchiolar cells are assumed to conform to the general mammalian pattern.

SOME CELL TYPES IN NORMAL LUNGS

EPITHELIAL CELLS

Airways	Alveoli
Ciliated	Type I pneumonocytes
Goblet	Type II pneumonocytes
Epithelial serous	Type III pneumonocytes
Brush	
K (including neuroepithelial body cells)	Alveolar macrophage
Basal	Interstitial cell (alveolar macrophage precursor)
Intermediate	
Special type	Fibroblast
Non ciliated bronchiolar secretory (Clara)	

CONNECTIVE TISSUE CELLS (UNSPECIALIZED)

Globule leucocyte

Lymphocyte

Plasma cell

Subepithelial mast cell

Connective tissue mast cell

Eosinophil

Table 1: List of some cell types in normal lungs, according to Breeze and Wheeldon, 1977.

1. BRONCHIOLAR EPITHELIUM

The cell types recognised in the bronchiolar epithelium are ciliated, brush, basal, Kultschnitzky (K)-type and Clara cells.

CILIATED CELLS

These are elongated columnar cells and have a tapering attachment to the basement membrane. Ciliated cells are joined to adjacent columnar cells at their apex by tight junctions and laterally by desmosomes and cytoplasmic interdigitations. Cilia projected from their luminal surface and are attached to the apical cytoplasm by basal bodies. Cilia, in cross-section, have the typical structure of nine paired peripheral microtubules (doublets), surrounding a central pair, thus making up the typical 9-plus-2 structure. The peripheral microtubules are continuous with the basal body.

In addition to cilia, the apical surface of ciliated cells contains abundant microvilli which project into the lumen between the cilia. Each microvillus contains fine filaments within its cytoplasm.

The cell cytoplasm is more electron-lucent than that of most non-ciliated epithelial cells because it does not contain

secretory products, granules or many ribosomes. In the lower part of the cell there are scattered profiles of rough surfaced endoplasmic reticulum (RSER), free ribosomes, a few tonofilaments, smooth surfaced vesicles, multivesicular bodies and, occasionally, lysosomes and pools of glycogen. The well-developed Golgi apparatus is situated above the nucleus, which contains a prominent nucleolus. Many mitochondria are found in the upper part of the cell, just below the apical row of basal bodies.

BRUSH CELLS

Brush cells are infrequently found in the epithelium of the bronchiole, are columnar in shape, rest on a basement membrane and reach the airway lumen. The nucleus is not lobulated, sometimes contains a nucleolus and is found in the lower part of the cell, below the Golgi zone. The cytoplasm is of moderate electron density and contains free ribosomes and glycogen granules, a few profiles of RSER and occasionally lysosomes. Many small vacuoles and vesicles are found among numerous mitochondria in the apical part of the cell. The characteristic features of brush cells are their content of filament bundles that occur throughout the cytoplasm and the dense population of microvilli on the luminal surface. The microvilli contains fine axial filaments, some of which appear to be continuous with filament bundles in the cytoplasm. Brush cells form

tight junctions and lateral cytoplasmic junctions with adjacent cells.

The function of brush cell is unknown. An absorptive function is postulated because of the pinocytic vesicles that are present in the apical cytoplasm. The filament bundle content has led to a stretch receptor role proposal. A chemoreceptor function has also been suggested.

BASAL CELLS

The ovoid cells form a single row along the basement membrane and are responsible for the pseudostratified appearance of the epithelium of larger bronchioles. There is a wide, irregular intercellular space around each cell and this is often crossed by long cytoplasmic processes, which contact adjacent cells and often form desmosomes. The nucleus is large and indented and fills most of the cell. Large numbers of ribosomes and tonofilaments are found in the cytoplasm, which also contain a small Golgi zone, a few mitochondria, glycogen granules, short profiles of RSER and occasionally lysosomes.

`K' CELLS

The K cells are usually found singly in the epithelium, although they may occur in clusters of three to five cells. The nucleus is round to oval, and the cells have a pyramidal or triangular shape. The apical cytoplasm is narrowed and may or may not reach the lumen. Interdigitations of the lower cell borders with neighboring cells without desmosomes have been observed and surface microvilli may be seen reaching the lumen. The cytoplasm contains a prominent Golgi zone, many free ribosomes, abundant smooth surfaced endoplasmic reticulum (SSER), many microfibril tubules, and characteristic granules that have a small electron dense core that is separated by a clear halo from the limiting trilaminar unit membrane.

The function of the K cells is unknown. It has been suggested that these cells might be involved in regulation of the pulmonary circulation, especially in the neonatal period. These cells are thought to govern smooth muscle tone in the bronchial wall and play a part in the production of amines or possibly the generation of kinins in the lung. It has been postulated that some K cells function as air chemoreceptors, especially in the terminal bronchial system of the newborn.

CLARA CELLS

Clara cells are taller than the ciliated cells. The apical cell cytoplasm contains profuse SSER; the mitochondria in this zone are often spherical and are unusual in that they are virtually devoid of cristae and have an abundant, pale matrix. Irregular, electron-dense, homogenous, membrane-bound inclusions, with proteinaceous material, are observed in the apical cytoplasm. The nucleus is found in the central part of the cell with a prominent Golgi apparatus and profiles of RSER. Numerous mitochondria with a few electron-dense membrane-bound inclusions and some lysosomes may be found in the basal cytoplasm. The Clara cells rest on the basement membrane, which covers the connective tissue of the bronchiolar wall and their lateral cell margins are characterized by complex interdigitations, closed by tight cell junctions or desmosomes, with the plasma membrane of adjacent epithelial cells.

There is general agreement that the nonciliated bronchiolar or Clara cell is secretory (Al-Tikriti and Henry, 1988). This function is indicated by the histochemical profile, the abundant mitochondria, and the extensive SSER and Golgi zone, both apparently sites of secretion, granule formation and release.

It has been postulated that nonciliated bronchiolar secretory cells provide a form of surface-active layer in the bronchioles which contribute to the hypophase in the alveoli or provide a watery medium for cilia in the bronchioles similar to that on which the mucus is carried in larger airways.

2. LAMINA PROPRIA

The lamina propria appears as a rather narrow layer of connective tissue that supports the cells of the epithelium. This layer separate the bronchiolar epithelium from the lung capillaries and alveoli. The width of the lamina propria varies depending on the size of the bronchiole. In smaller bronchioles only a narrow layer of connective tissue separates the bronchiolar epithelium from the endothelium of lung capillaries. Only a few isolated fibroblasts are seen within this layer. In larger bronchioles, the lamina propria is wider and consists of numerous fibroblasts, collagen fibres and elastic fibres. Smooth muscle fibres many of which contain glycogen are found among the layers of the connective tissue. Lymphocytes, plasma cells and occasional mast cells are present beneath the epithelium. The muscularis mucosa are four to six cells thick in the larger bronchioli, but decreased to one to two cells thick in the distal bronchioli. In the respiratory bronchioli the muscularis consists only of a single discontinuous layer of smooth muscle.

3. PERIBRONCHIOLAR TISSUE

The bronchioli are surrounded by a peribronchiolar connective tissue layer containing numerous small blood vessels, lymphocytes, plasma cells and occasional mast cells.

4. ALVEOLAR EPITHELIUM

The ultrastructure of the pulmonary alveoli of the calf has been described in detail by Epling (1964); Breeze (1973); Rybicka and others (1974a) and Bryson (1980). Kikkawa and others (1965), and Atwal and Sweeny (1971) gave a similar account of the ultrastructure of the lungs in lambs and goats.

TYPE 1 PNEUMONOCYTES

The cell body of the type 1 pneumonocyte is squamous or occasionally cuboidal in form and its thin cytoplasm attenuates laterally covering most of the alveolar surface. Over much of the alveolar area, the cytoplasm of type I pneumonocyte and the cytoplasm of endothelial cells lining the underlying pulmonary capillaries are closely opposed, with fusion of the epithelial and endothelial basement membrane. The Golgi apparatus and occasional mitochondria are found near the elongated or oval nucleus which

frequently contains one or more nucleolus. Organelles are infrequently seen in the cytoplasm. A moderate number of SSER and infrequently RSER are scattered in the cytoplasm, in which free vesicles, occasional small dense inclusions and free ribosomes are found. A large number of pinocytic vesicles may be found both at the luminal and basal borders of the cell. The cell outline is generally smooth, but occasionally short, stumpy processes with microvilli are seen singly on the free border. The type I pneumonocytes join to each other and to type II pneumonocytes by tight junctions.

TYPE II PNEUMONOCYTES

The type II pneumonocytes are cuboidal or columnar cells which are often found in their characteristic positions at the junction of the alveolar septa and in this position they often overlaid interstitial cells in the alveolar wall. However, type II cells can be found in any position on the alveolar walls. The spherical nucleus often contains one of more nucleoli which is found towards the base of the cell. The free surface of the type II pneumonocyte is thrown into a variable number of irregular short microvilli with the exception of the basal parts, which are covered by overlapping cytoplasmic extremities of the type I cells, forming tight junctions. Organelles and inclusions are common in the cytoplasm. Mitochondria are abundant and the

SSER are well-developed. The Golgi apparatus is found at the base of the cell in a perinuclear position and in some instances is also apparent at the apex. Small multivesicular bodies can be distinguished in close association with the vesicles of the Golgi zone. RSER is present to a lesser degree than SSER. Free ribosomes are found in large numbers especially in the apical cytoplasm.

The characteristic feature of the type II pneumonocyte is the presence of membrane-bound inclusions within the cytoplasm. The larger inclusions are seen near the apex of the cell and the smaller inclusions towards the base. Inclusions are frequently empty or they contain different amounts of electron dense material which is homogenous or arranged in complex whorls. Type II pneumonocytes are the site of disaturated lecithin synthesis (Smith and Kikkawa, 1978), particularly dipalmatoyl lecithin, which is the most important surface-active compound in the lung.

TYPE III PNEUMONOCYTES

The type III pneumonocyte or alveolar brush cell was first described in the rat by Meyrick and Reid (1968). The cell is found on the alveolar epithelium in contact with the basement membrane. The cell shape varies from cuboidal to columnar. The alveolar surface of the cell may be large,

but only a fraction, that studded with microvilli, contributes to the free surface. The remaining alveolar edge is covered by cytoplasmic prolongations from a type I pneumonocyte. The type III pneumonocyte is taller that the type I or type II pneumonocyte. The microvilli protruding into the alveolar space are blunt and contain fine filaments originating in the cytoplasm. The nucleus is not lobulated and occupies one-third to one-half of the base of the cell. An occasional nucleolus may be seen. Mitochondria are situated below the microvilli with the Golgi apparatus in a supranuclear position. Other cytoplasmic organelles present include vacuoles and vesicles, centrioles, lysosomes, fine filaments and free ribosomes. Glycogen is abundant throughout the cytoplasm, often in clumps. Cell junctions are usually smooth and tight, but where the cell is adjacent to a type II pneumonocyte, the membrane may be folded. The type III pneumonocyte has not been described in the alveoli of sheep or cattle; however its presence has been suggested in the horse (Nowell and Tyler, 1971).

5. ALVEOLAR MACROPHAGES

Alveolar macrophages are free cells found in varying numbers in the alveolar lumen. They occasionally rest on epithelial cells or remain attached, but do not form cell to cell junctions. Most macrophages have an irregular outline with many pseudopodia. The have an eccentrically

placed bean-shaped nucleus in which a nucleolus is often prominent. The cytoplasm of the alveolar macrophage are tightly packed with organelles. Many mitochondria are present with a discrete Golgi zone close to the nucleus. RSER is spare but many free ribosomes, small vacuoles and vesicles are found in the cytoplasm. Membrane bound phagosomes of uneven size with pleomorphic material may be found. Lamellar material similar to that observed in the inclusions of type II pneumonocytes may be seen in some phagosomes. The plasma membrane of the cell is often enfolded in many places. A feature of the alveolar macrophage is the presence of small, round lysosomes, which are membrane bound and have homogenous, electron dense contents.

6. INTERSTITIUM

The interstitium of the alveolar wall has capillaries that contain red blood cells, intravascular macrophages, monocytes and polymorphonuclear leukocytes. The capillaries are lined by endothelial cells. Intravascular macrophages have also been described recently in several species including sheep (Wheeldon and Hansen-Flaschen, 1986; Warner and others, 1986; Warner and others, 1987) and cattle (Rybicka and others, 1974b; Warner and Brain, 1984). The other cells formed in the alveolar septum include pericytes, interstitial cells, plasma cells, lymphocytes, mast cells

and polymorphonuclear leukocytes. Varying amounts of connective tissue consisting of bundles of collagen, reticulin, elastic fibres, fibroblasts and smooth muscle fibres are also found.

CHAPTER 3

MATERIALS AND METHODS

MATERIALS AND METHODS

The procedure for obtaining the animals to be studied, the selection of lung samples at post-mortem examination as well as the methods used to process these samples are described below.

1. ANIMALS

The sheep and cattle examined in this study were housed in the Department of Veterinary Medicine and the Department of Veterinary Parasitology until the time for euthanasia.

The clinical cases and the animals with normal respiratory tracts were maintained for several days and examined daily to ascertain the relevant diagnosis or clinical normality of the respiratory system prior to post-mortem examination and the collection of samples for TEM and LM.

The calves on the lungworm experiment were kept in the Department of Veterinary Parasitolopgy and monitored daily throughout the experiment.

2. SELECTION OF SAMPLES FOR TRANSMISSION ELECTRON MICROSCOPY (TEM) AND LIGHT MICROSCOPY (LM)

All animals were killed by shooting with a captive bolt pistol and immediately exsanguinated by jugular section. The lungs were removed as quickly as possible and small blocks of tissue 1-3mm in size were excised from the cranial, middle and caudal lobes of the right lung of each animal for TEM. Lung smaples for TEM from the calves infected with lungworms were taken from the caudal lobes of both the right and left lung. The small blocks of tissue were placed in drops of chilled fixative, consisting of 1.3% paraformaldehyde and 1.6% glutaraldehyde, on blocks of wax and chopped into pieces 0.5mm in thickness using grease-free razor blades. The lung samples were then transferred into vials containing the chilled fixative at $4^{o}C$. The small pieces were left to fix for 4-6 hours in paraformaldehyde/glutaraldehyde. Samples for LM were taken from areas adjacent to those sampled for TEM and fixed in 10% buffered neutral formalin for 24-48 hours.

3. PROCESSING OF LUNG TISSUE SAMPLES FOR TEM AND LM

Lung tissues for TEM were then rinsed in quick succession in 0.1M cacodylate buffer containing 0.1M sucrose and left overnight. They were then post fixed in 0.5% osmic acid in Millonig's buffer for one hour.

Dehydration was carried out through an ascending series of 30%, 60%, 90%, 100% acetone followed by impregnation through graded mixtures of araldite/acetone. The tissues were then embedded in BEEM capsules containing fresh araldite and polymerised in the oven at 60^{o}C for 48 hours.

When the blocks were hardened, thick sections (1 micron) were cut with glass knives on an LKB ultratome III and stained with a mixture containing equal parts of 1% methylene blue, 1% azure II and 1% borax so that specific areas could be selected for electron microscopic examination. Ultrathin sections (50nm) were cut on an LKB Mark III Ultratome, mounted on copper mesh grids and double stained with saturated uranyl acetate in methanol and lead citrate. Stained sections were examined in an AEI 6B electron microscope.

For LM, all the lung samples after fixation were trimmed to 3mm thick and processed by a standard method. The tissues were dehydrated, cleared and finally embedded in a synthetic wax "Paraplast" and blocked into plastic moulds. Sections were cut at 5-8 microns on a rotary microtome, mounted on a glass slide and stained with haematoxylin and eosin.

CHAPTER 4

A TRANSMISSION ELECTRON MICROSCOPIC STUDY OF NORMAL SHEEP LUNGS AND BRONCHIOLAR-ALVEOLAR REACTIONS IN SHEEP WITH PULMONARY ADENOMATOSIS

NORMAL SHEEP LUNGS AND BRONCHIOLAR-ALVEOLAR REACTIONS IN SHEEP WITH PULMONARY ADENOMATOSIS

The study of bronchiolar-alveolar reactions in sheep was carried out in two stages. In the first stage sheep with normal lungs were studied by TEM and in the second phase sheep with pulmonary adenomatosis (SPA) were investigated by TEM.

1. Materials and methods

The lungs from adult sheep with clinically normal respiratory tracts were studied and the lungs from sheep with SPA were also examined.

NORMAL SHEEP

The two normal adult sheep studied were submitted to the veterinary school and had no clinical history of pulmonary disease. This was confirmed by clinical examination carried out by the staff at the Department of Veterinary Medicine where the animals were housed.

ANIMALS WITH SHEEP PULMONARY ADENOMATOSIS

Three adult ewes with sheep pulmonary adenomatosis (SPA) were obtained from the University of Glasgow Veterinary

School Cochno Farm Field Station. The animals had overt clinical signs and had been investigated thoroughly by clinicians in the Department of Veterinary Medicine.

The samples collected from the lungs and the methods used to process the samples for TEM and LM have been described in Chapter 3.

2. Results

NORMAL SHEEP

The lungs from both sheep were grossly normal and no abnormalities were detected by LM examination of the samples from the lobes of the right lung in each case.

Two types of epithelial cells were found to line the bronchioli and they were identified as ciliated cells and nonciliated or Clara cells (Fig. 1). The epithelial cells rested on a continuous basement membrane overlying the lamina propria.

The ciliated cells formed about one-third of the cellular population of the bronchiolar epithelium. The cytoplasm of the ciliated cells were less electron dense than the Clara cells (Fig. 1) and possessed cilia that were attached to the apical cytoplasm by basal bodies (Fig. 2). The ciliated

cells varied in shape from low cuboidal to columnar and were joined to adjacent cells at the apex by tight junctions (Fig. 3). In addition to the cilia, the apical surface contained numerous microvilli, some of which were branched (Fig. 3). The mitochondria were numerous and were often concentrated at the apical cytoplasm beneath the basal bodies (Fig. 3). Profiles of SSER and RSER were also noted in the apical cytoplasm. The nuclei were round to oval and often contained a nucleoli.

The non-ciliated Clara cells comprised about two-thirds of the epithelial cell populations (Fig. 1). The cytoplasm of the Clara cells was more electron dense than the ciliated cells. The nucleus were large and often centrally placed (Fig. 4). The surface of the Clara cells was often thrown into small projections (Fig. 4). A prominent Golgi-apparatus, numerous round or elongated mitochondria, SSER and electron dense bodies were commonly seen in the cytoplasm of Clara cells (Figs. 4 and 5). Membrane-bound granular material was noted in the cytoplasm of some Clara cells (Figs. 4 & 5).

The alveoli of normal sheep were lined by type I and type II pneumonocytes but only type II pneumonocytes were easily identified by LM (Fig. 6). The squamous type I pneumonocyte had a prominent nucleus (Fig. 7) and its cytoplasm attenuated laterally from the nuclear region to form long

and thin cytoplasmic extensions which covered most of the surface of the alveoli (Fig. 8). Cytoplasmic organelles including the mitochondria and Golgi-apparatus were occasionally noted in the cytoplasm. Type II pneumonocytes were cuboidal in shape and had a large centrally placed nuclei in which nucleoli were sometimes prominent (Fig. 8). Characteristic membrane-bound inclusions which were often empty were found in the cytoplasm of the type II pneumonocytes (Fig. 9). The inclusions varied in size and number in individual type II pneumonocytes. Numerous microvilli were present at the surface of the cell (Fig. 9).

The capillaries within alveolar walls were lined by a layer of endothelium which rested on a continuous basement membrane. The endothelial cells often protruded into the capillary lumen (Fig. 10). Within alveolar capillaries, erythrocytes, monocytes and intravascular macrophages were sometimes observed (Fig. 10). Lymphocytes and polymorphonuclear leukocytes were less frequently seen. Intravascular macrophages were large cells with a large nuclei of variable shape and contained abundant cytoplasm. Numerous pseudopodia were seen at the cell surface (Fig. 10). The intravascular macrophages were closely opposed to the capillary endothelium and contained numerous mitochondria, Golgi-apparatus and phagosomes of various sizes (Fig. 10).

The interalveolar septa contained varying amounts of connective tissue consisting of bundles of collagen (Fig. 8), fibroblasts, smooth muscle cells, interstitial cells and occasional mast cells.

Alveolar macrophages were seldom found in the normal animals. These were large cells with a rough and irregular cell surface and contained a large nucleus (Fig. 11). A normal complement of cytoplasmic organelles were present.

ANIMALS WITH SHEEP PULMONARY ADENOMATOSIS (SPA)

All three sheep had extensive consolidation of both lungs by SPA and contained copious amounts of lung fluid. Tissues examined by LM had the characteristic features of the tumour (Fig. 12). Groups of alveolar macrophages associated with the tumour were readily found (Fig. 13).

The tumour cells were predominantly large, varied in density and were seen either in solid acinar (Fig. 14) or as irregularly cuboidal to columnar cells lining the alveolar spaces (Fig. 15) Some of the tumour cells were found in the lumen. Many cytoplasmic microvilli were evident on the free surfaces of the cells towards the alveolar lumen (Figs. 15 and 16). The length and number of microvilli varied between tumour cells and some which projected into the intercellular spaces appeared to interdigitate. Cell borders with

prominent junctional complexes could be easily identified especially in groups of tumour cells (Fig. 14). The tumour cells rested on a basement membrane and in some lesions dense bands of collagen fibres and fibroblasts surrounded them (Fig. 15).

Many of the tumour cells had cytoplasmic inclusions (Fig. 16). These inclusions appeared to have increased in number in tumour cells as compared to normal type II pneumonocytes. The oval to round inclusions varied in number and size from cell to cell, were bounded by a membrane and were located mainly in the supranuclear position facing the luminal surface. These inclusions usually remained empty (Fig. 16) although some enclosed amorphous electron-lucent material (Figs. 15 and 17).

The mitochondria varied in structure, number and distribution. They were more numerous in densely cellular areas and were oval, spherical or elongated in shape (Fig. 14). In some tumour cells they were well preserved with prominent cristae while in others they were partially or completely disrupted. Ribosomes were uniformly scattered throughout the cytoplasm of the tumour cells.

The smooth endoplasmic reticulum was normal in some cells while in others it was morphologically abnormal being greatly distended into irregularly shaped vacuoles (Fig.

17). Most of the tumour cells had prominent Golgi-apparatus that often appeared to be multiple (Fig. 17). Glycogen granules were encountered occasionally (Figs. 16 and 17).

The nuclei in the tumour cells were usually large, round or oval although some had indentations. Perinuclear spaces were prominent in some cells (Fig. 17).

A consistent finding in the alveolar spaces was a pronounced cellular infiltration consisting predominantly of alveolar macrophages (AM), surfactant material and necrotic cellular debris (Figs. 13 and 18). As many as ten AM's were sometimes found in a single foci amongst lesser numbers of polymorphonuclear leukocytes (Fig. 19) and plasma cells (Fig. 20) in the alveolar spaces.

The AM's displayed a great variation in size and irregularity in shape (Figs. 21 to 25). They were generally larger than normal AM's and frequently possessed an eccentric single nucleus. The nuclei were mostly indented although some were round or oval. Nuclear pores were an uncommon finding. Extensive ruffling of the cell surface resulted in many cytoplasmic extensions or pseudopodia (Figs. 21 to 25) which were occasionally in close association with the alveolar epithelium (Fig. 21).

The overall cell density in the AM's had increased and the number of cytoplasmic organelles were also generally increased. Large numbers of mitochondria (Figs. 21 and 22), well developed Golgi-apparatus with multiple stacks of lamellae (Fig. 21) and membrane-bound electron dense lysosomes were often seen (Figs. 23 and 25). Strips of RSER and abundant free ribosomes were a common finding. Vacuoles of different shapes and sizes were frequently seen (figs. 22 and 24). Membrane-bound phagocytized material, including cellular debris and surfactant material were occasionally observed in AMs (Figs. 24 and 25). In some AM's, numerous lamellar structures were seen within the cytoplasm (Fig. 23) while in others electron-lucent material similar to those seen in the type II inclusions were present (Fig. 21). Copious amounts of material usually considered to be surfactant because of its lamellated or lattice structure was seen in many alveoli (Figs. 18 and 26). Phagosomes were regularly seen in AM's (Figs. 24 and 25) and sometimes contained material similar to surfactant.

DISCUSSION

The general morphology and cellular composition of the bronchiolar epithelium of the normal sheep was basically similar to those of other mammals. However, certain cell types that have been described in other species were not observed in the sheep studied. These included the brush, basal and Kultschnitzky-type cell of mammals (Breeze and others, 1976; Breeze and Wheeldon, 1977) and the goblet and brush cells that were occasionally seen in small numbers in the epithelium of the large bronchioles in cattle (Bryson, 1980) and in pigs (Baskerville, 1970a). The failure to detect these cells in the bronchioles was probably due to the fact that only two animals were examined. In this study, ciliated and non-ciliated (Clara) cells were the only cell types identified in the bronchiolar epithelium. This finding compared favourably with other studies in the mouse (Karrer, 1956), pigs (Baskerville, 1970b), hamsters and horses (Nowell and Tyler, 1971) and in cattle (Iovannitti and others, 1985).

The structure of the bronchiolar ciliated cell and Clara cell in a number of mammals have been reviewed (Breeze and others, 1976; Breeze and Wheeldon, 1977) and the present study revealed no major deviations in the case of the sheep. Many studies on the Clara cells have been carried out. These investigations included the comparative

ultrastructural studies in mammals (Smith and others, 1973; Smith and others, 1979; Plopper and others, 1980a), quantitative evaluation of Clara cells in mammals (Plopper and others, 1980b) and the differentiation capacity of the Clara cell (Brody and others, 1987). Despite the numerous electron microscopic studies, the function of the Clara cells in the bronchioles remains largely obscure (Kuhn and others, 1974; Brody and others, 1987). The Clara cells are thought to synthesise, store and secrete the protein component of the extra cellular lining layer of the bronchiole (Massaro, 1989). Contrary to what has been reported in calves (Bryson, 1980), there was no evidence of apocrine secretion into the bronchiolar lumen although membrane-bound granules were seen in the cytoplasm of some Clara cells. The brush or type III pneumonocyte that has been described in the rat (Meyrick and Reid, 1968; Chang and others, 1986) was not observed in the alveolar epithelium of the sheep in this study.

Macrophages were rarely found in the alveolar lumina in the normal sheep that were studied. These alveolar macrophages (AM's) were large cells with an irregular cell surface. The cytoplasm contained few mitochondria and small electron-dense lysosomes. There has been conflicting observations regarding the occurrence of the AM's in the literature.

Meyrick and Reid (1970) concluded that AM's were a common finding in the human lung. The observations in this study on sheep regarding the rarity of AM's were concordant with other studies in cattle (Rybicka and others, 1974a; Mariassy and others, 1975) and in pigs (Baskerville, 1970a).

Pulmonary intravascular macrophages (PIM) have been previously reported in normal animals including the sheep (Warner and others, 1986; Wheeldon and others, 1986; Wheeldon and Hansen-Flaschen, 1986). The morphological and cellular characteristics of the PIM's in this study resembled those of the mature PIM's in sheep (Wheeldon and Hansen-Flaschen, 1986) and in pigs (Winkler and Cheville, 1985).

Sheep pulmonary adenomatosis (ovine pulmonary carcinoma, jaagsiekte) is a retrovirus induced pulmonary neoplasm of sheep of economic importance (De Martini and others, 1988) and is classified histologically as a bronchioloalveolar carcinoma (Stunzi and others, 1974).

The proliferating tumour cells in this study had morphologic features of type II pneumonocytes and were characterised by the presence of numerous microvilli, tight junctions and intracellular membrane-bound inclusions. Similar observations have been reported by other investigations in both natural (Nisbet and others, 1971;

Perk and others, 1971, Wandera and Krauss, 1971; Hod and others, 1974; Hod and others, 1977; Rosadio and others, 1988a) and experimental cases (Payne and Verwoerd, 1984; Rosadio and others, 1988b; De Martini and others, 1988). Recently, in New Zealand, Dalefield and Alley (1988) reported a case of a well differentiated type II pneumonocyte adenocarcinoma in a 13-year-old Romney ewe with ultrastructural features similar to SPA.

There is general disagreement as to whether the bronchiolar cells formed an integral part of the neoplastic epithelial proliferation. Some neoplastic growths were reported to involve bronchiolar epithelial cells (Rosadio and others, 1988b) and, more specifically, Clara cells (Rosadio and others, 1988a), ciliated cells (Hod and others, 1977) and also Clara cells, ciliated cells, goblet cells and brush cells (Nisbet and others, 1971). In contrast, the findings of this study and others (Wandera and Krauss, 1971; Perk and others, 1971; Payne and Verwoerd, 1984) revealed that the bronchiolar epithelium were not associated with the proliferation of the tumour cells.

Large masses of glycogen have been reported in many tumour cells in SPA (Perk and others, 1971; Wandera and Krauss, 1971). This observation contradicts the finding of Payne and Verwoerd (1984) who found such granules only on rare occasions. The results of this study are in agreement with

Nisbet and others (1971) in that a small portion of the tumour cells contain glycogen. It is interesting to note than in the study of adenomatosis in mice, glycogen was also found (Brookes, 1968). The appearance of glycogen in a cell in which it is not normally present suggest a biochemical alteration (Perk and others, 1971) with probable reversion to fetal cell epithelium in which glycogen is normally apparent (Cutlip, 1985).

The tumour cells in this study had other abnormalities such as increased free ribosomes, dilation of the endoplasmic reticulum, mitochondrial degeneration, widened perinuclear space and increased numbers of intra-cytoplasmic inclusions and microvilli on their free surface. These findings have been earlier reported in natural cases of SPA (Wandera and Krauss, 1971).

While morphological, immunological and other studies implicate a type B or type D retrovirus as the etiological agent of SPA, the virus has not yet been cultured (De Martini and others, 1988).

No viral particles were seen in this study and in several other investigations (Wandera and Krauss; Nisbet and others, 1971; Cutlip, 1985).

The most frequent cell type found in the alveolar spaces was the AM's which were mainly seen contiguous to the neoplastic cells. Lesser numbers of AM's were apparent in close proximity to the epithelium of normal or tumour cells. Plasma cells and polymorphonuclear leukocytes were noted occasionally. The infiltration of the AM's surrounding the neoplastic foci is a consistent feature of SPA, both in naturally occurring (Tustin, 1969; Wandera, 1971; Hod and others, 1977) and experimentally induced cases (Payne and Verwoerd, 1984; De Martini and others, 1987).

In this study it was evident that SPA caused morphological alterations in the AM's as compared to normal AM's. The AM's could be classified into two populations. The first group comprised AM's that were less electron dense, had increased numbers of cytoplasmic organelles and numerous phagosomes and contained vacuoles of differed shapes and sizes. The second category of AM's consisted of the more electron dense AM's with a high degree of development of cytoplasmic structures, notably lysosomes, mitochondria and RSER. Both the groups displayed a pleomorphic cell shape and possessed extensive finger-like pseudopods. These morphologic changes represent AM activation (Adams, 1976; North, 1978; Allison, 1978).

A prominent ultrastructural characteristic, micropinocytosis vermiformis, has been noted in some AM's. The formation of

this structure is considered to be due to either the invagination of plasma membrane or the approximation of the pseudopodium to the cell membrane (Warner and others, 1987) and has been reported only in cell types of the mononuclear phagocyte system, including the spleenic macrophage, hepatic kupffer cells and the lymph node macrophage (Winkler and Cheville, 1985). Tubular structures of micropinocytosis vermiformis are signs of stimulated receptor-mediated endocytosis (Winkler and Cheville, 1985; Winkler, 1988).

The cytoplasm of the first group of AM's contained many small irregularly shaped translucent vacuoles, which were presumably pinocytic or they could represent lipid vacuoles as described by Shibuya and others (1986). Using histochemical studies they confirmed that the foam cells seen in the alveolar spaces in cases of lung neoplasms were actually lipid-laden macrophages. Destruction of the pulmonary tissue results in the release of endogenous lipid with accumulation of foam cells (Beaver and others, 1963).

The potential role of the AM's in the pathogenesis of SPA is unknown (Rosadio and others, 1988b). The excessive surfactant material which is produced by the transformed type II pneumonocytes may serve as a stimulus for the accumulation of macrophages. Alveolar macrophages have also been shown to be capable of inducing DNA synthesis in alveolar type II cell cultures in rats (Leslie and others,

1985) and may do so in neoplastic epithelial cells as well. Recently, SPA-derived, transformed alveolar type II cell lines have been demonstrated to produce a macrophage chemotactic factor which may act to recruit AM's into the SPA affected lungs (Myer and others, 1987). In addition, the massive accumulation of AM's may potentiate tumour progression, as suggested to occur in some non-pulmonary tumours. (Nelson and others, 1981).

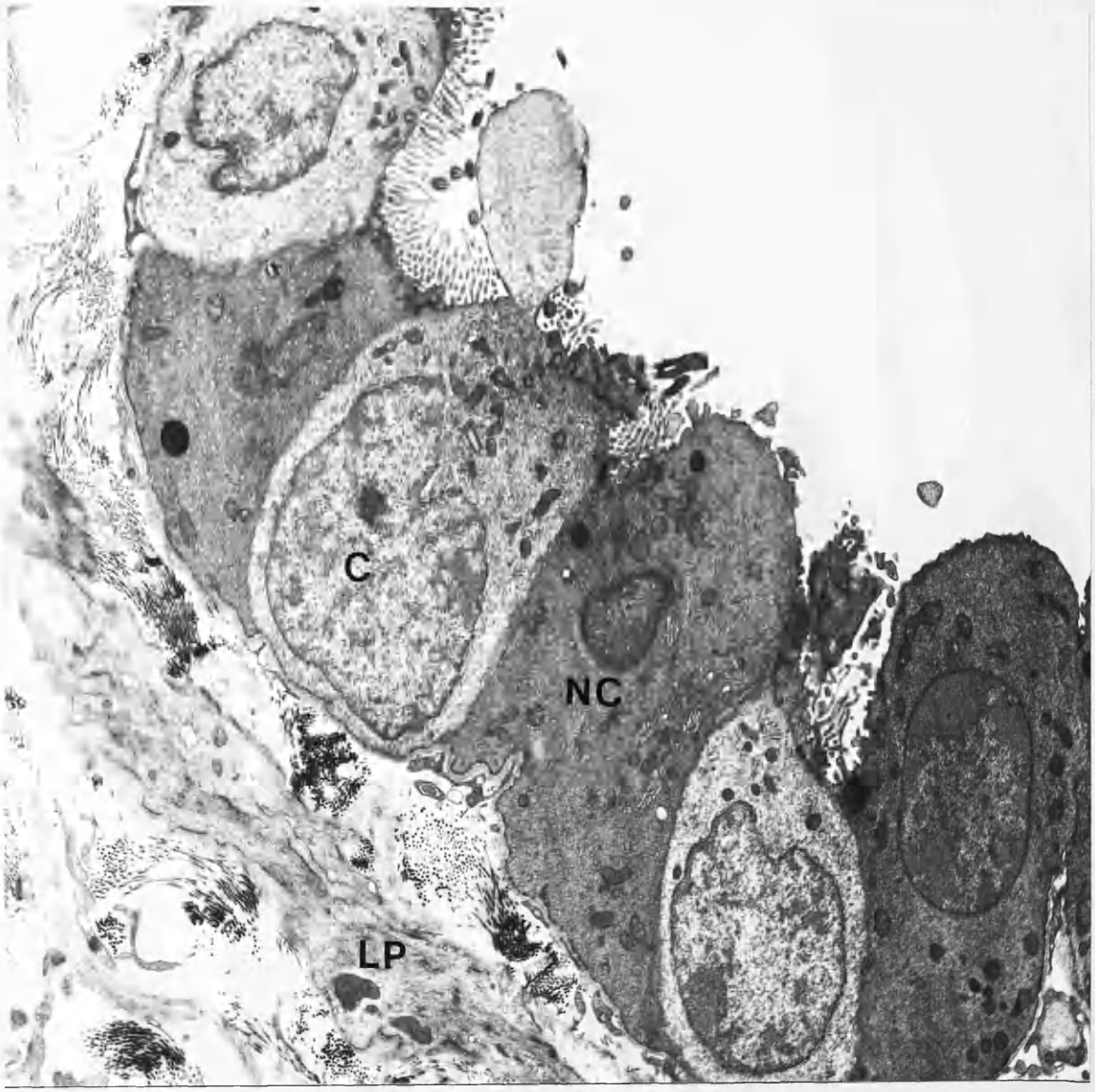

Fig. 1 Ciliated cell (C) and nonciliated cell (NC) in the bronchiolar epithelium of a normal sheep. Note the difference in electron density between the cells. LP = bronchiolar lamina propria. (x 8,000)

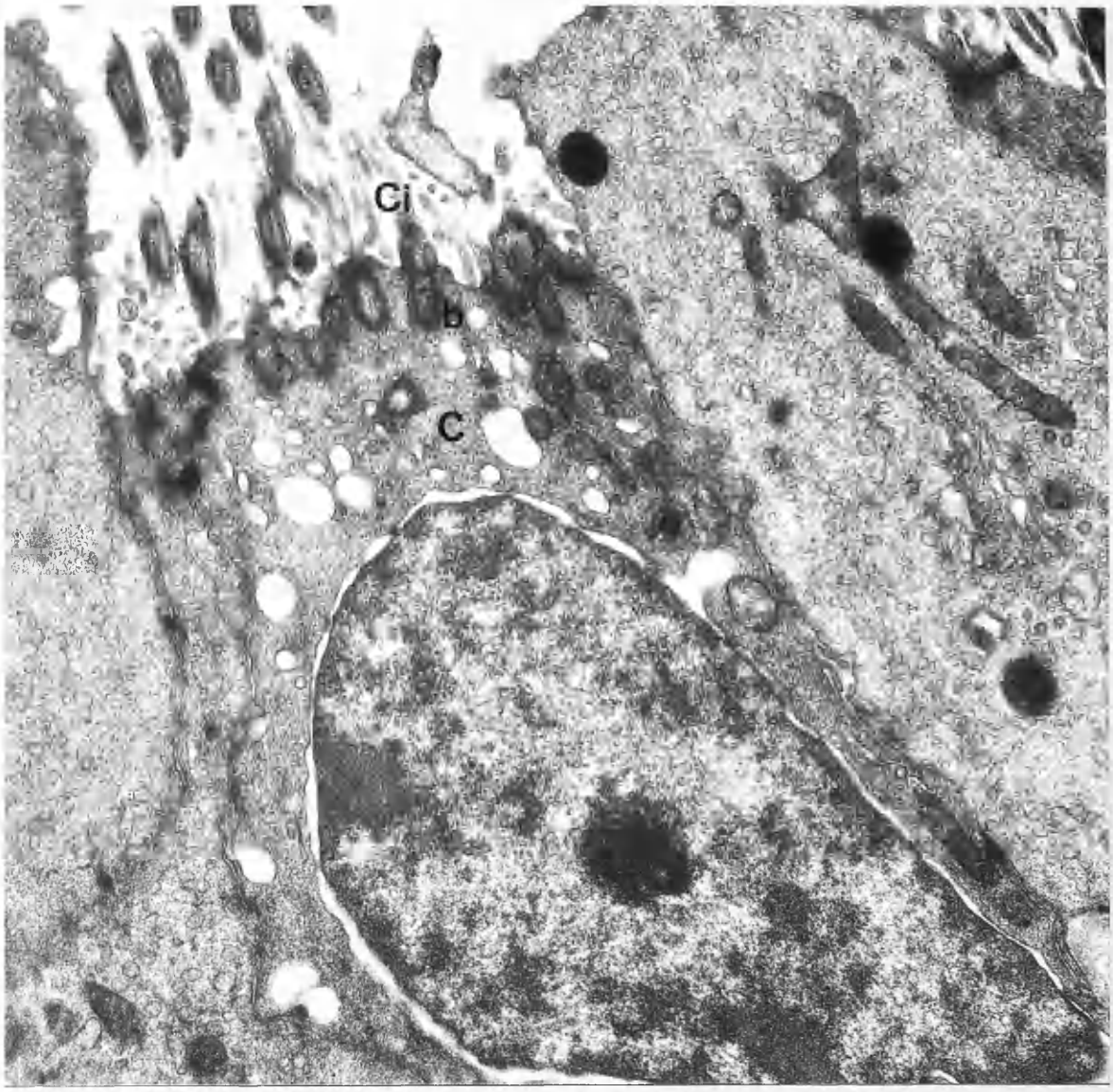

Fig. 2 Apex of a bronchiolar epithelium of a normal sheep showing cilia (Ci) projecting from a ciliated cell (C). Cilia are attached to the apical cytoplasm by basal bodies (b).
(x 20,000)

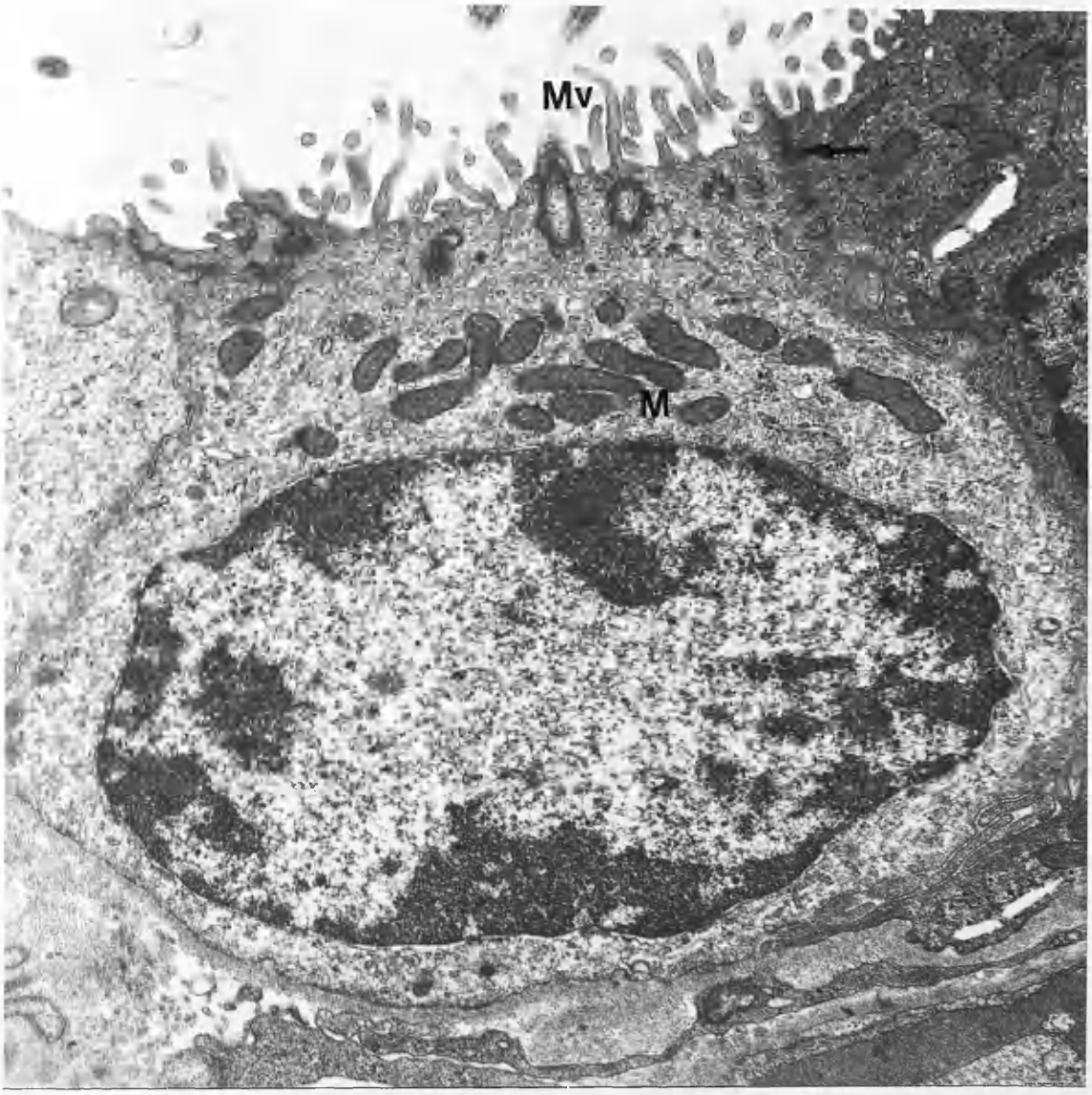

Fig 3. Ciliated cell in bronchiolar epithelium of a normal sheep. The mitochondria (M) are located in the apical cytoplasm beneath the basal bodies. Numerous microvilli (Mv) are seen projecting from the ciliated cell. Some of the microvilli are branched. Note tight junction (arrow) between adjacent cells.
(x 20,000)

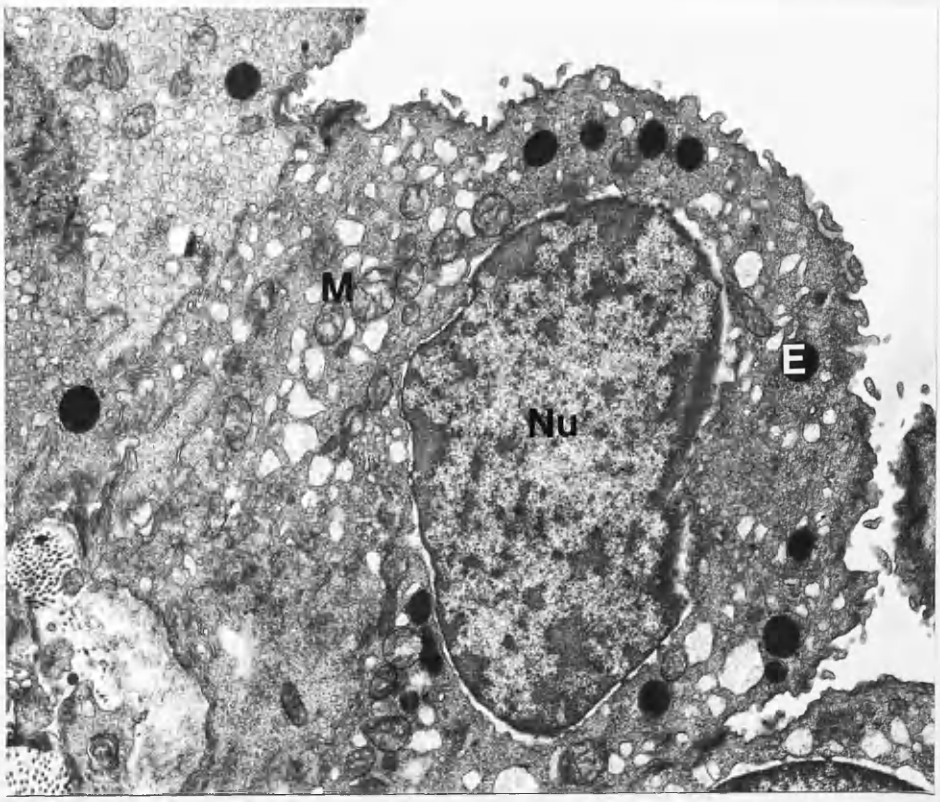

Fig. 4 Apex of a Clara cell projecting into the lumen of a bronchiole in a normal sheep. The nucleus (Nu) is large and centrally placed. Mitochondria (M) and electron dense bodies (E) are seen in the cytoplasm. Note the numerous microvilli on the cell surface.
(x 12,500)

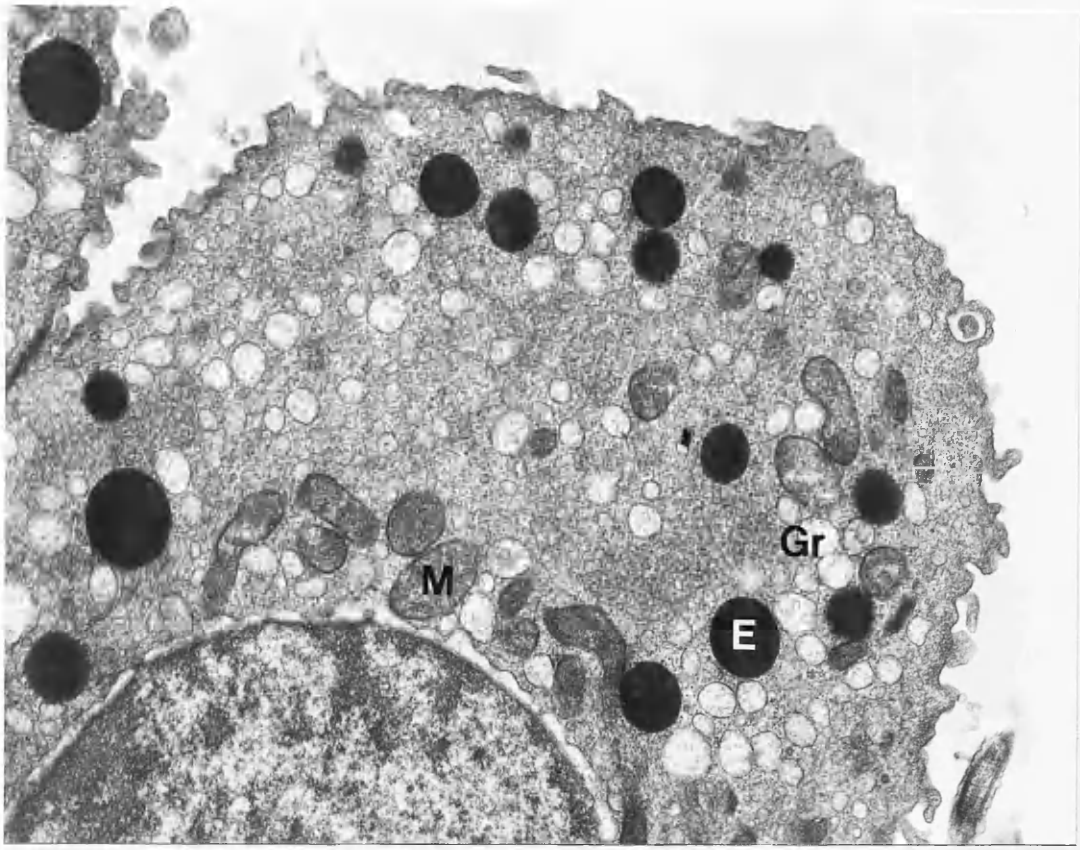

Fig. 5 Clara cell in a bronchiole of a normal sheep. The apex of the cell projects into the bronchiolar lumen. Mitochondria (M), electron dense bodies (E) and membrane-bound granules (Gr) are seen in the cytoplasm.

(x 20,000)

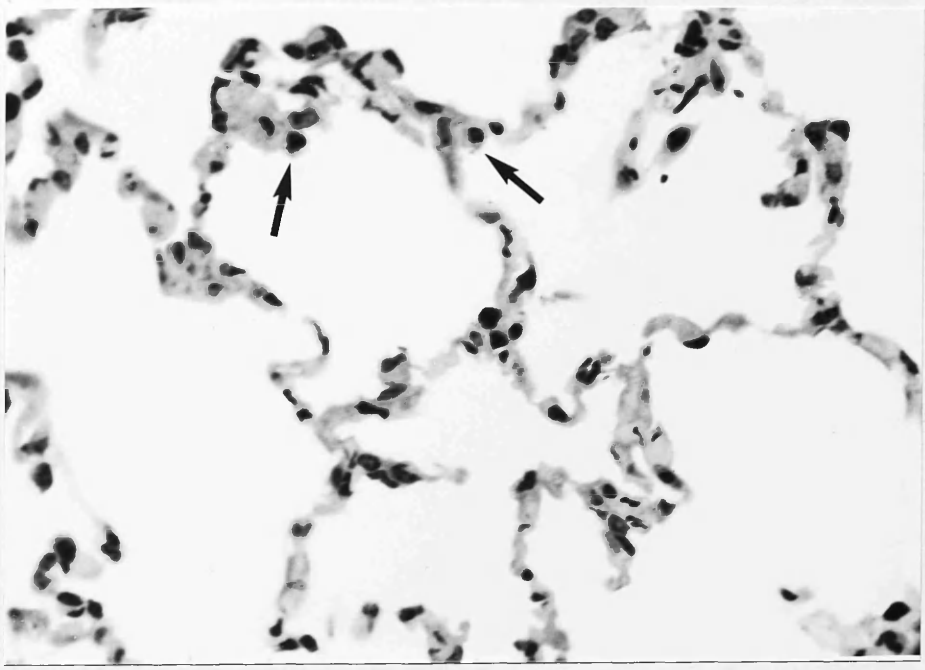

Fig. 6 Alveolar walls with Type II pneumonocytes in a normal sheep. With the light microscope these cells have a large nucleus and vacuolated cytoplasm (arrows). Methylene blue, azure II and borax stain.

(X 700)

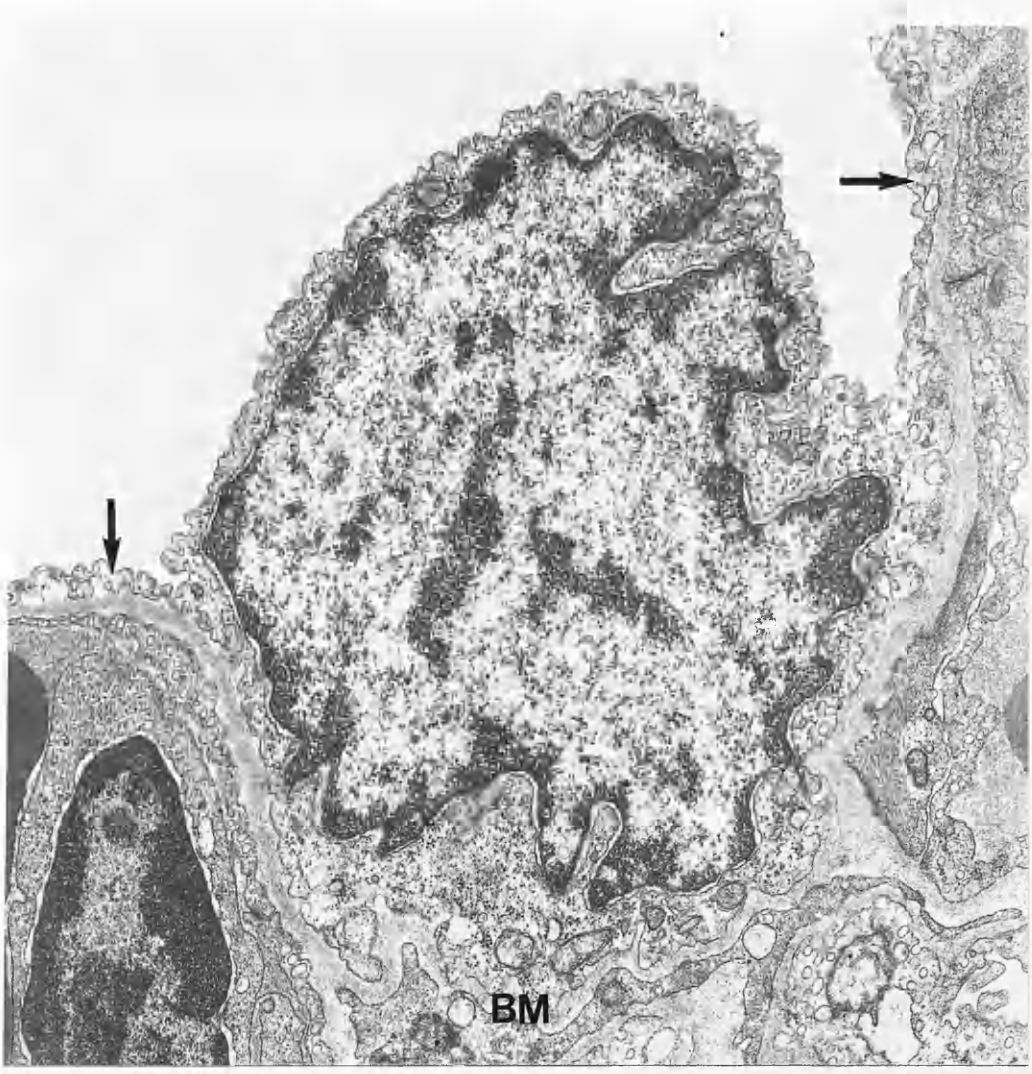

Fig. 7 The I pneumonocyte resting on a continuous basement membrane (BM) in a normal sheep. The cell body is cuboidal and its cytoplasm attenuated laterally (arrows).
(x 20,000)

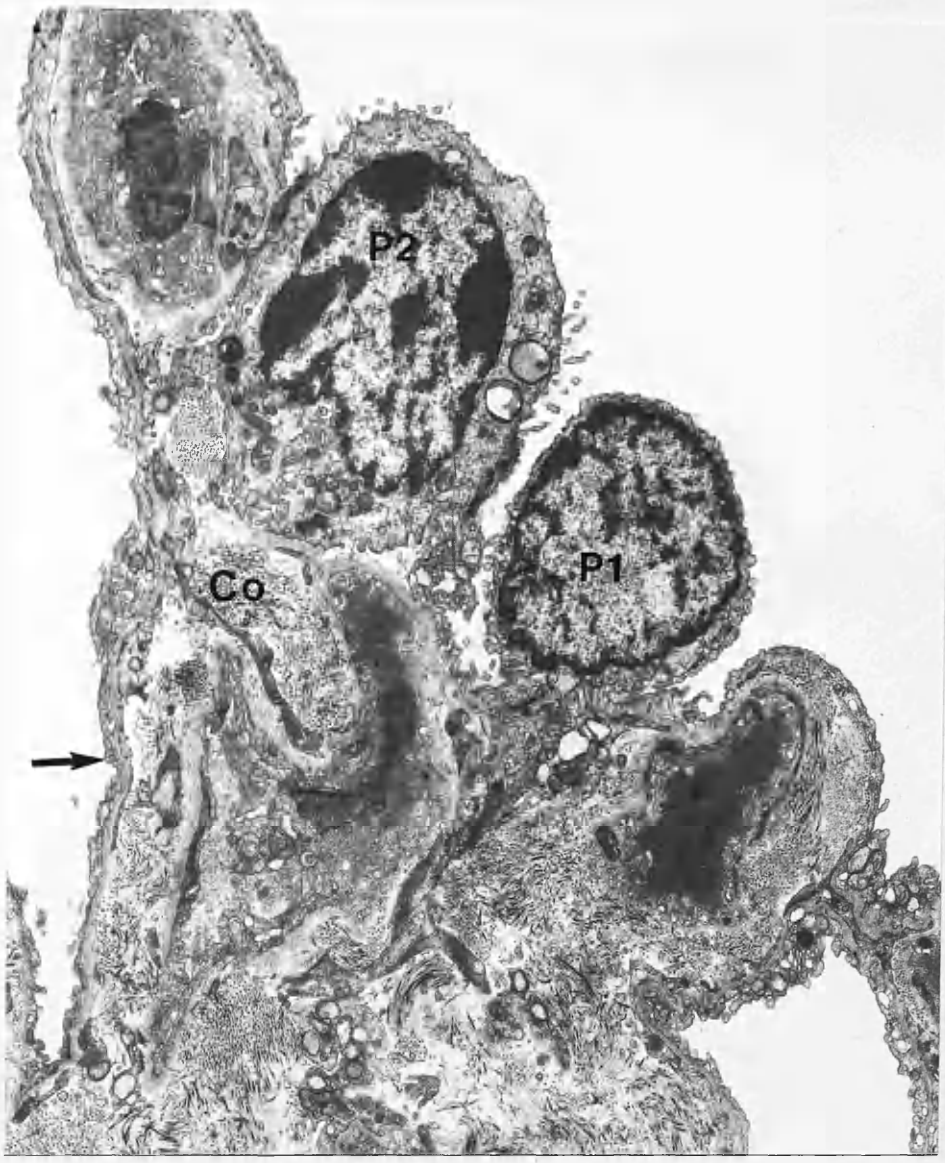

Fig. 8 Type I pneumonocyte (P1) and type II pneumonocyte (P2) on an alveolar in a normal sheep. Collagen fibrils (Co) are also present. Note the extensive attenuation of the type I pneumonocyte cytoplasm (arrow).

(x 8,000)

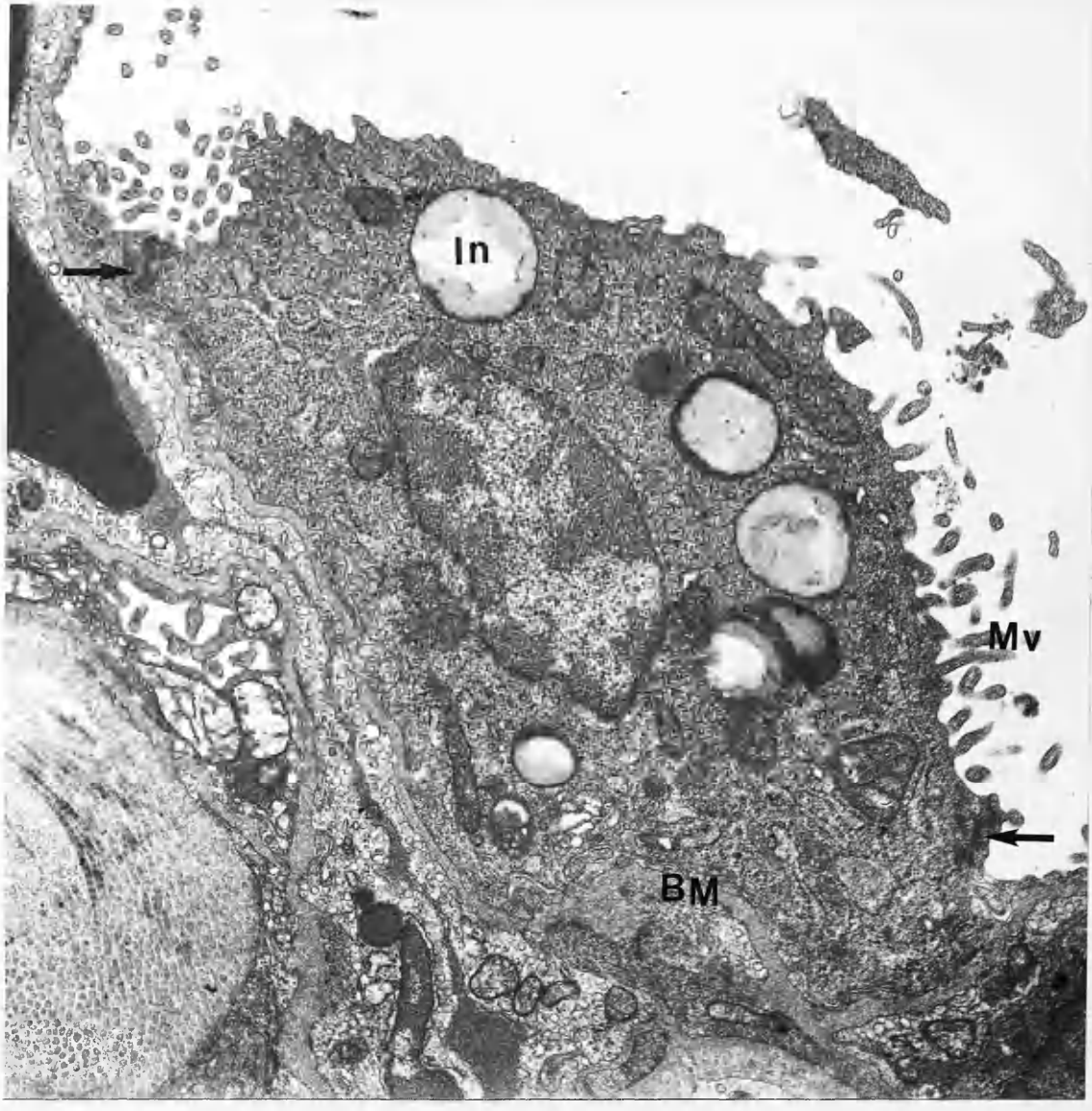

Fig. 9 Type II pneumonocyte of a normal sheep. The cell contains many microvilli (Mv) and the characteristic inclusions (In) which are empty. Note the continuous basement membrane (BM) and the tight junctions (arrows).
(x 20,000)

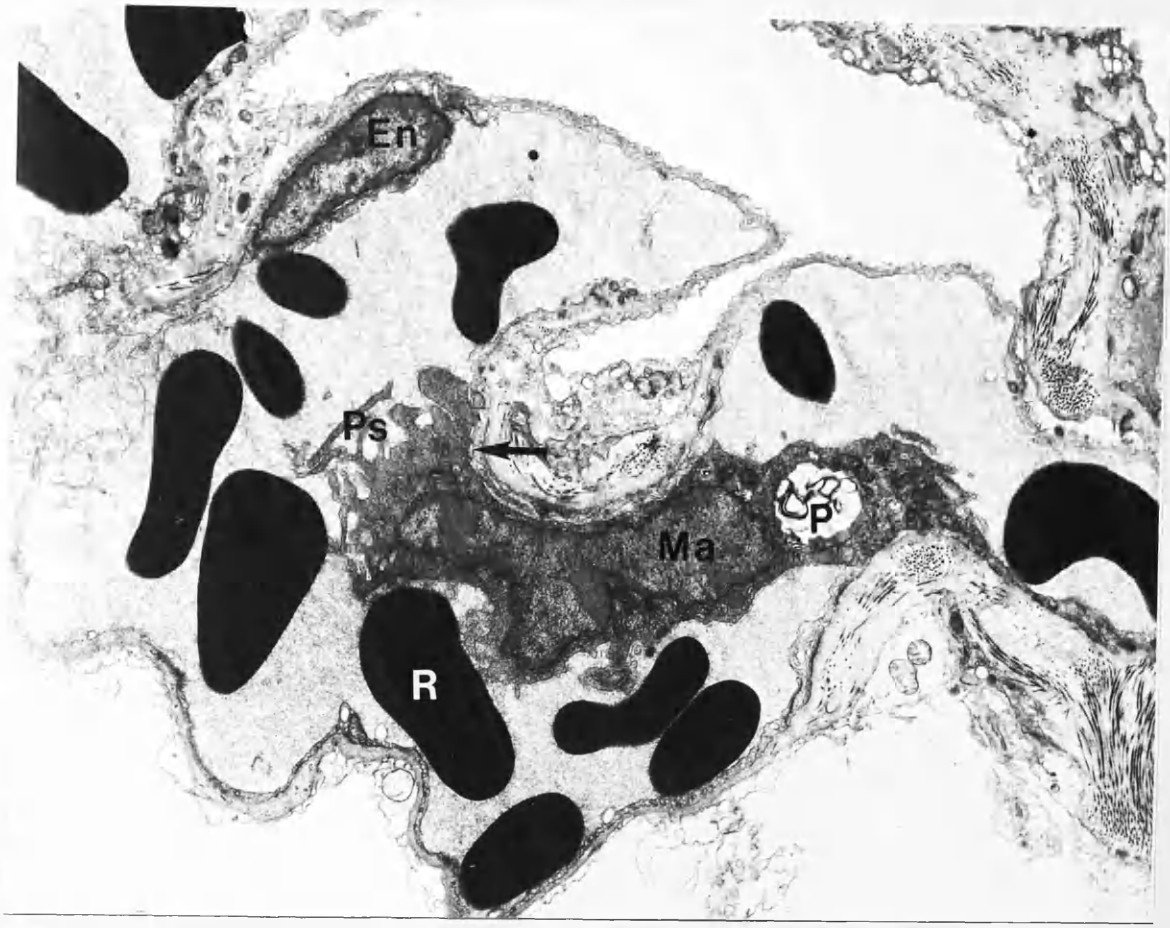

Fig. 10 Portion of a pulmonary capillary in the alveolar wall of a normal sheep. An endothelial cell (En) with its nucleus is visible. The alveoli are lined by long thin cytoplasmic extensions of the type I pneumonocyte. An intravascular macrophage (Ma) is seen in the alveolar capillary with numerous pseudopodia (Ps) and a phagosome (P). Note the zone of close opposition of the intravascular macrophage and endothelial cytoplasm (arrow). R = red blood cell.

(x 8,000)

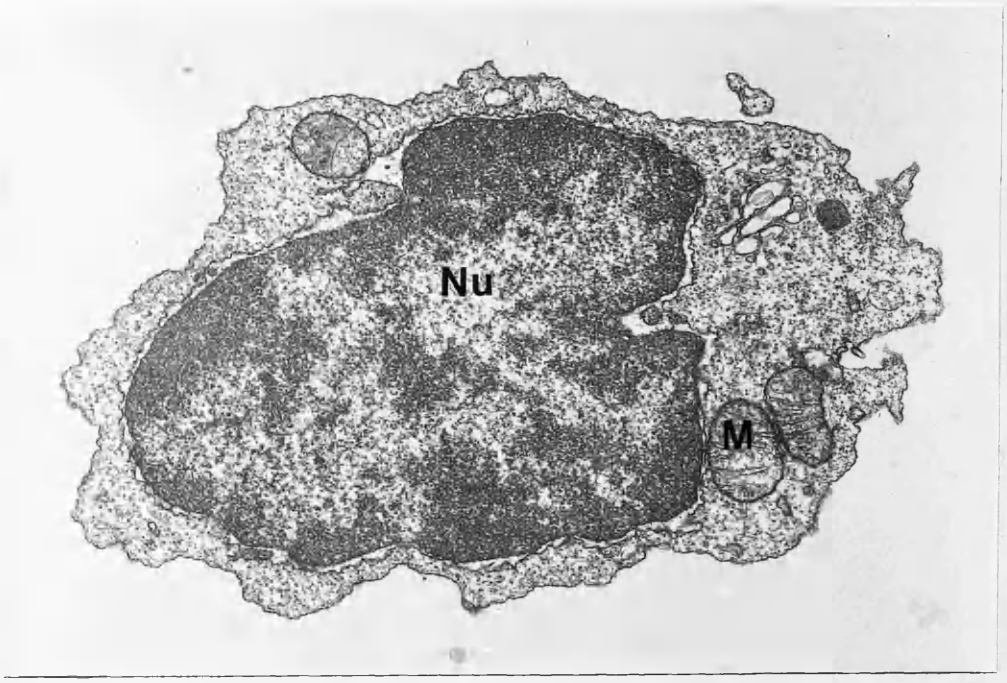

Fig. 11 Alveolar macrophage in a normal sheep with a large nucleus (Nu) and irregular cell surface. Numerous mitochondria (M) are present.
(x 20,000)

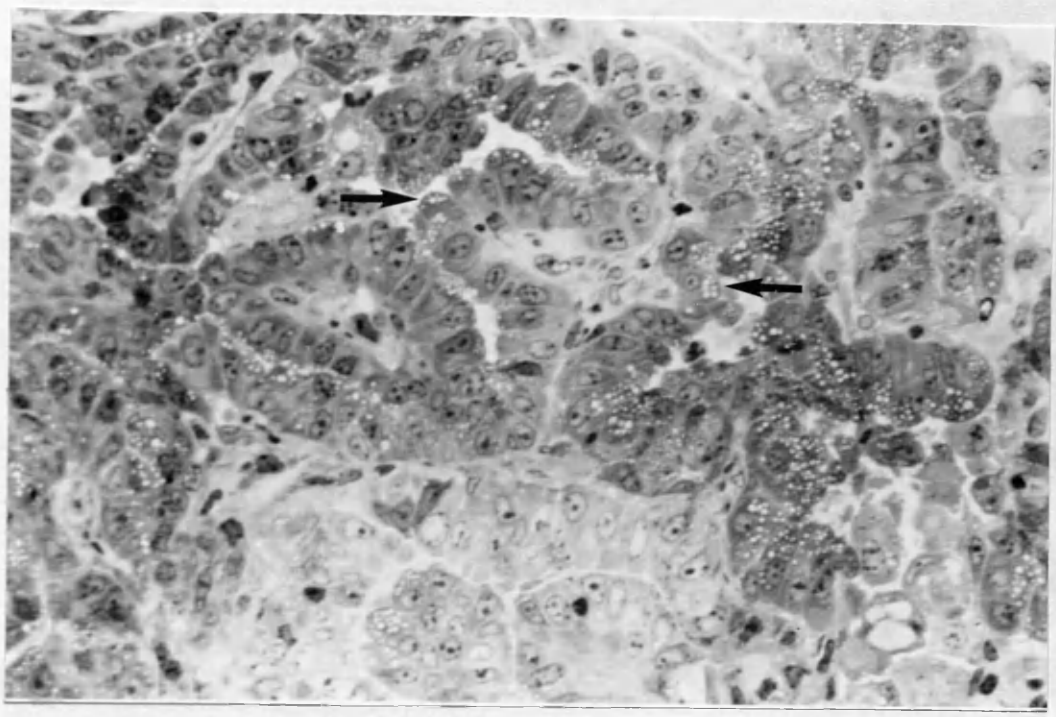

Fig. 12 Group of SPA tumour cells. Vacuoles (arrows) seen under the light microscope correspond to Inclusions in Figs. 15 and 16. Methylene blue, azure II and borax stain.

(X 700)

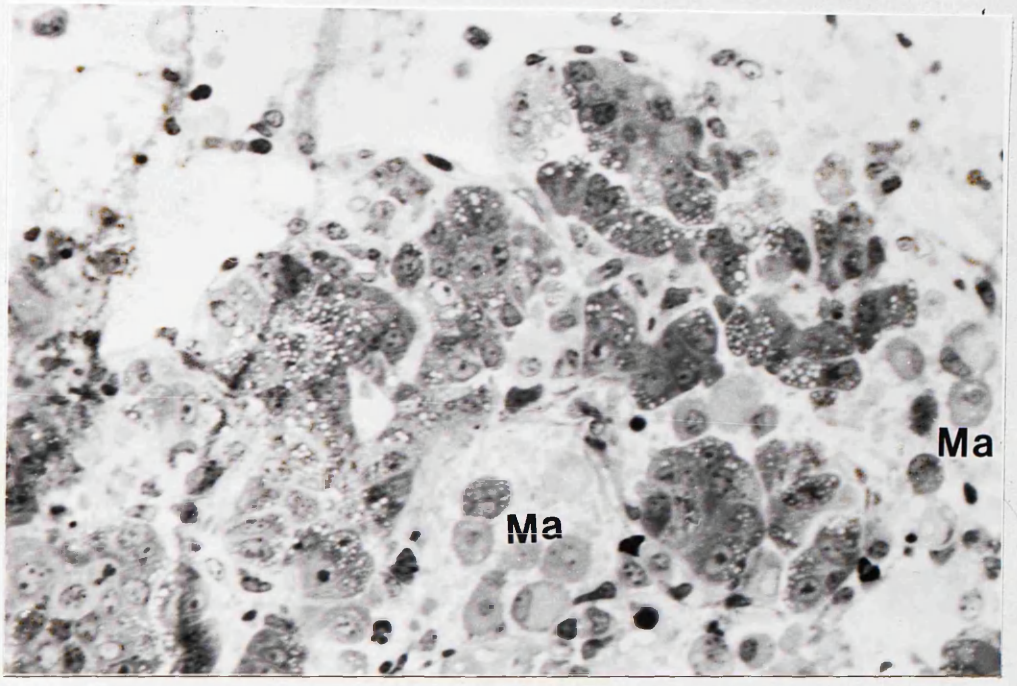

Fig. 13 Alveoli containing neoplastic type II cells in SPA. Groups of alveolar macrophages (Ma) can be seen in adjacent alveolar spaces. Methylene blue, azure II and borax stain.
(x 700)

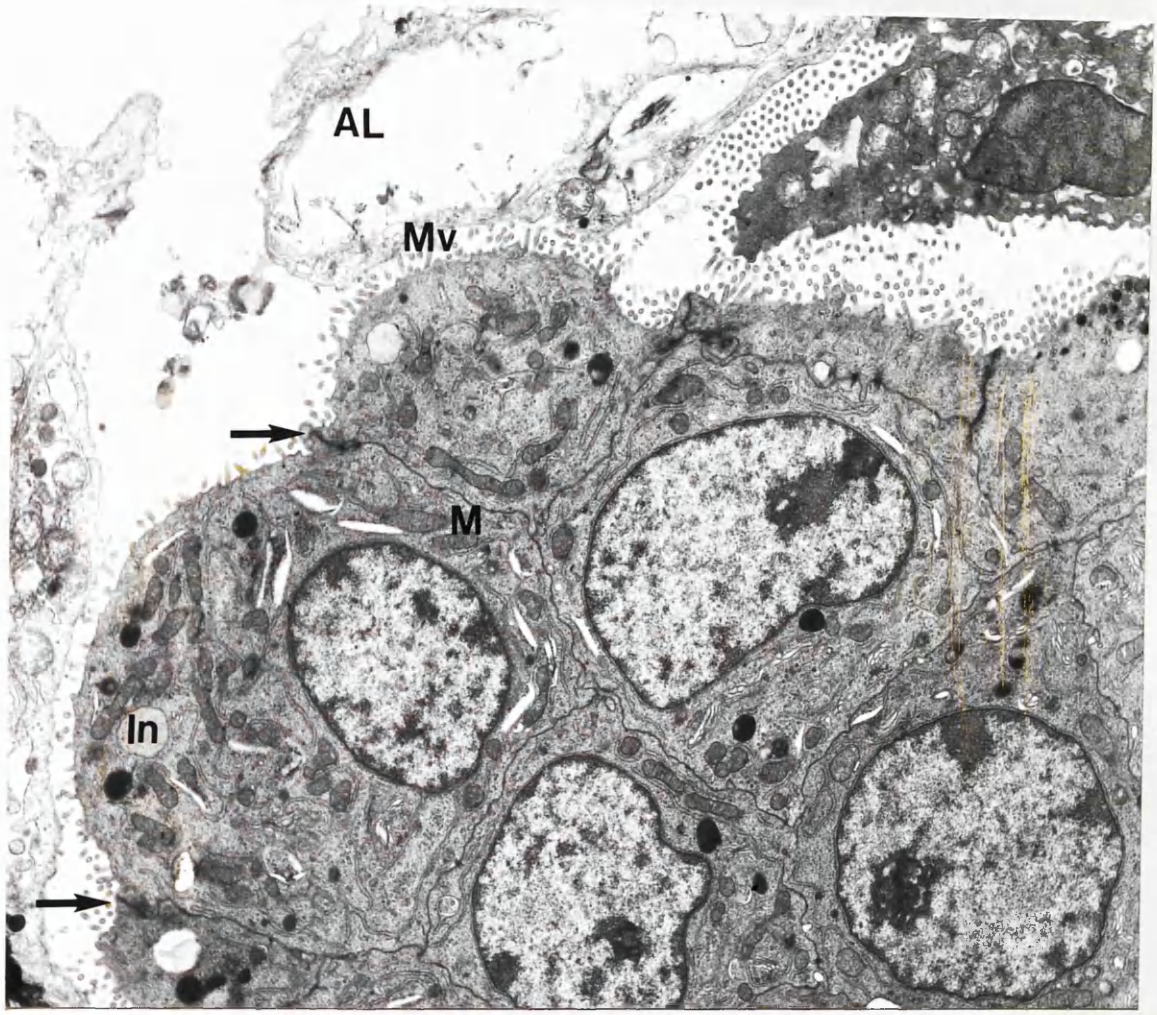

Fig. 14 Sheet of SPA tumour cells. Note microvilli (Mv), mitochondria (M), Inclusions (In) and junctional complexes (arrows).

AL = Alveolar lumen.

(x 8,000)

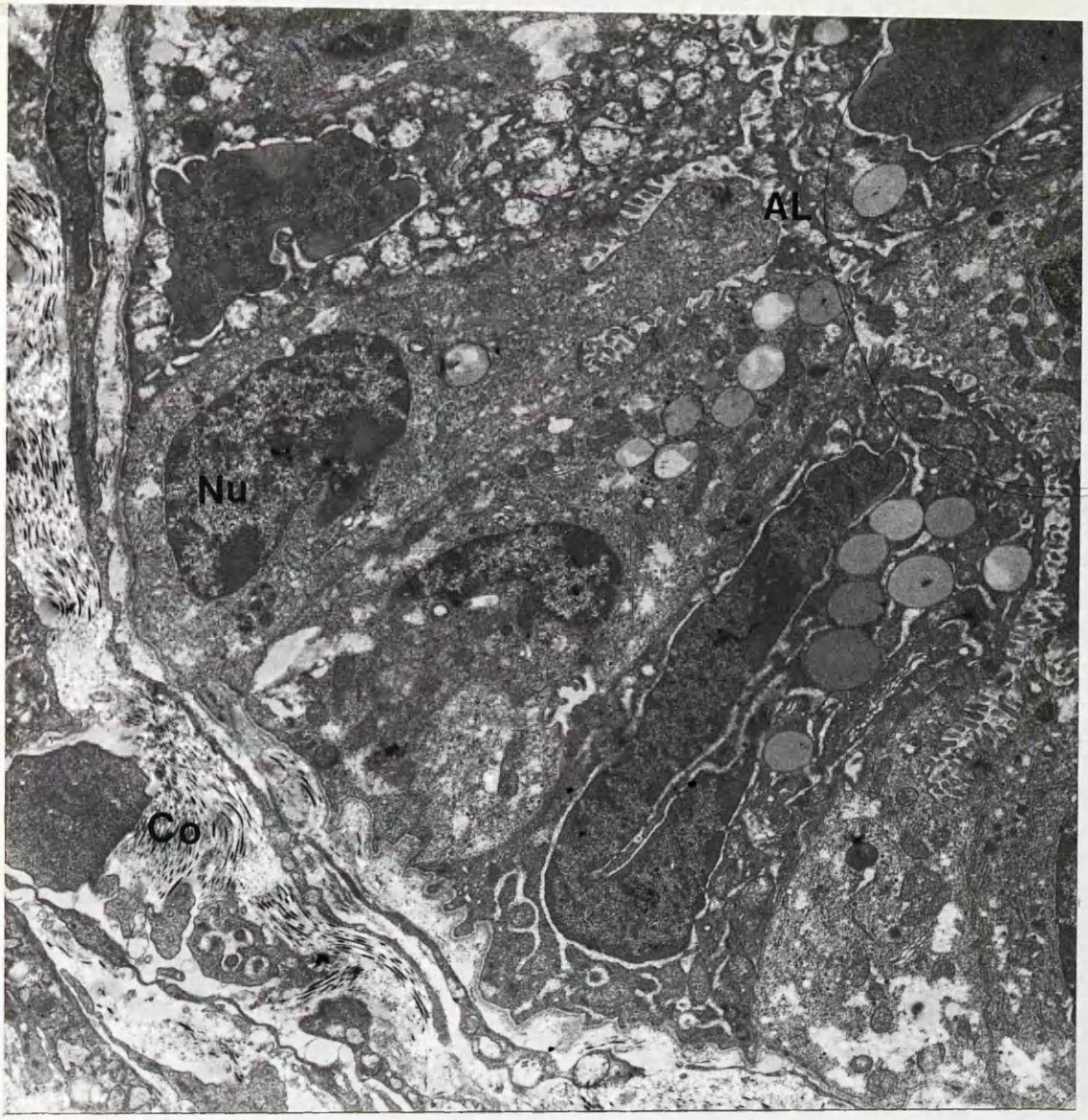

Fig. 15 Compact SPA tumour cells around an alveolar space (AL). Note the numerous collagen fibres (Co) at the base of these cells.

Nu = Nucleus.

(x 8,000)

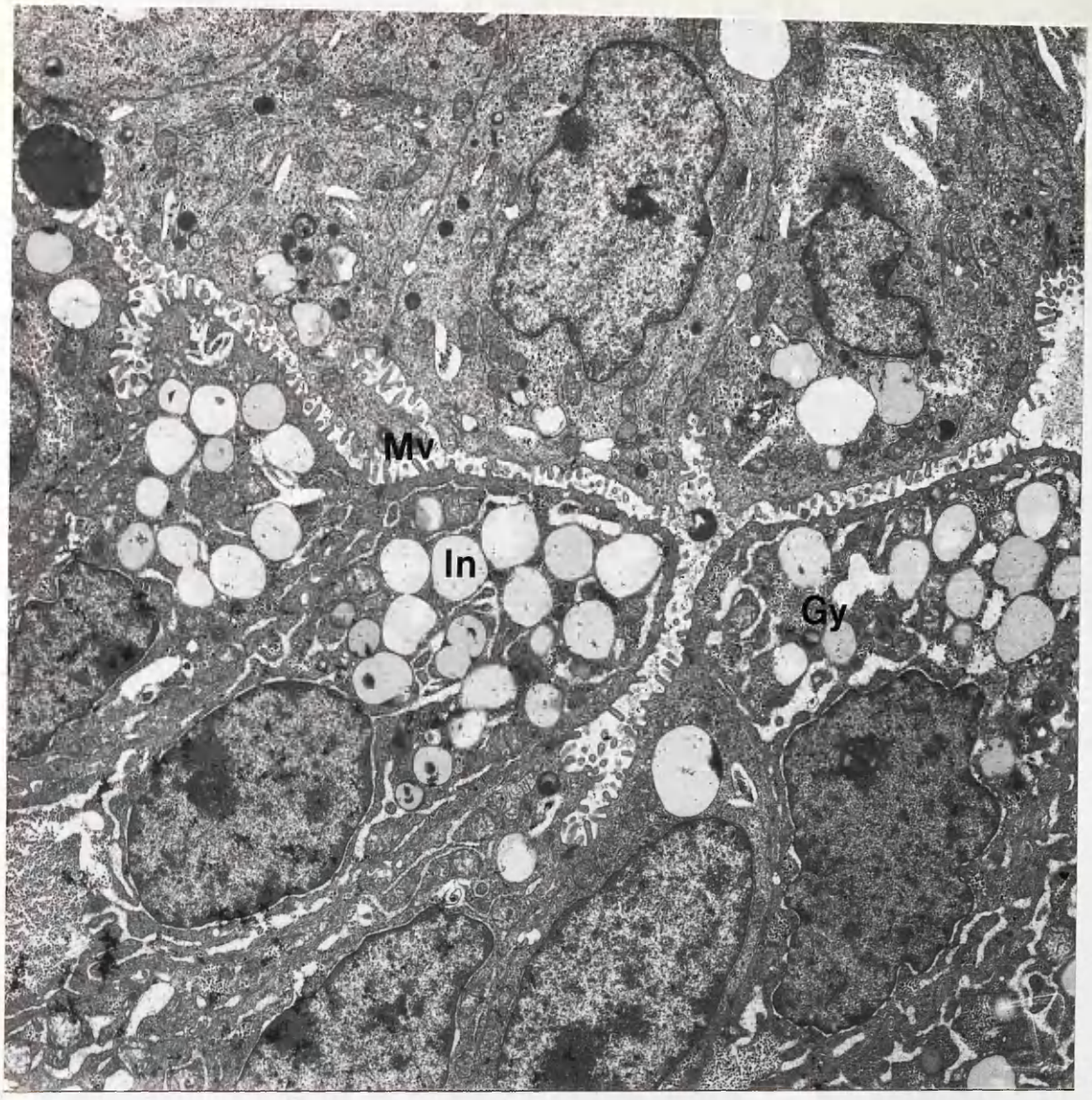

Fig. 16 The alveolar lumen is lined by SPA tumour cells. Note the microvilli (Mv), numerous inclusions (In), glycogen deposits (Gy) and marginated nucleoli. (x 8,000)

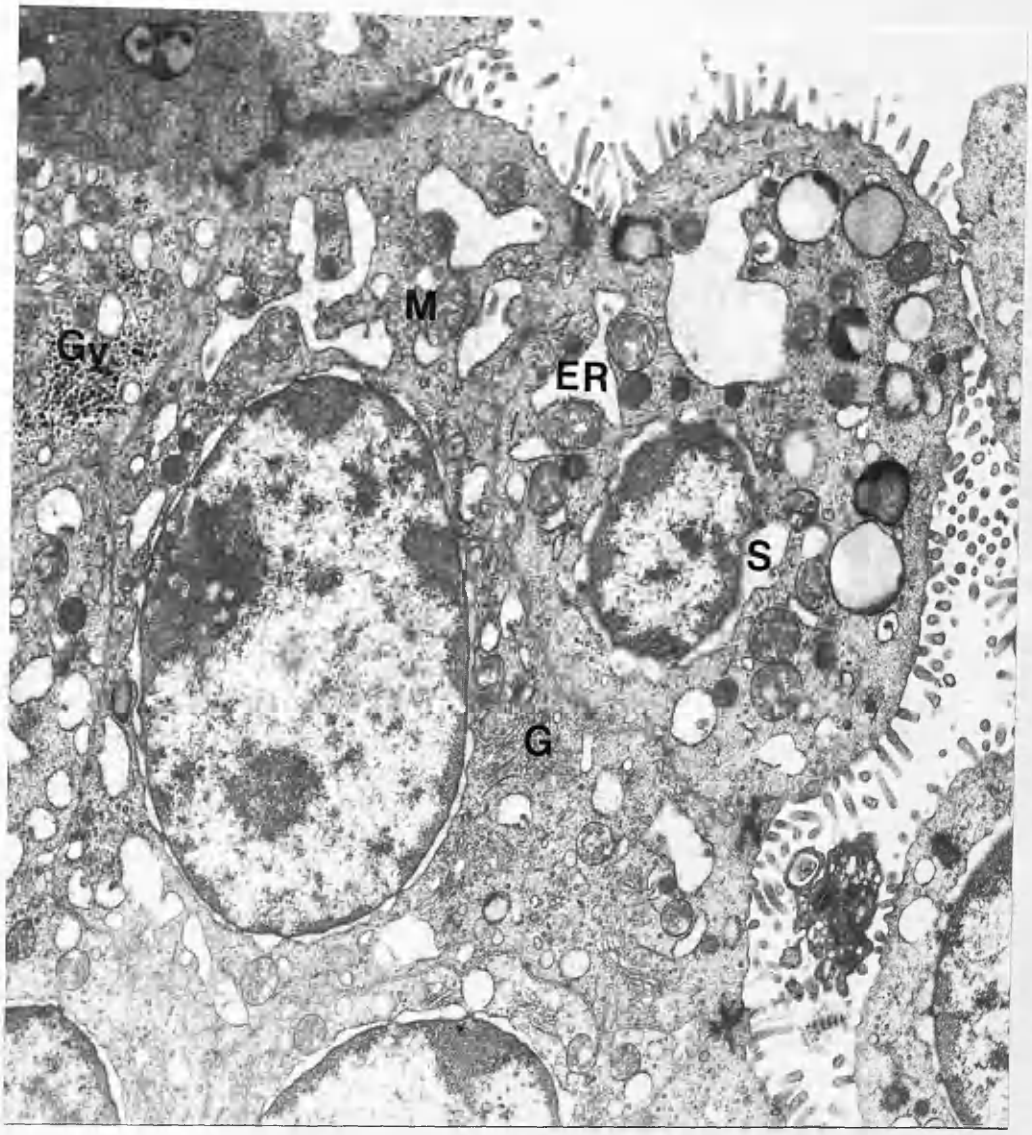

Fig. 17 Tumour cells with distended smooth surfaced endoplasmic reticulum (ER) and widened perinuclear space (S) in SPA.

Gy = Glycogen deposits; M = Mitochondria;

G = Golgi-apparatus.

(x 12,500)

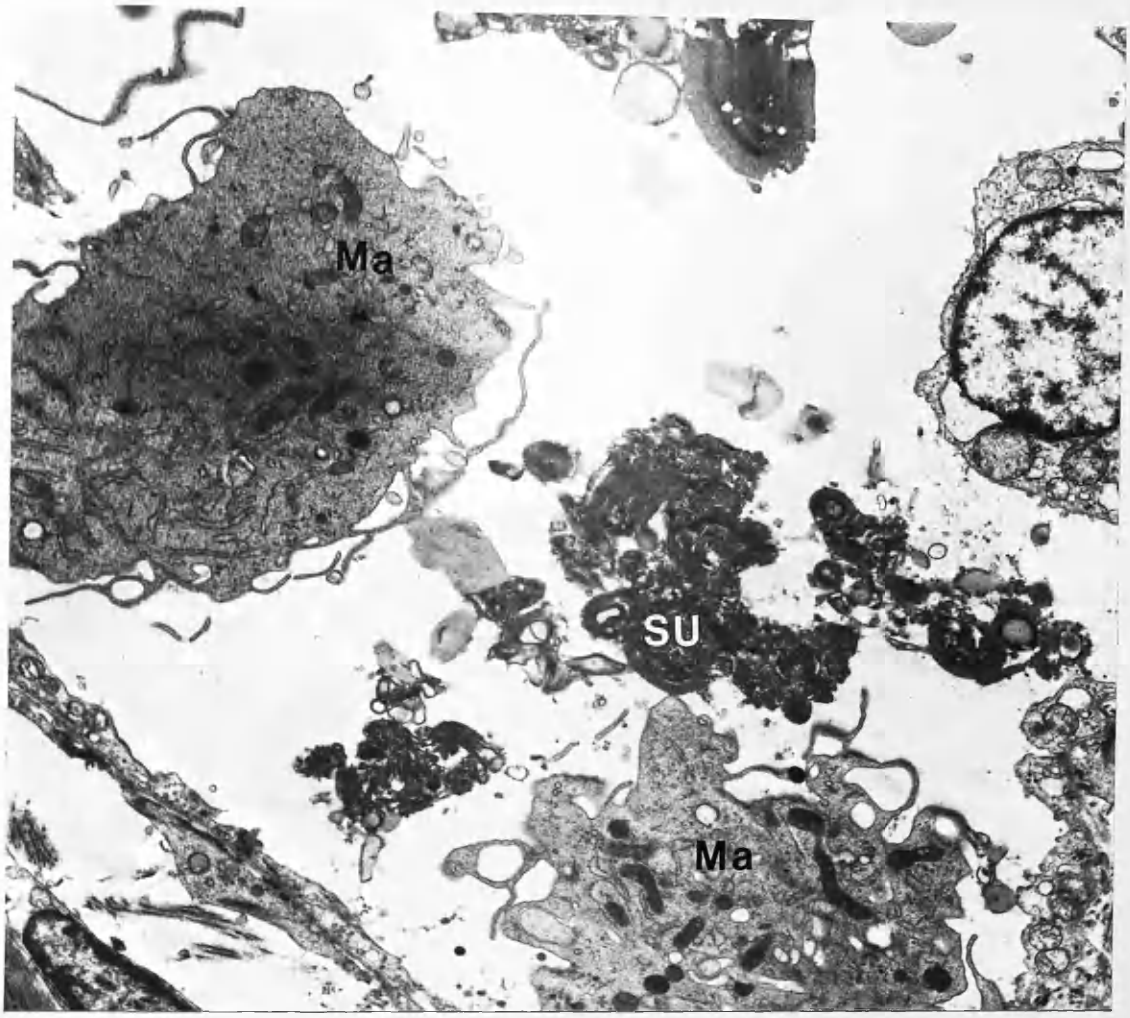

Fig. 18 Two alveolar macrophages (Ma) in the alveolar lumen and surrounding a large accumulation of surfactant material (SU) in SPA.
(x 8,000)

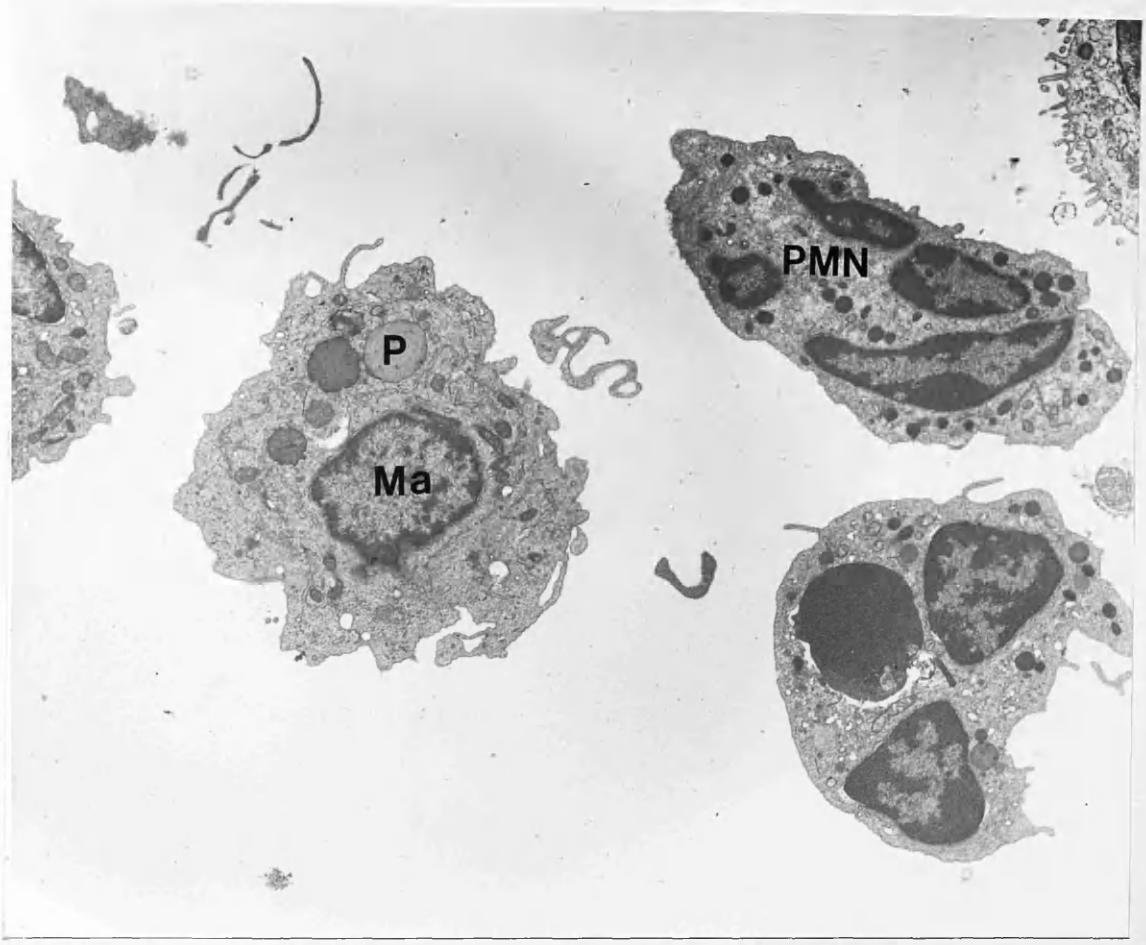

Fig. 19 Sheep pulmonary adenomatosis. Alveolar lumen containing an alveolar macrophage (Ma) and two polymorphonuclear leukocytes (PMN).
P = phagosome.
(x 8,000)

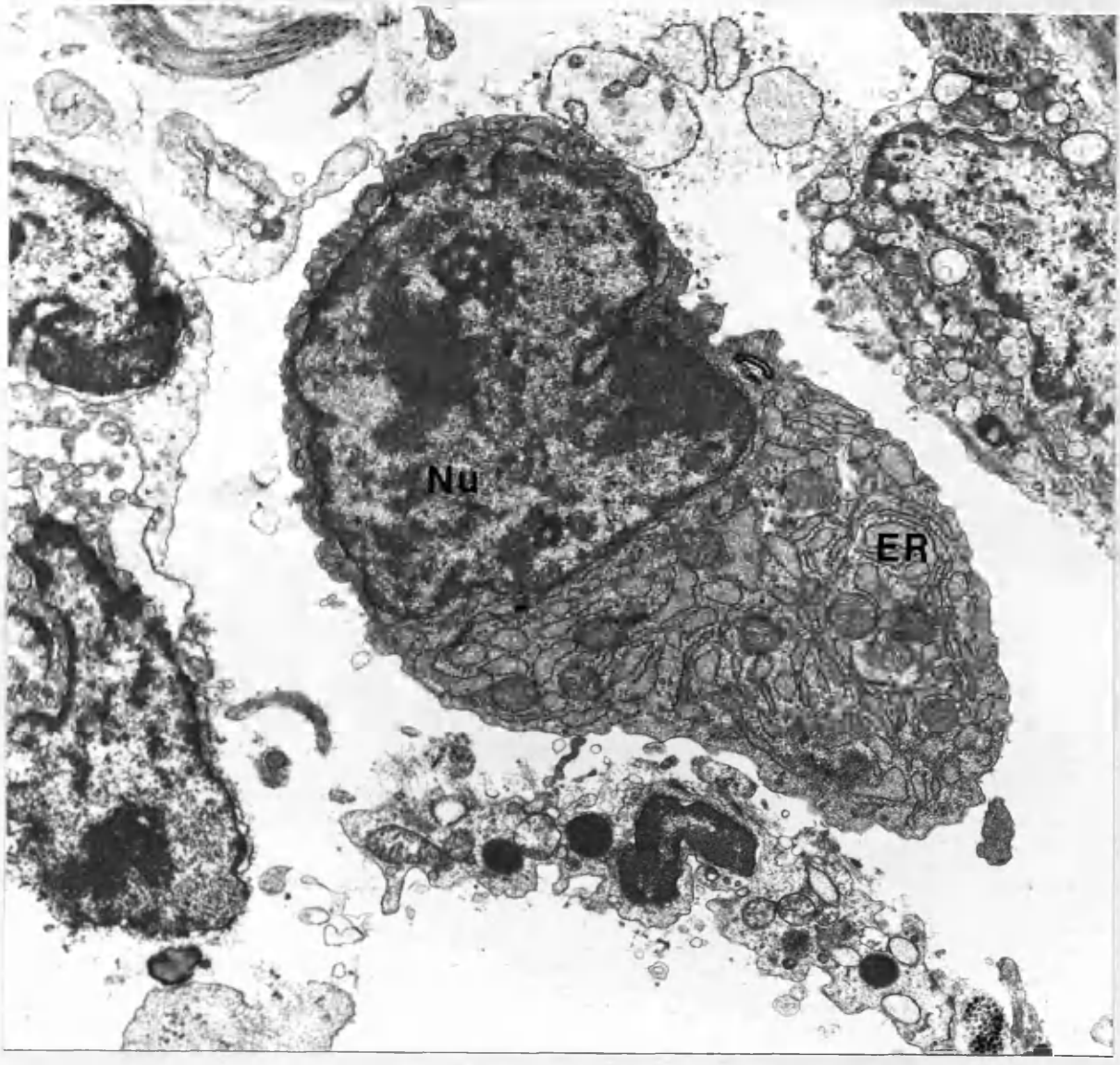

Fig. 20 Sheep pulmonary adenomatosis. A plasma cell in the alveolar lumen containing abundant rough surfaced endoplasmic reticulum (ER) and a large nucleus (Nu) that is eccentrically placed. (x 12,500)

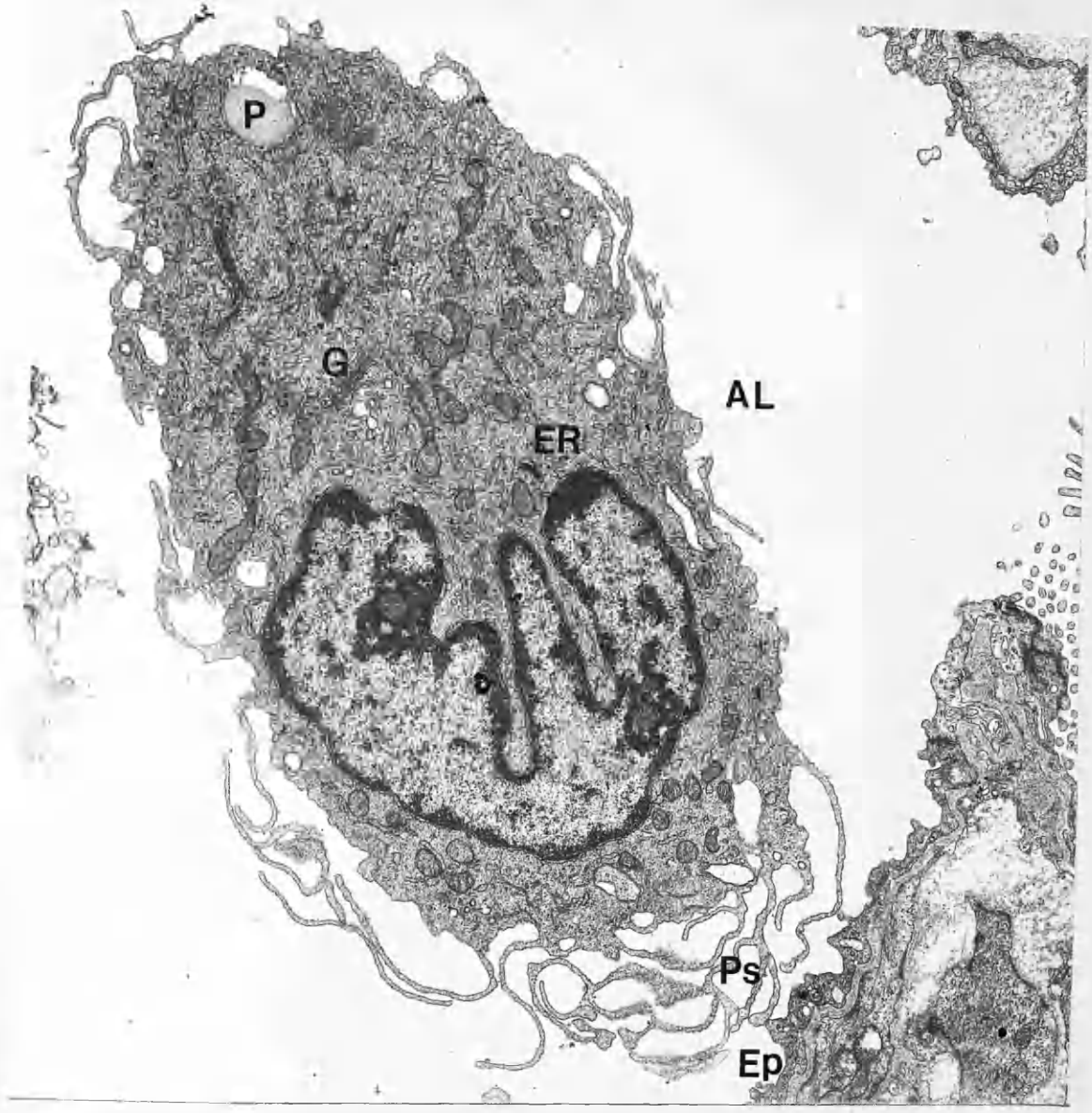

Fig. 21 Sheep pulmonary adenomatosis. Alveolar macrophage with extensive pseudopodia (Ps) and lying close to the alveolar epithelium (Ep). Note abundant rough surfaced endoplasmic reticulum (ER), ribosomes, Golgi-apparatus (G) and a phagosome (P).

AL = Alveolar lumen.

(x 12,500)

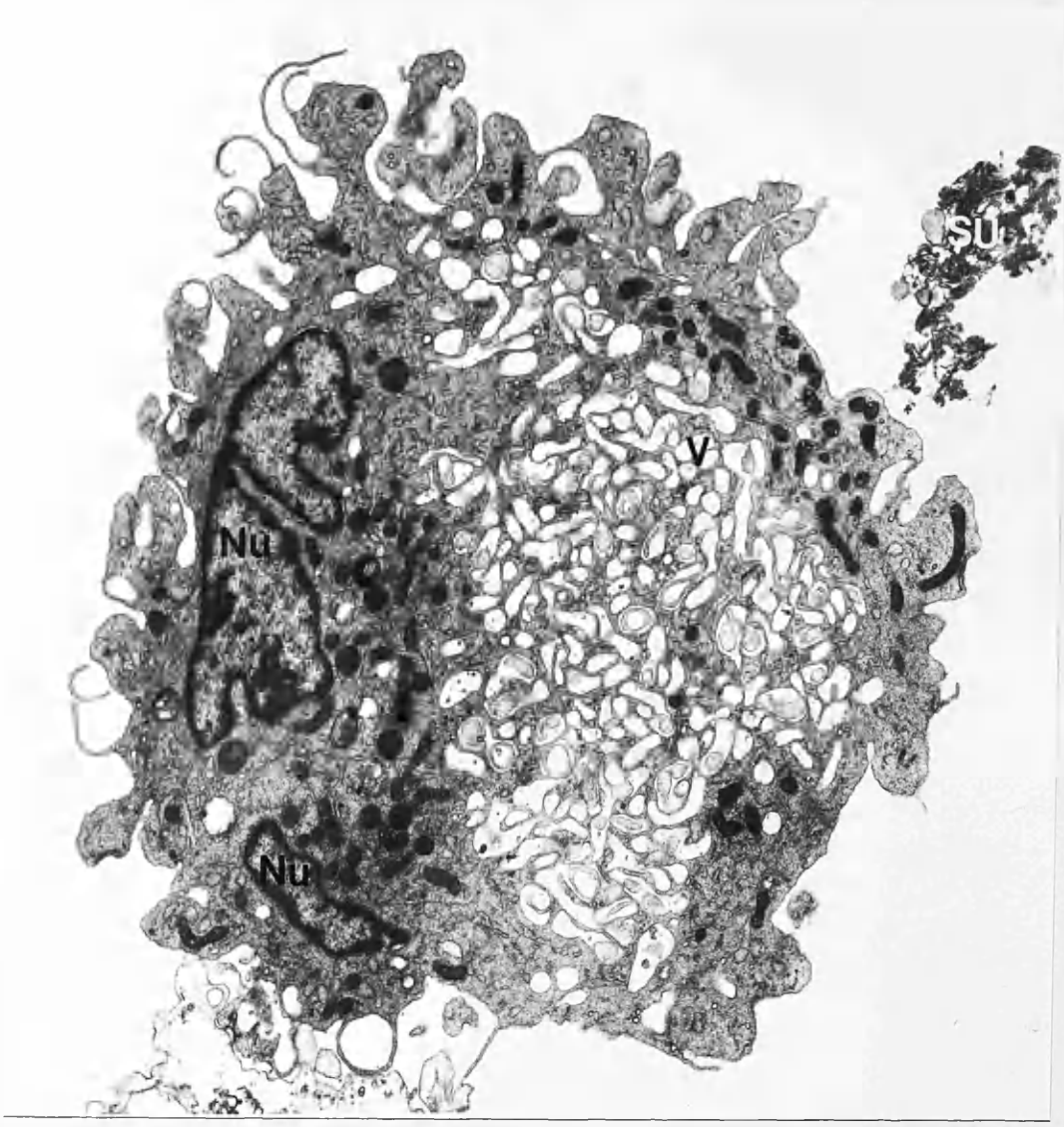

Fig. 22 Sheep pulmonary adenomatosis. Alveolar macrophage with two nuclear profiles (Nu), abundant mitochondria, lysosomes and vacuoles (V).
Su = Surfactant material.
(x 12,500)

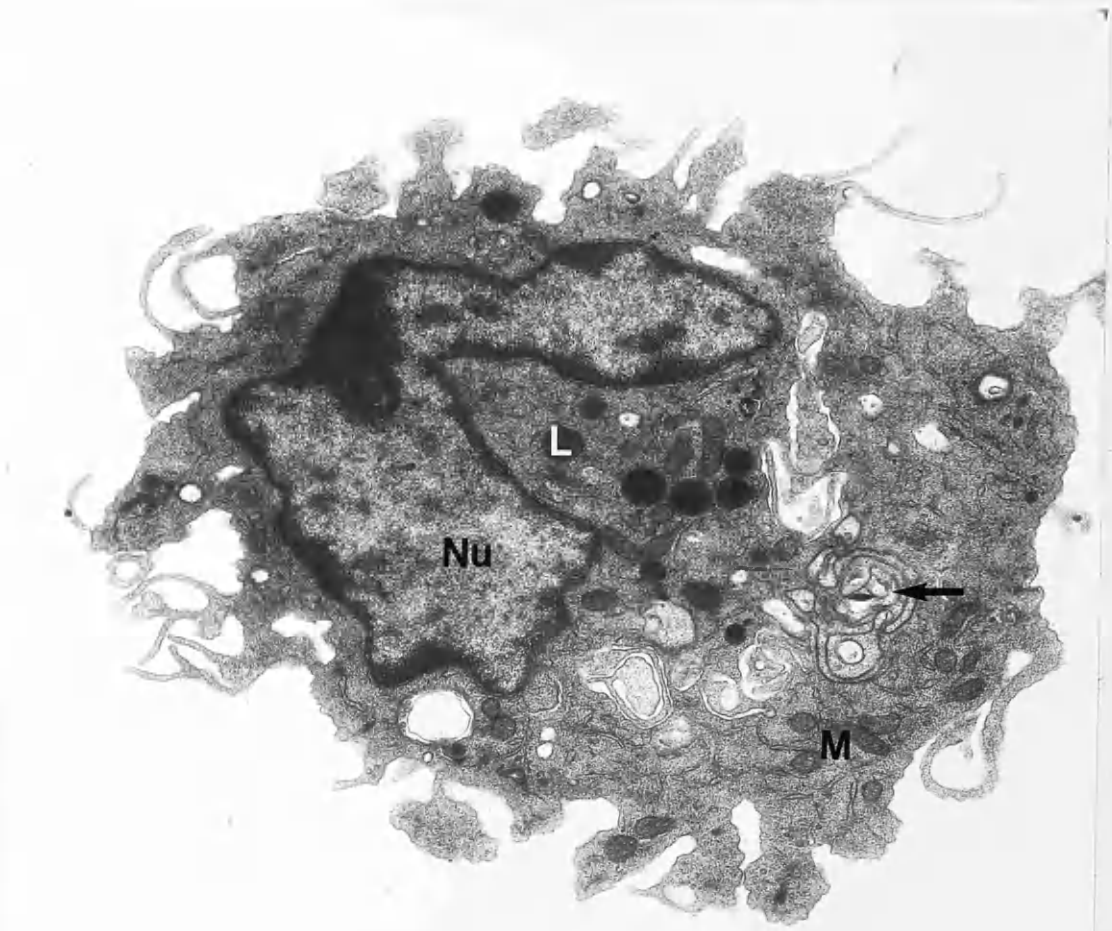

Fig. 23 Sheep pulmonary adenomatosis. Alveolar macrophage with an indented nucleus (Nu), lysosomes (L), mitochondria (M) and lamellar structures resembling micropinocytosis vermiformis (arrow). (x 12,500)

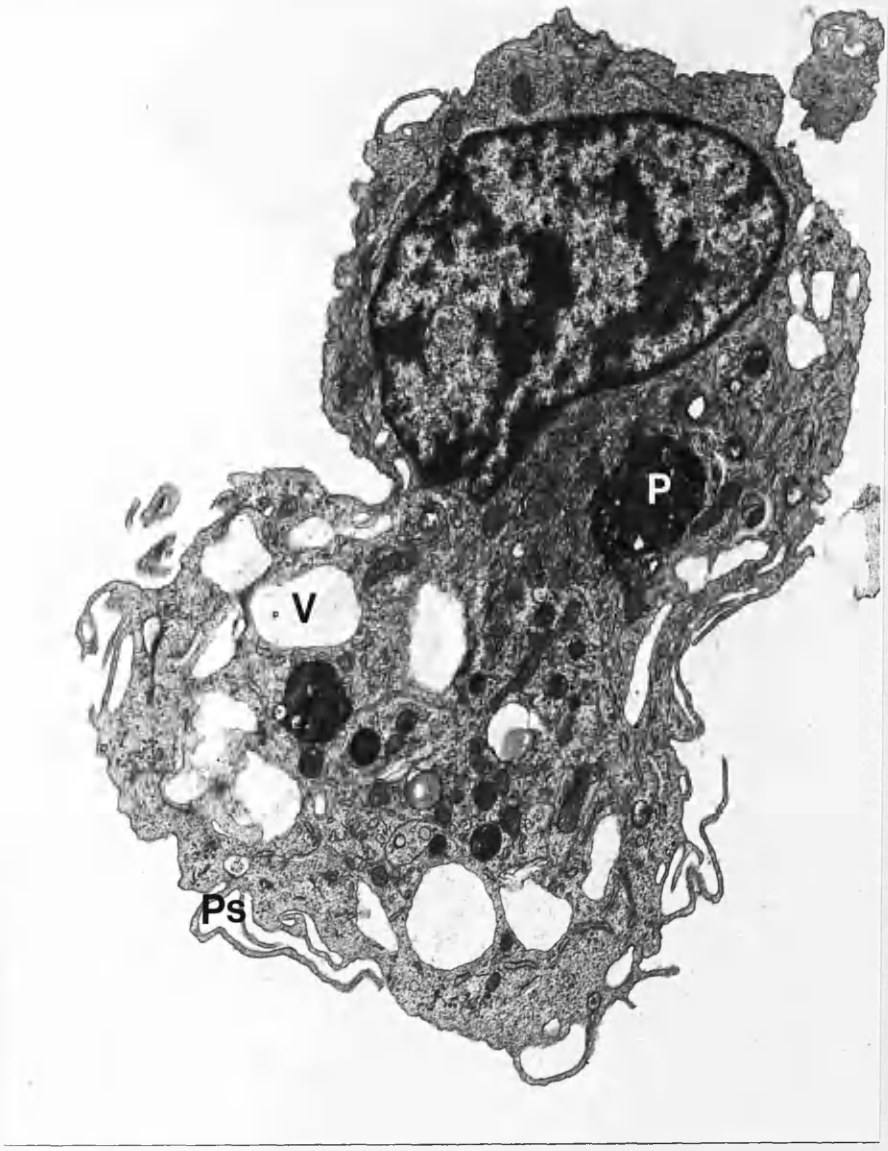

Fig. 24 Sheep pulmonary adenomatosis. Alveolar macrophage with pseudopodia (Ps), phagosomes (P) and vacuoles (V).
(x 8,000)

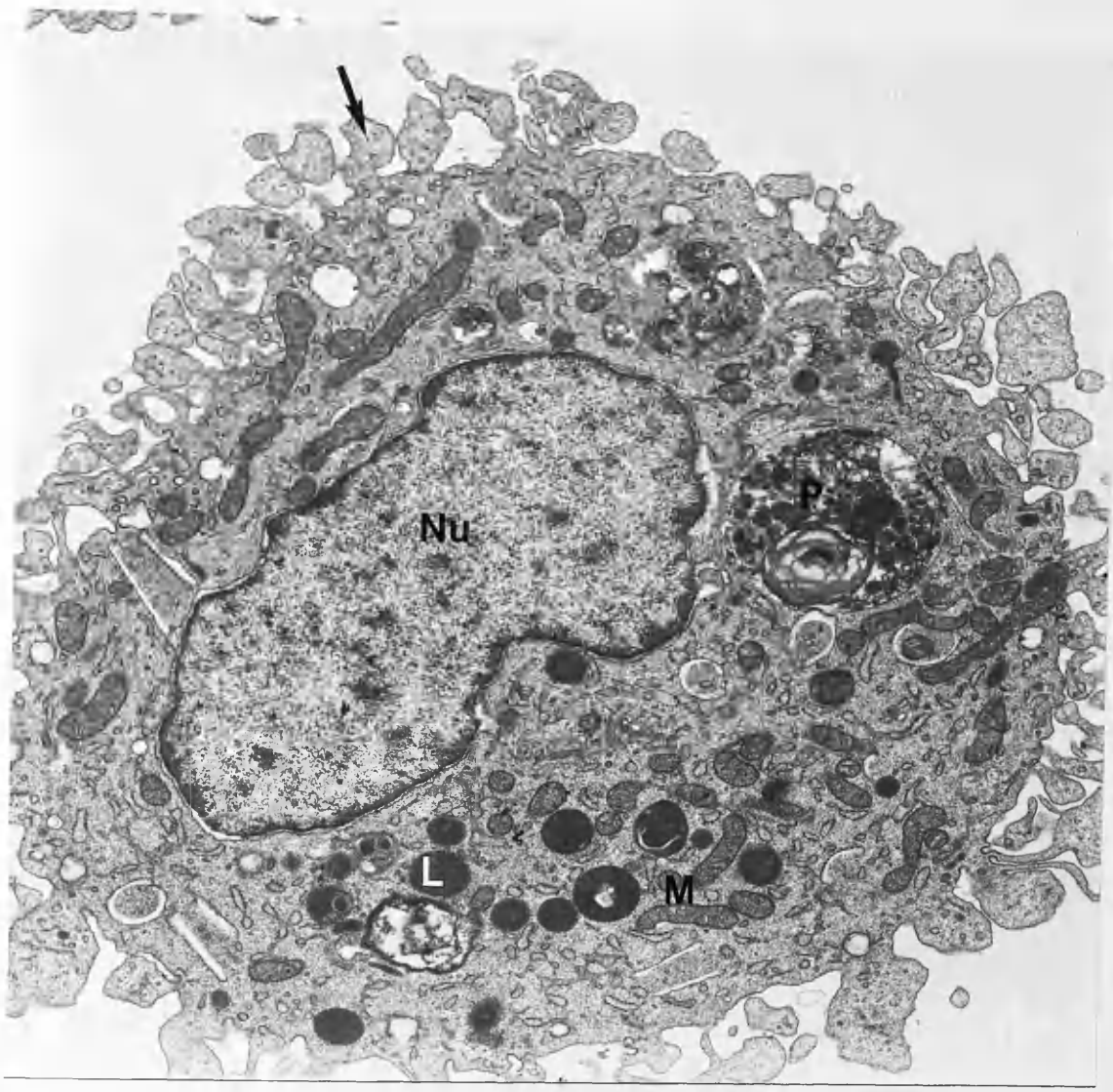

Fig. 25 Sheep pulmonary adenomatosis. Alveolar macrophage with increased projections (arrow) on the cell surface. Note also increased numbers of mitochondria (M), lysosomes (L) and a phagosome (P) containing surfactant material.

Nu = Nucleus.

(x 12,500)

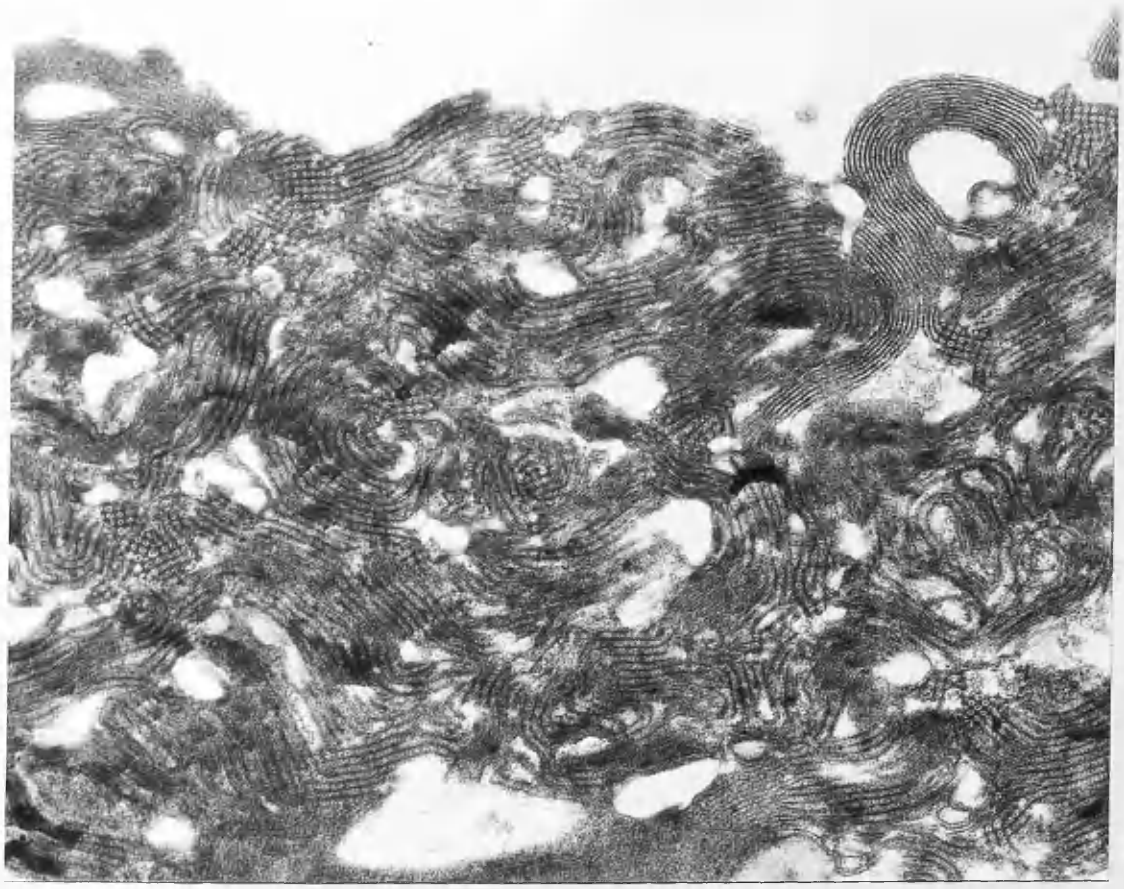

Fig. 26 Sheep pulmonary adenomatosis. High power of surfactant material as seen in Fig. 18. Note the lamellar configuration.
(x 40,000)

CHAPTER 5

A TRANSMISSION ELECTRON MICROSCOPIC STUDY OF NORMAL CATTLE LUNGS AND BRONCHIOLAR-ALVEOLAR REACTIONS IN CALVES INFECTED WITH LUNGWORMS.

NORMAL CATTLE LUNGS AND BRONCHIOLAR-ALVEOLAR REACTIONS IN CALVES INFECTED WITH LUNGWORMS

The study of bronchiolar-alveolar reactions in cattle was again carried out in two stages. In the first stage, cattle with normal lungs were studied by TEM and in the second phase calves with experimental lungworm infection were investigated by TEM.

1. Materials and methods

The lungs from adult cattle with clinically normal respiratory tracts were studied and the lungs from calves with experimentally infected lungworms were also examined.

NORMAL CATTLE

The two adult cattle studied were submitted to the veterinary school and had no clinical history of pulmonary disease. This was confirmed by clinical examination carried out by the staff at the Department of Veterinary Medicine where the animals were housed. The samples collected from the lungs and the methods used to process the samples for TEM and LM have been described in Chapter 3.

CALVES WITH EXPERIMENTAL LUNGWORM INFECTION

The six male Friesian cross calves, three to four months of age, that were obtained for the study were experimentally infected with Dictyocaulus viviparus. The calves had been infected by staff of the Department of Veterinary Parasitology and the details of the experimental design are given in Table 2 .

		DAY 1	DAY 24	DAY 45	DAY 71
GROUP	ANIMALS	1st VACCINATION	2nd VACCINATION	CHALLENGE	P.M. EXAM.
1	R5 and R15	1,000 larvae orally (Irradiated 40 Kr)	1,000 larvae orally (Irradiated 40 Kr)	8,000 larvae orally	+
2	014 and 025	2,500 larvae intravenously (Irradiated 100 Kr)	2,500 larvae intravenously (Irradiated 100Kr)	8,00 larvae orally	+
3	G10 and G11	-	-	8,000 larvae orally	+

Table 2: Schedule of experimental lungworm infection.

P.M. Exam. = Post Mortem Examination

2. Results

NORMAL CATTLE

The lungs from both cattle were grossly normal and no abnormalities were detected by LM examination of the samples from the lobes of the right lung in each case.

The cell types found in the bronchiolar epithelium and the alveolar epithelium were essentially similar to those seen in the sheep.

In the bronchiolar epithelium, ciliated cells and non-ciliated Clara cells were found in approximately equal numbers (Fig. 27). All the cells rested on a basement membrane.

The ciliated cell was columnar in shape, possessed a large nucleus, many mitochondria, Golgi-apparatus and numerous cilia that were attached to the cytoplasm by basal bodies.

The Clara cell was also columnar in shape and the luminal edge extended over neighbouring cells. The large nucleus was centrally located, lobulated and had deep invaginations (Fig. 28). Glycogen was uniformly distributed throughout the cytoplasm (Fig. 28).

The alveoli were lined by type I pneumonocytes and type II pneumonocytes (Fig. 29). The nucleus of the type I pneumonocyte was large and its cytoplasm extended over the alveolar surface and capillaries. Pinocytic vesicles and small vacuoles were sometimes seen (Fig. 30). The type II pneumonocyte was a large cuboidal cell with numerous short microvilli at the free surface and had characteristic membrane-bound inclusions (Fig. 31). The cytoplasm contained many mitochondria and had numerous free ribosomes, as well as those associated with the endoplasmic reticulum.

The alveolar septa contained varying amounts of connective tissue mostly collagen fibres, capillaries, fibroblasts and interstitial cells (Fig. 29). Within the alveolar capillaries, erythrocytes, monocytes and intravascular macrophages were sometimes observed. Intravascular macrophages contained abundant cytoplasm and possessed numerous pseudopodia at the cell surface. The cytoplasm of the intravascular macrophages contained numerous mitochondria, a Golgi apparatus, vesicles and lysosomes. The nuclei of intravascular macrophages were large and of variable shape.

Alveolar macrophages were occasionally seen in the alveolar lumen. These were large irregularly shaped cells, with a large nucleus, Golgi-apparatus and many mitochondria (Fig. 32)

CALVES INFECTED WITH LUNGWORMS

In the calves infected with lungworms, alterations were observed in both the bronchiolar and in the alveolar epithelium.

The ciliated cells of the bronchioles were markedly swollen and had reduced electron density due to the formation of large numbers of vacuoles (Fig. 33) and moderate distension of SSER. The topographic distribution of injured ciliated cells within the bronchioles was random and damaged cells were often located contiguous to normal ciliated cells. Loss of cilia was extensive and although stumps of cilia were sometimes seen at the cell apex, in the majority of the affected cells only basal bodies remained as evidence of the previous presence of cilia (Fig. 33). In addition, injured cells had swollen mitochondria with disorganised cristae (Fig. 33). Exfoliated ciliated cells were occasionally found in the bronchiolar lumen.

The Clara cells were as equally affected as the ciliated cells. Like the ciliated cells, the Clara cells had lost some of their cytoplasmic organelles with the resulting formation of vacuoles and distension of SSER (Fig. 34). Exfoliation of Clara cells into the bronchiolar lumen was also occasionally noted.

The basement membrane of the bronchiolar epithelium remained intact despite the ultrastructural changes in the ciliated and Clara cells. In addition, eosinophils and plasma cells were present in the lamina propria of some bronchioles.

In the bronchiolar lumen, abundant inflammatory cells, predominantly polymorphonuclear leukocytes, macrophages and eosinophils were present (Fig. 35). This cellular influx was accompanied by large amounts of necrotic cellular debris.

Alveolar macrophages, eosinophils, plasma cells, polymorphonuclear leukocytes and smaller numbers of mast cells, lymphocytes and desquamated type II pneumonocytes were observed in the alveolar lumina by both LM (Figs. 36 and 37) and TEM (Figs 38 and 39). This cellular infiltration was accompanied by abundant aggregates of fibrin (Fig. 40). Very often the eosinophil granules were more electron lucent than normal eosinophils.

The alveolar macrophages were large cells, possessed numerous pseudopodia on their cell surface and displayed a great variation in cell shape (Figs. 41 and 42). The cell cytoplasm was usually electron dense and rich in cytoplasmic organelles, notably mitochondria, Golgi apparatus, lysosomes, ribosomes and RSER (Figs. 41 and 42). A characteristic feature of the AM's is the presence of

abundant phagosomes of different sizes (Figs. 42 and 43), some of which occupied almost half of the cytoplasmic volume (Fig. 44).

Significant changes were also noted in the type I and type II pneumonocytes of the alveolar epithelium and in the alveolar interstitium. Swelling of the thin cytoplasmic segments of the type I pneumonocytes was observed (Fig. 45). The swelling was usually patchy, but in some areas it was widespread and the pinocytic vacuoles became markedly conspicuous (Fig. 45) However, there was no evidence of necrosis of the swollen type I epithelium.

Many alveoli were lined by a layer of cuboidal to columnar epithelial cells (Fig. 46). These cells rested on a basement membrane, formed tight junctions with adjacent cells and many short microvilli projected from the free, luminal border of each cell, particularly on the lateral surfaces. Within the cytoplasm, there were numerous organelles consisting of numerous mitochondria, glycogen granules and RSER. In addition, numerous membrane-bound electron dense cytoplasmic inclusions were evident mostly in the apical portions of the cells (Fig. 46). The nuclei were large and were located at the base of the cell.

Within the alveoli, severe degeneration of the type II pneumonocyte was commonly seen. Large cytoplasmic vacuoles

were often present in the cells, mitochondria were distorted and disintegrating, microvilli numbers and lamellated inclusions were markedly reduced and the cytoplasm was virtually devoid of most cytoplasmic organelles (Fig. 47).

The thickening of the alveolar septa was a consistent finding (Figs. 36, 37 and 48) and was mainly the result of the presence of large numbers of interstitial cells, eosinophils, mast cells, occasional polymorphonuclear leukocytes and increased amounts of collagen. Alveolar capillaries in these areas were usually filled with eosinophils and tightly packed red blood cells. Occasionally, polymorphonuclear leukocytes, platelets, intravascular macrophages and electron-dense material presumably serum proteins were seen. Interstitial oedema of the alveolar septa was an uncommon finding.

DISCUSSION

In the bronchiolar epithelium of normal cattle, ciliated and Clara cells were present. The goblet cells and brush cells as described by Bryson (1980) in the bronchiolar epithelium of cattle were not found in this study.

The morphology of the alveolar wall of the normal cattle was essentially similar to that described in other mammals (Meyrick and Reid, 1970; Greenwood and Holland, 1972) and previously in cattle (Epling, 1964; Rybicka and others, 1974a).

The alveoli of the normal cattle were lined by type I and type II pneumonocytes. The type III pneumonocyte which was described in rats (Meyrick and Reid, 1968; Chang and others, 1986) was not seen in this study.

Using scanning electron microscopy (SEM), AM's were commonly found in the lungs of cattle (Iovannitti and others, 1985). This observation conflicts with those of Epling (1964) who did not report AM's in the 20 mature cattle that were studied by TEM. Alveolar macrophages have been reported by several workers using SEM and TEM, in the horse and hamster (Nowell and Tyler, 1971), goat (Atwal and Sweeny, 1971), mice (Greenwood and Holland, 1972) and in cattle (Breeze, 1973; Bryson, 1980) although their frequency was not

mentioned. In this study, AM's were only occasionally observed.

Both in this study and those of others (Rybicka and others, 1974b; Bryson, 1980) PIM's have been seen but they were not reported in cattle by Breeze (1973).

As far as the author is aware, the only ultrastructural study in cattle infected with lungworms is that of Iovannitti (1984) who used the SEM. She observed adult parasites and eggs in the bronchial lumen. In addition, a severe cellular infiltration accompanied the worm eggs and larvae in the alveolar lumina. Similarly in this study, a marked cellular infiltration was noted in the alveolar lumina. However, no eggs or larvae could be identified and this may be a reflection of the fact that only a small and limited area of lung tissue could be examined by TEM compared with SEM.

Many of the pulmonary alveoli were lined by a continuous layer of type II pneumonocytes and the term 'epithelialisation' was used to describe this lesion (Jarrett and others, 1954). Three types of epithelialisation have been found in the bovine depending on the etiological agent (Jarrett and others, 1954). The epithelialisation seen in this study consists of a sheet of cuboidal to columnar epithelium and has been reported in

cattle experimentally infected with the lungworm D. viviparus (Jarrett and others, 1954, 1957; Michel and others, 1965). Jarrett and others (1957) further concluded that alveolar epithelialisation occurred as early as 13 days after infection and remained as late as 70 days post infection. Alveolar epithelialisation has also been reported in other bovine respiratory diseases, including fog fever (Breeze and others, 1975), atypical pneumonia (Jarrett, 1954), atypical interstitial pneumonia (Omar and Kinch, 1966) and diffuse alveolar fibrosis (Pirie and Selman, 1972). Other conditions in which alveolar epithelialisation has been described include oral dosing with L-tryptophan (Dickinson and others, 1967; Carlson and others, 1968), 3-methyl indole toxicity (Pirie and others, 1976) and experimental infection with parainfluenza-3 virus (Bryson and others, 1983). In goats, alveolar epithelialisation has been observed in natural cases of verminous pneumonia (Nimmo, 1979) and with intravenous or oral administration of 3-methyl indole (Huang and others, 1977; Bradley and Carlson, 1980). Alley and Manktelow (1971) have identified alveolar epithelialisation in chronic enzootic pneumonia in sheep. Although the term epithelialisation has been used alveolar epithelial hyperplasia is now the preferred term.

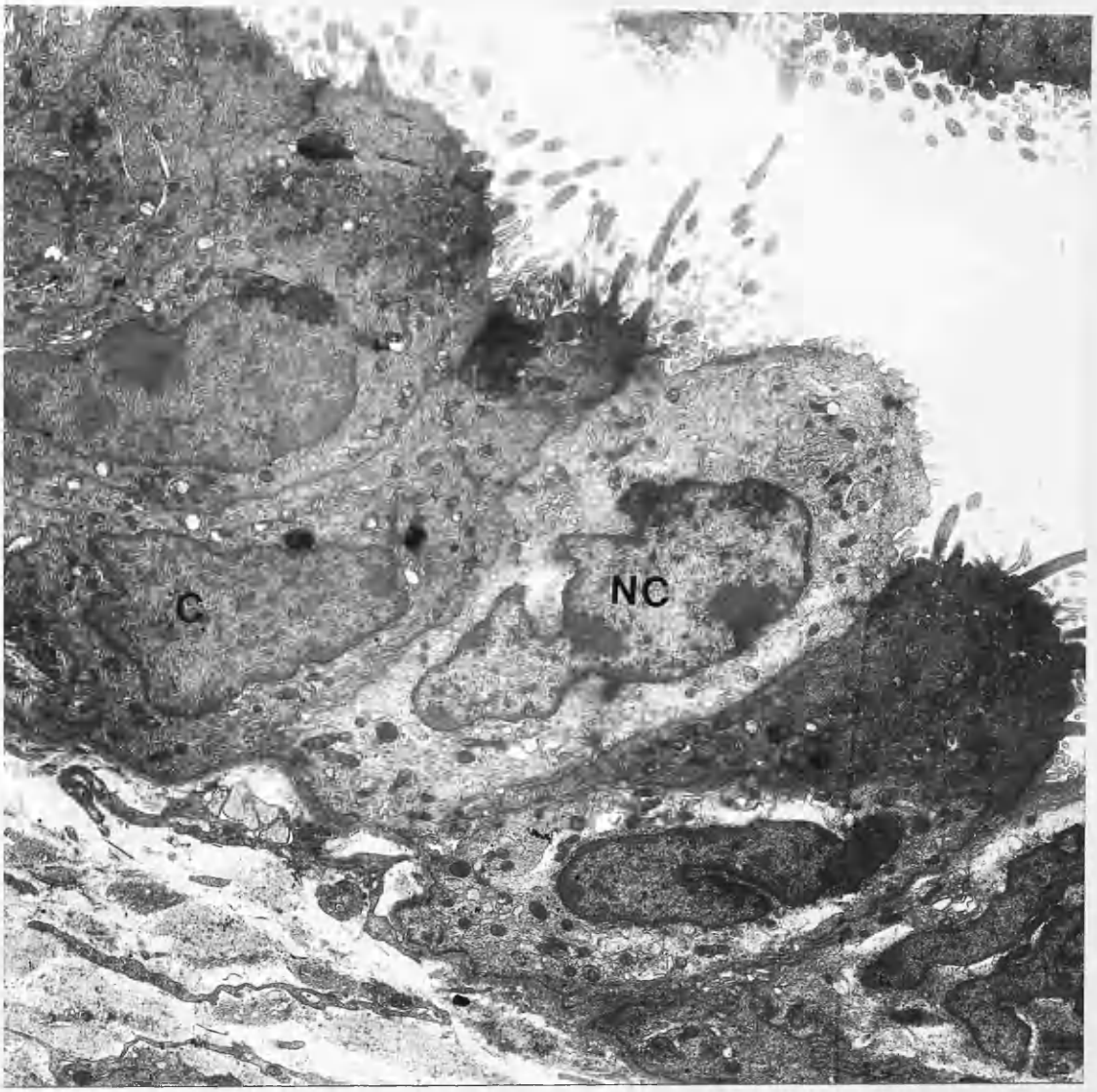

Fig. 27 Ciliated cell (C) and nonciliated cell (NC) in the bronchiolar epithelium of a normal cow.
(x 8,000)

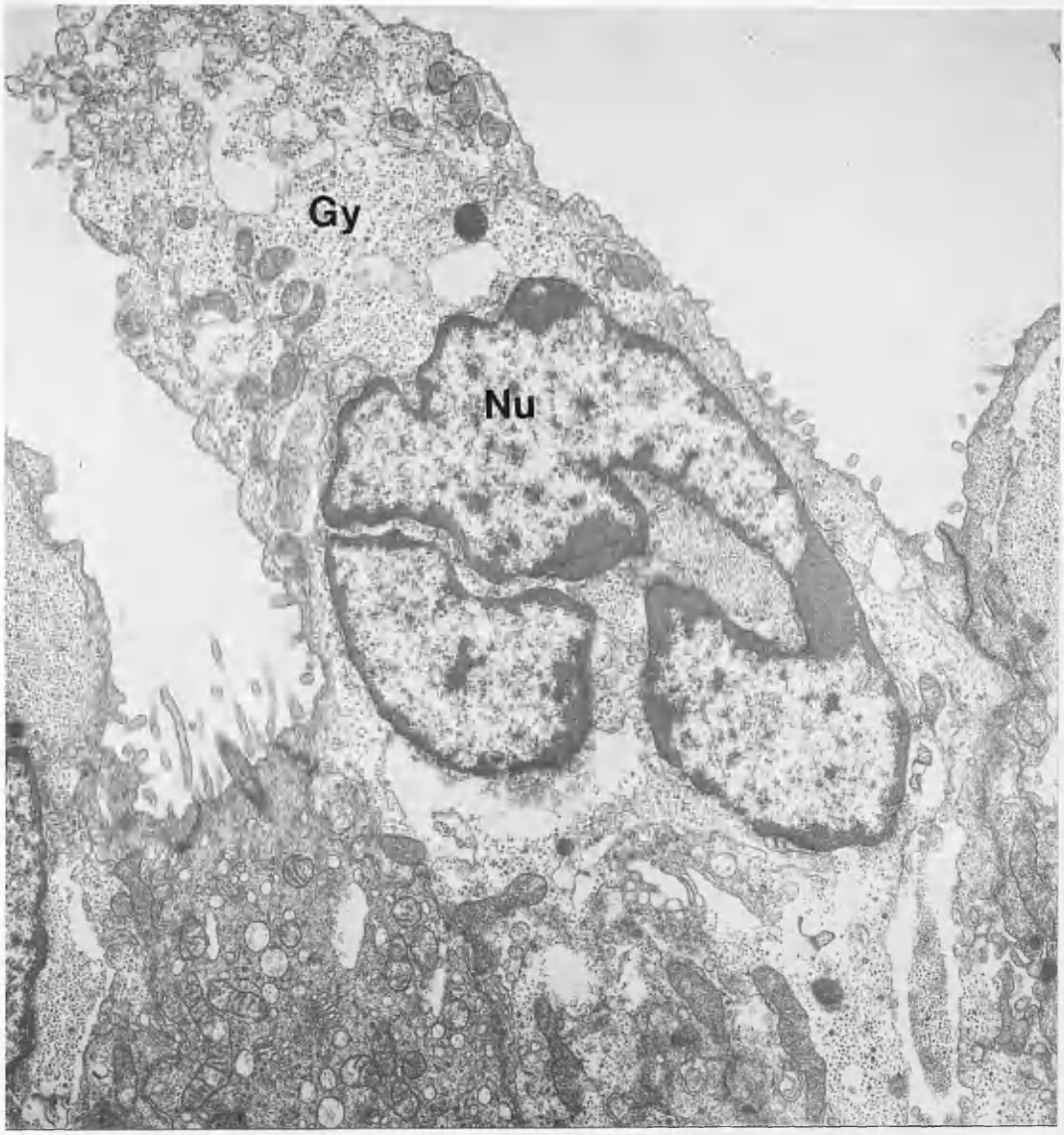

Fig. 28 Clara cell in the bronchiolar epithelium of a normal cow. Note the nuclear lobulation and invagination with abundant glycogen (Gy) in the cytoplasm. Nu = nucleus.
(x 12,500)

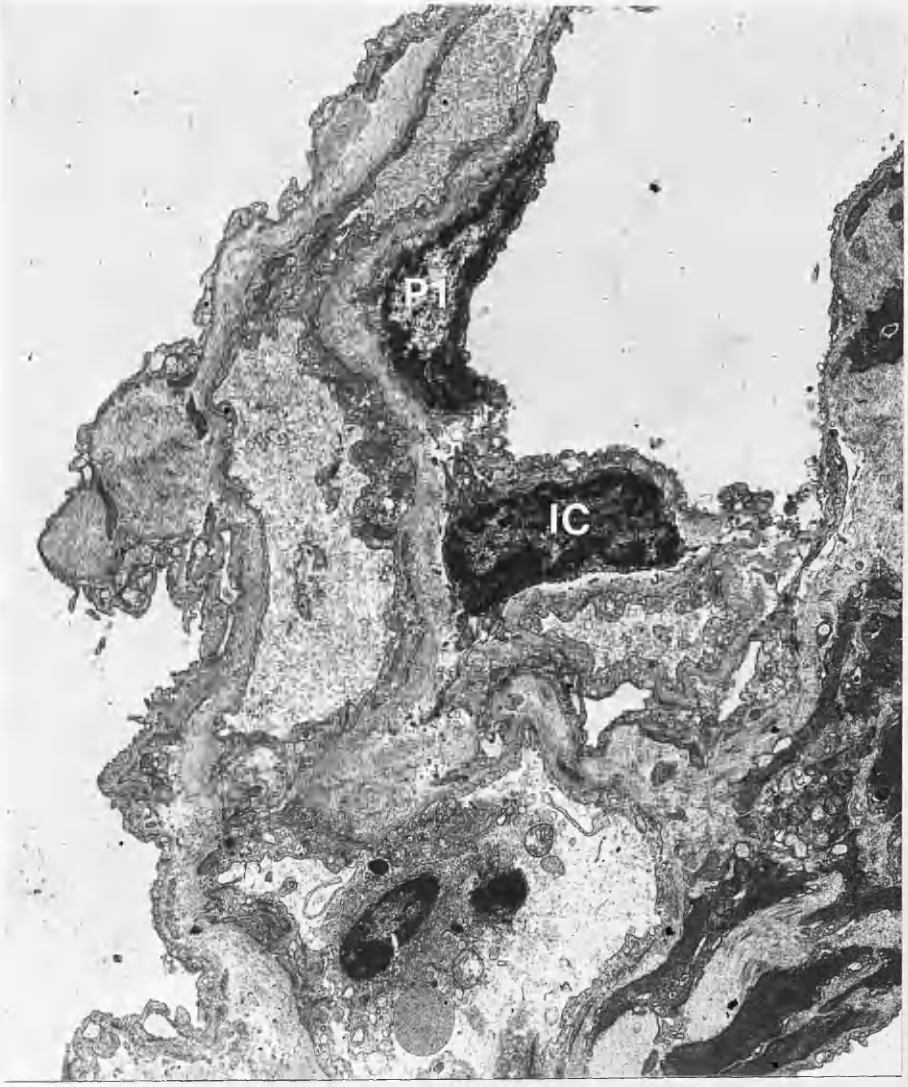

Fig. 29 Alveolar wall with a type I pneumonocyte (P1) in a normal lung of a cow. An interstitial cell (IC) is present in the interstitium.
(x 8,000)

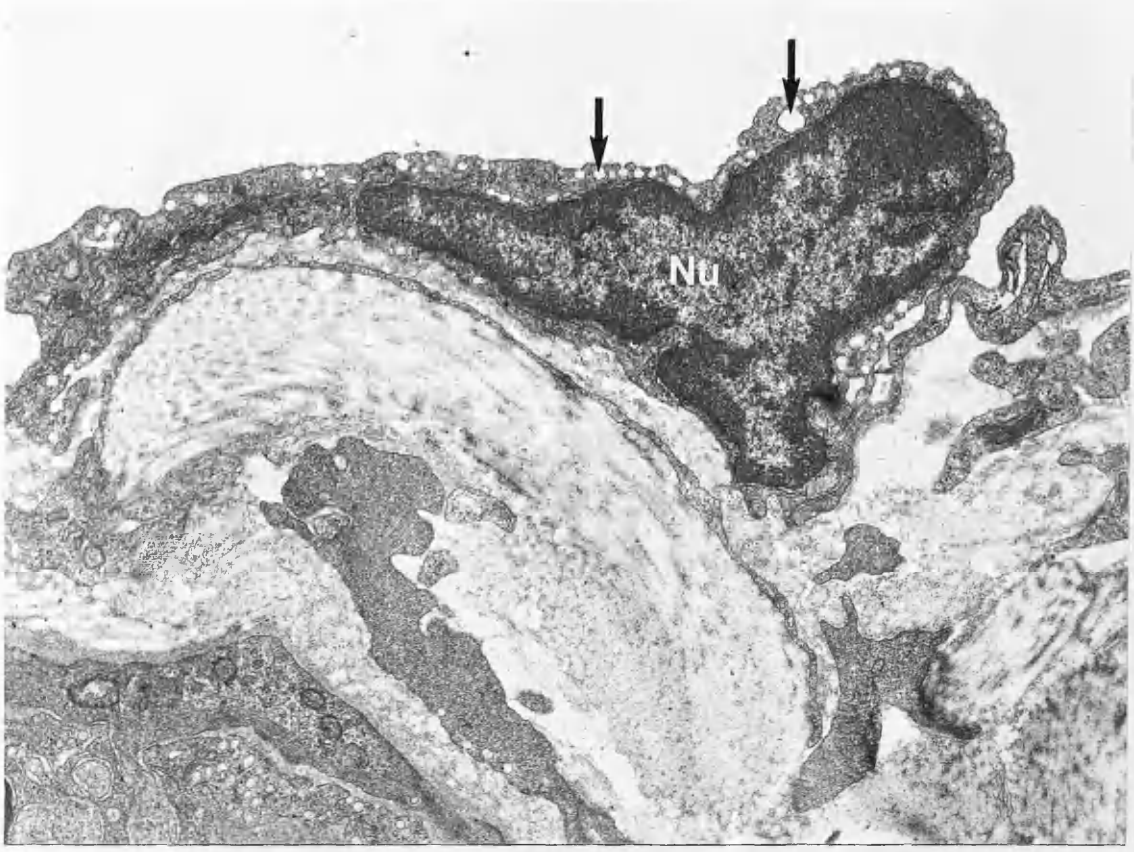

Fig. 30 A type I pneumonocyte in a normal cow. Note the numerous pinocytic vesicles and vacuoles in the cytoplasm (arrows). Nu = nucleus.
(x 20,000)

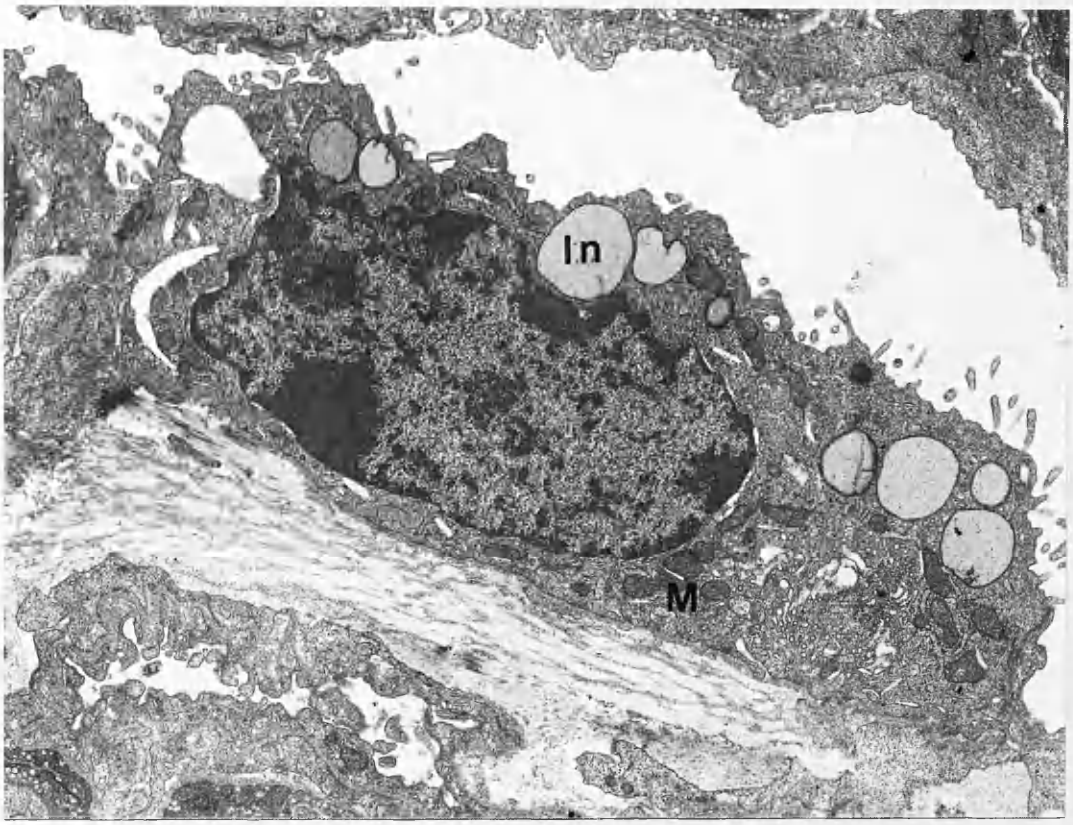

Fig. 31 A type II pneumonocyte in a normal cow. Note the numerous mitochondria (M) and membrane-bound inclusions. (In).
(x 12,500)

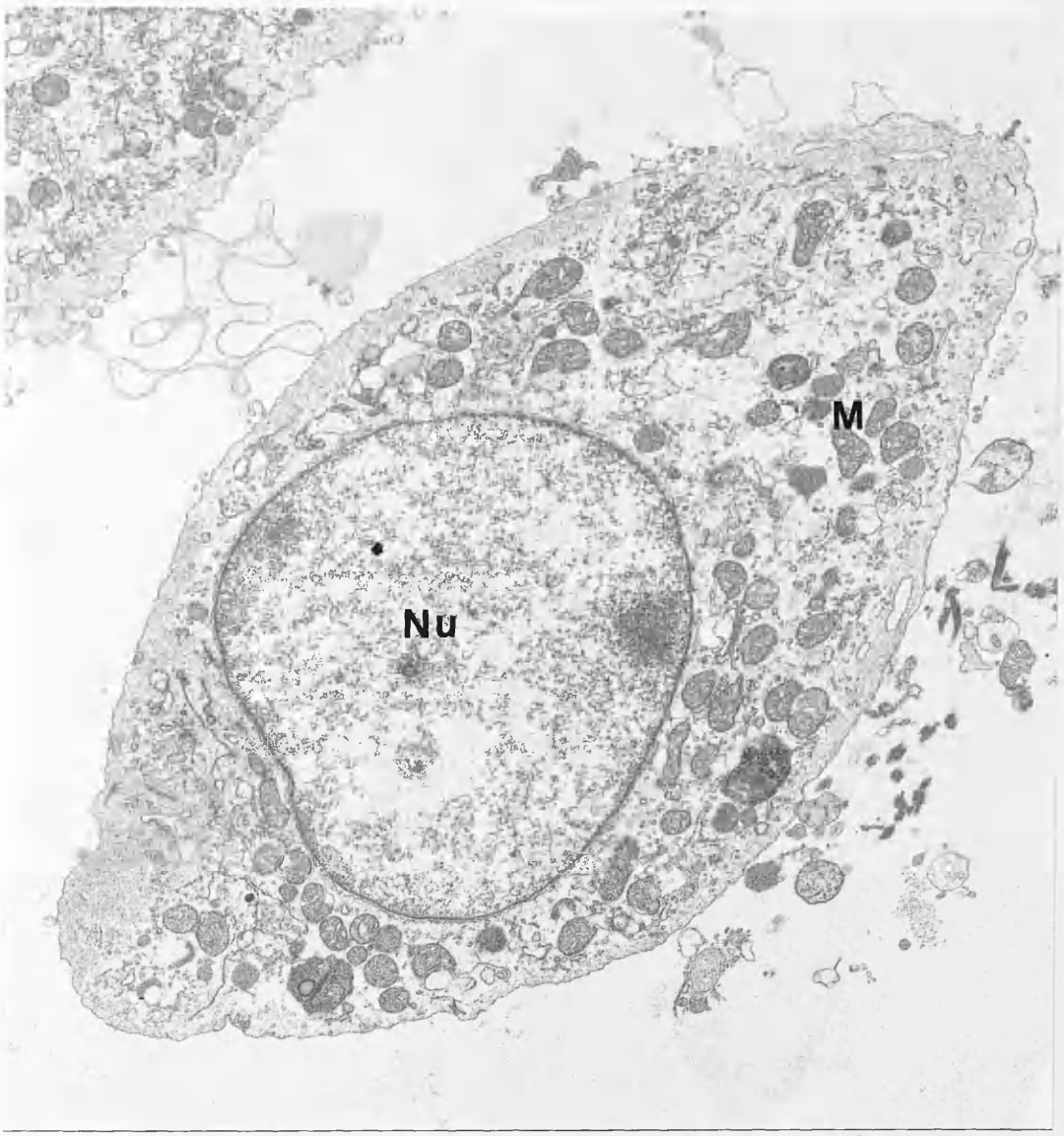

Fig. 32 Alveolar macrophage in a normal cow with a large nucleus (Nu) and many mitochondria (M). Note the absence of lysosomes.
(x 12,500)

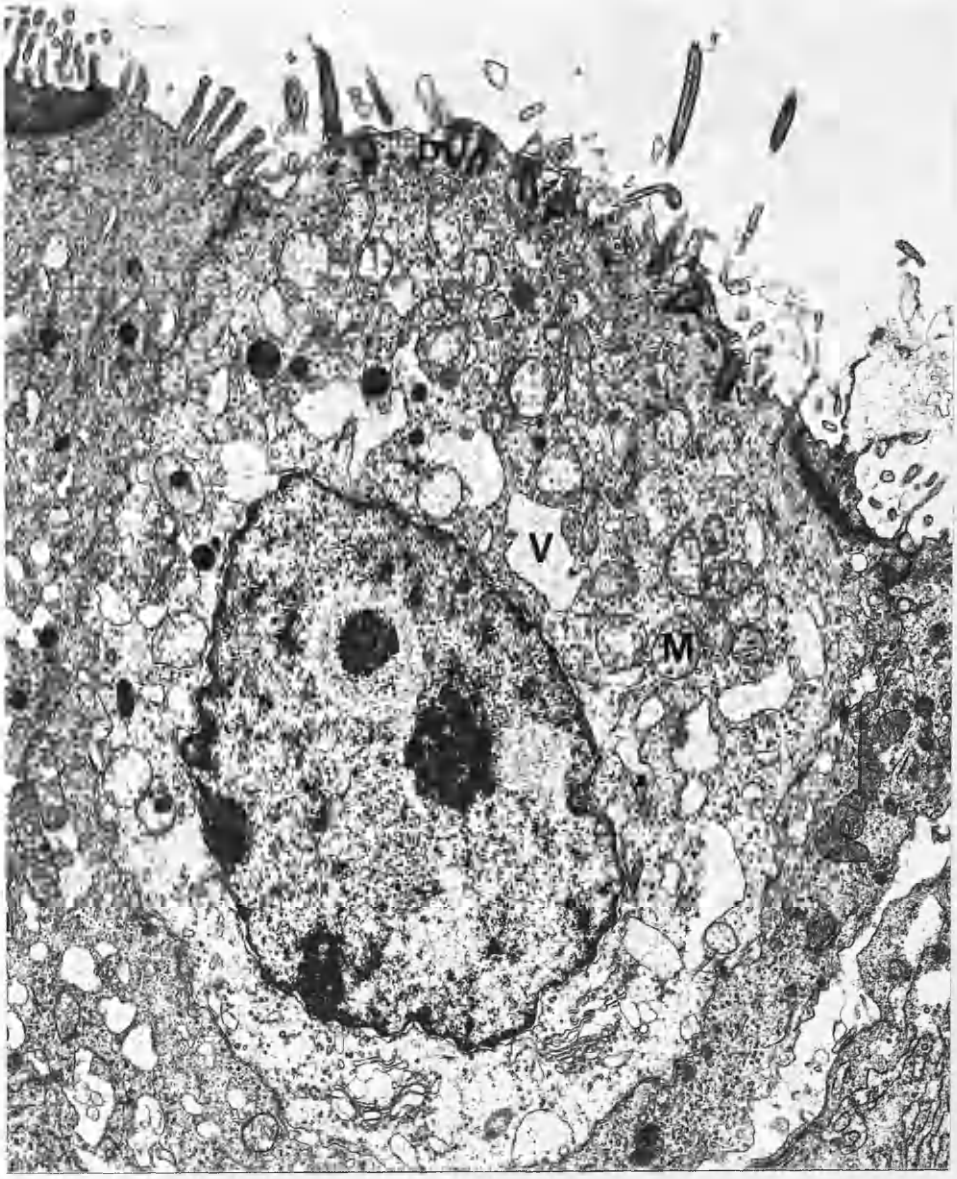

Fig. 33 A bronchiolar ciliated cell from a calf infected with lungworms. Note the swollen mitochondria (M) with disorganised cristae, large cytoplasmic vacuoles (V) and a general loss of cilia although basal bodies (b) are present.
(x 12,500)

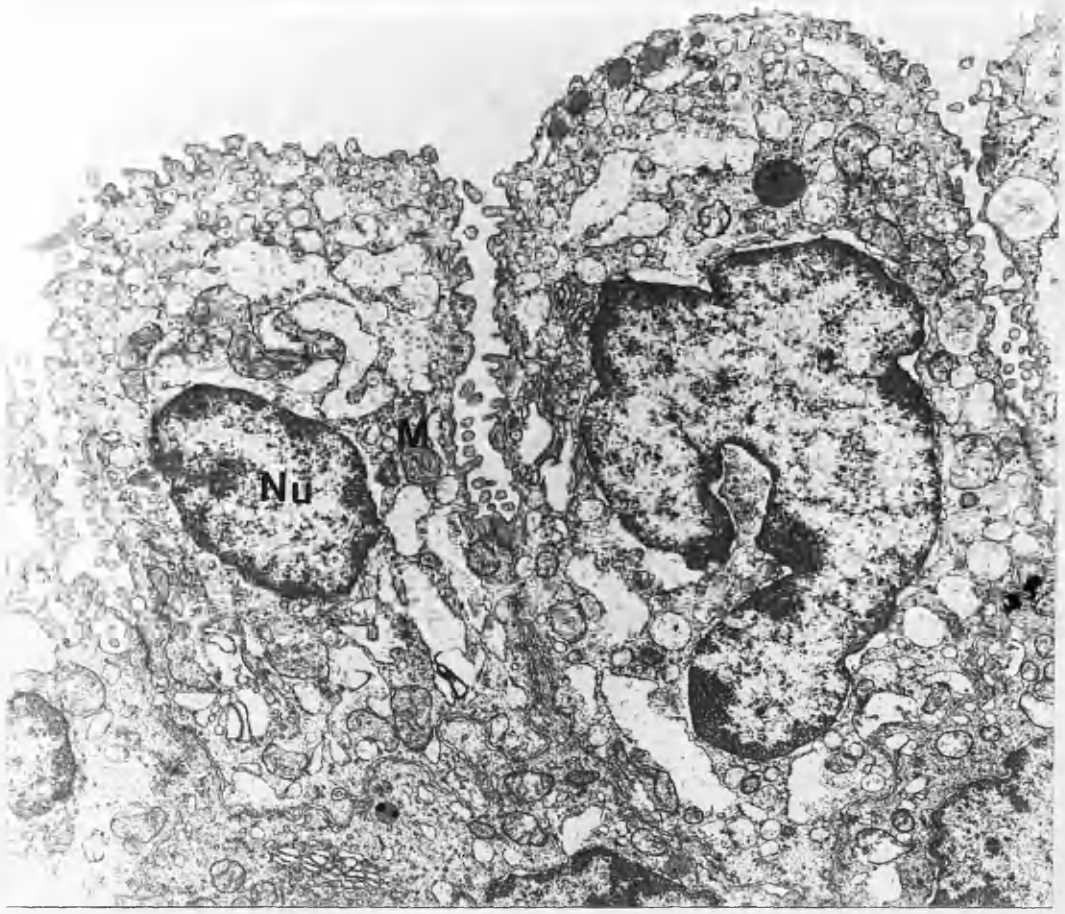

Fig. 34 Bronchiolar Clara cells from a calf infected with lungworms. Note the numerous vacuoles and distension of SSER. Nu = nucleus; M = mitochondria. (x 12,500)

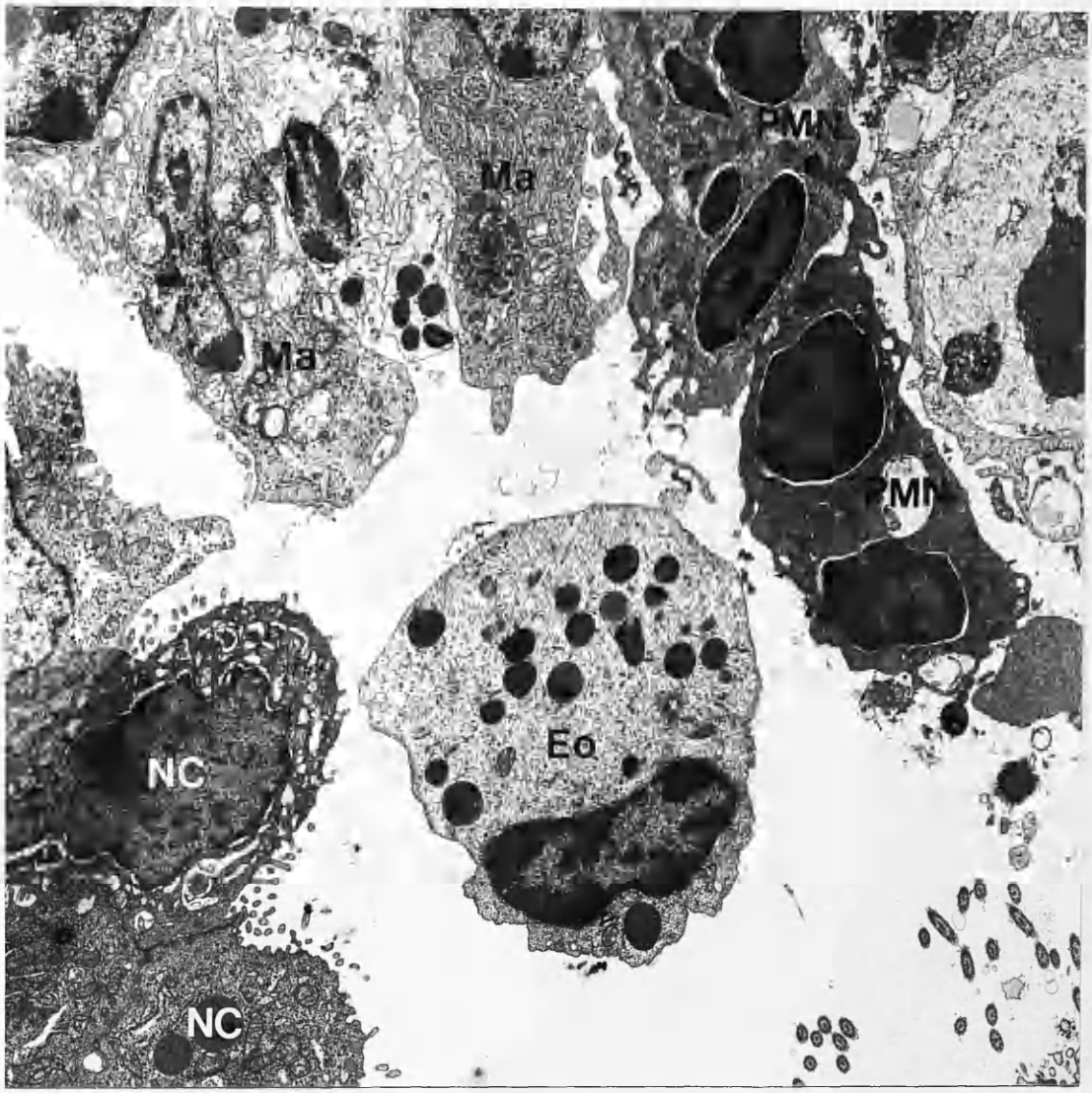

Fig. 35 Calf infected with lungworms. The lumen of a bronchiole with alveolar macrophages (Ma), polymorphonuclear leukocytes (PMN) and an eosinophil (Eo). NC = nonciliated Clara cell. (x 8,000)

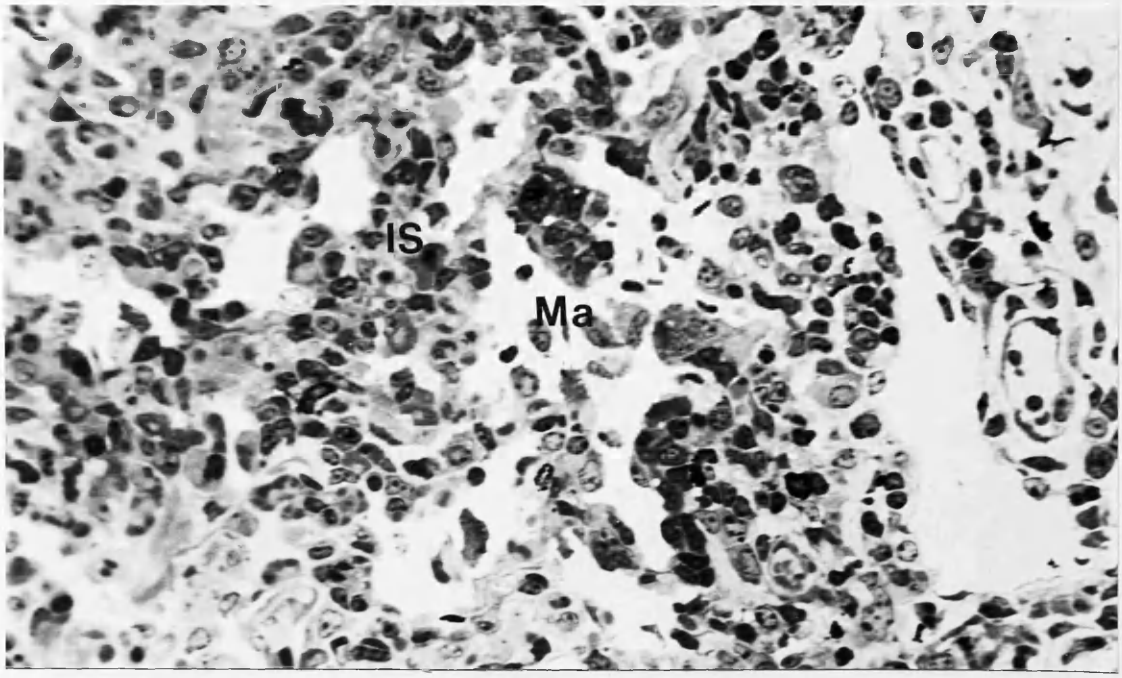

Fig. 36 A group of alveolar macrophages (Ma) can be identified in the alveolar space from a calf infected with lungworms. Note the thickened interalveolar septa (IS). Methylene blue, azure II and borax stain.
(x 700)

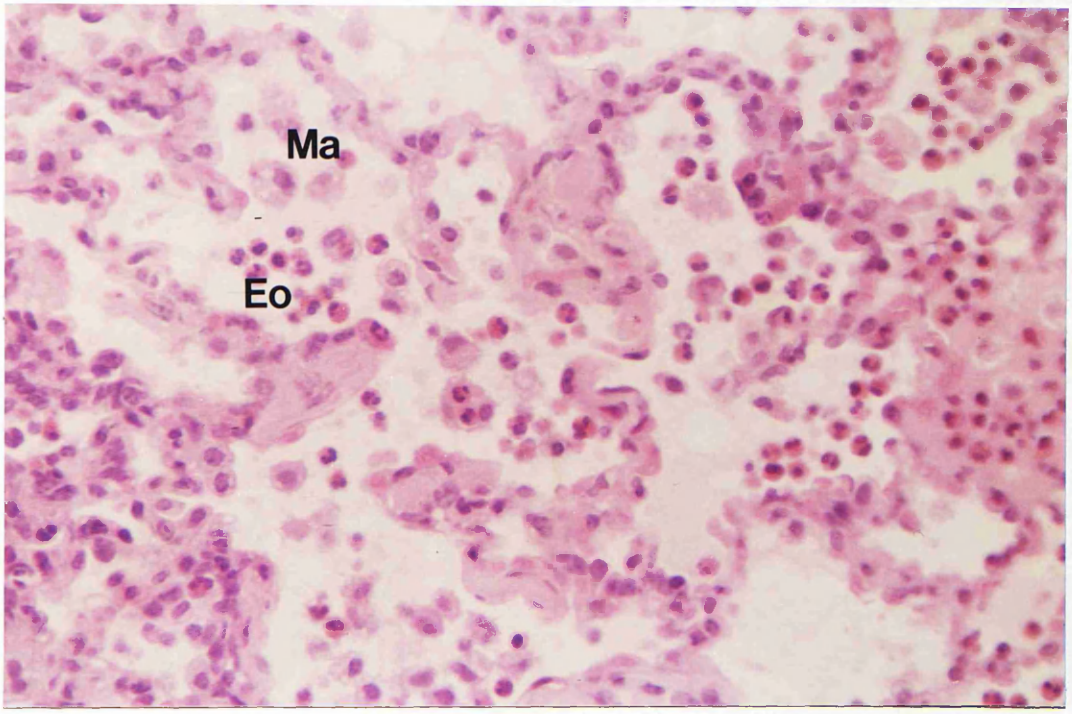

Fig. 37 Thickened interalveolar septa with macrophages (Ma) and eosinophils (Eo) within the alveoli of a calf infected with lungworms. H & E stain. (x 700)

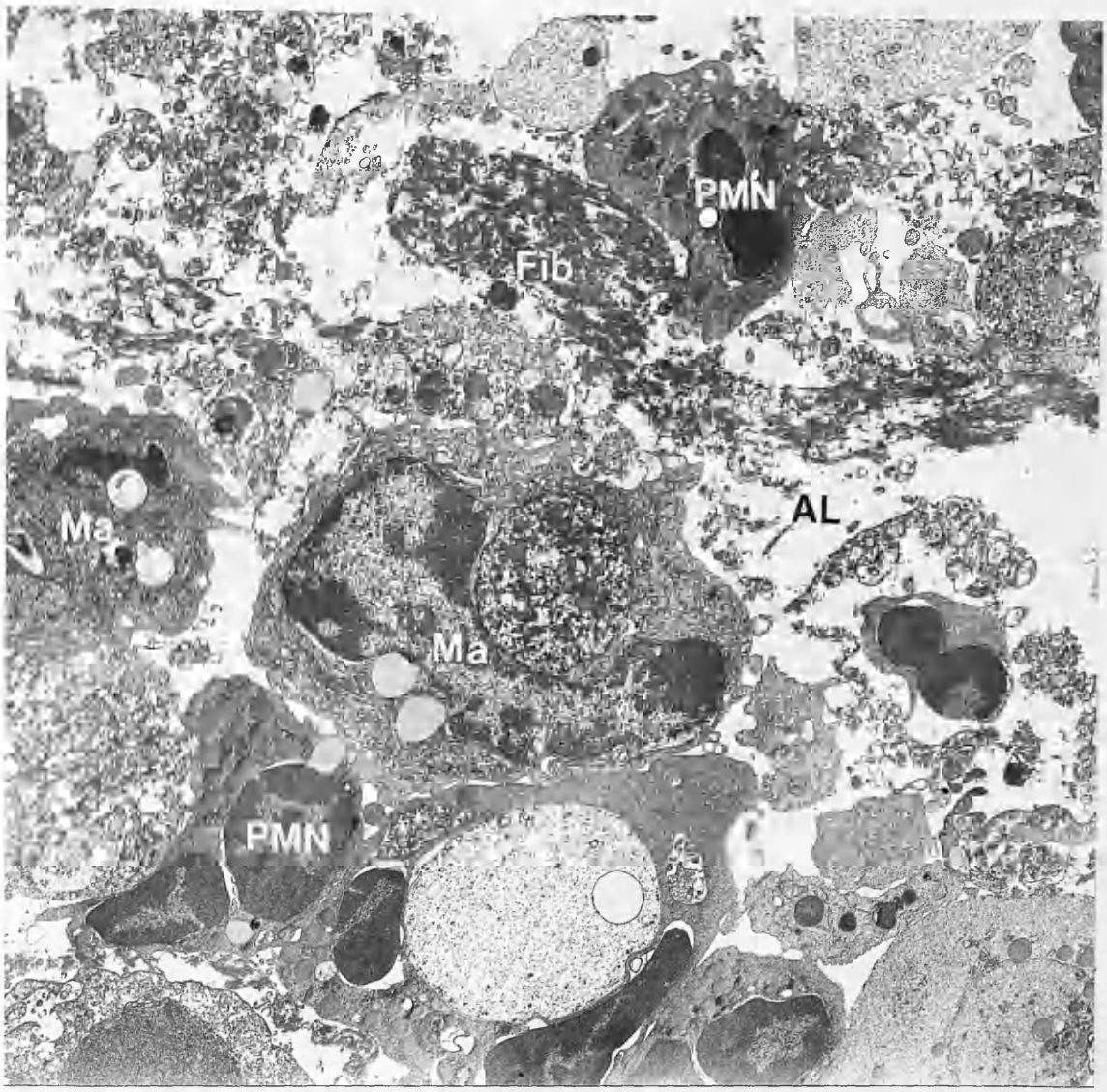

Fig. 38 Alveolar lumen (AL) containing inflammatory cells and exudate in a calf infected with lungworms. Note the alveolar macrophages (Ma), polymorphonuclear leukocytes (PMN), fibrin (Fib) and cellular debris.
(x 8,000)

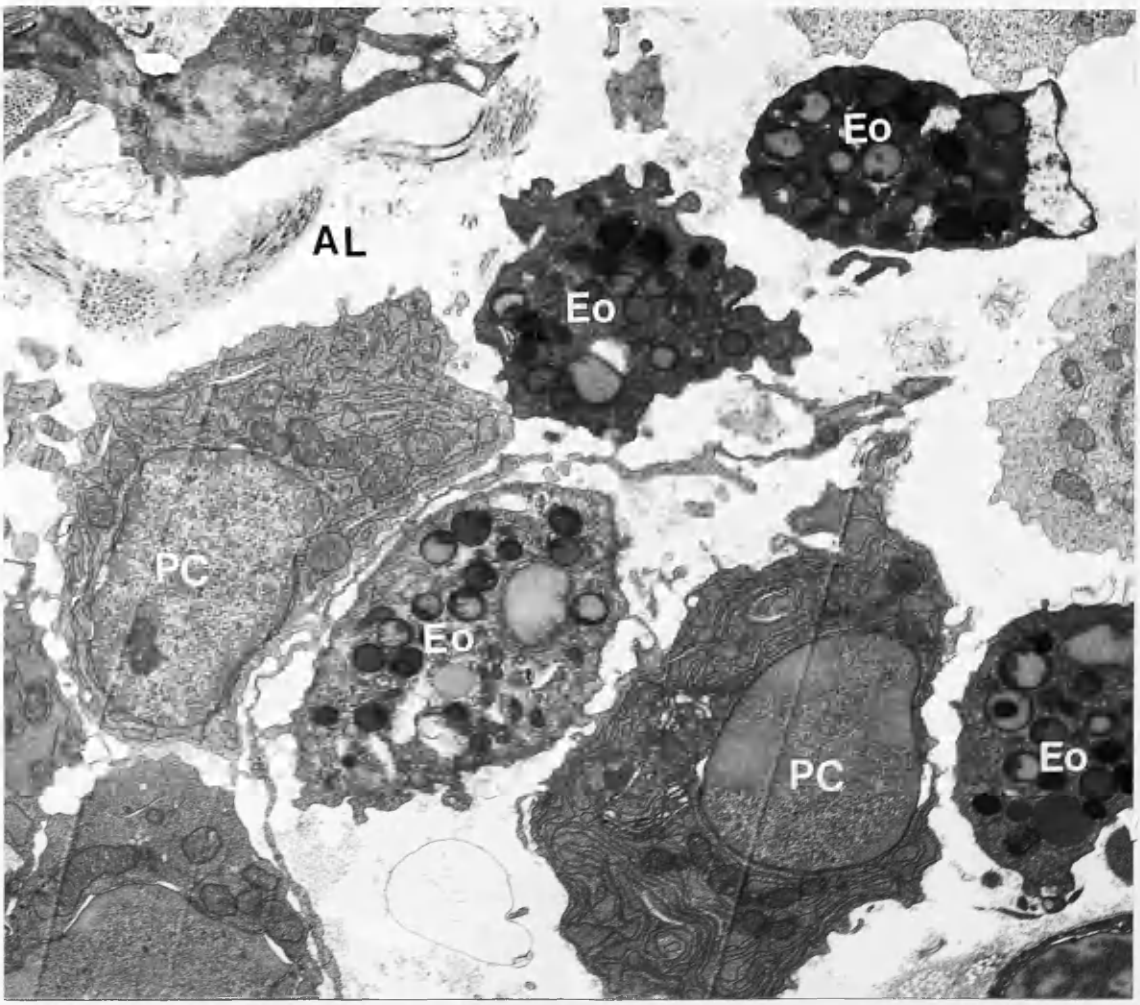

Fig. 39 Alveolar lumen (AL) containing inflammatory exudate in a calf infected with lungworms. Plasma cells (PC) and numerous eosinophils (Eo) are found amongst cellular debris.
(x 8,000)

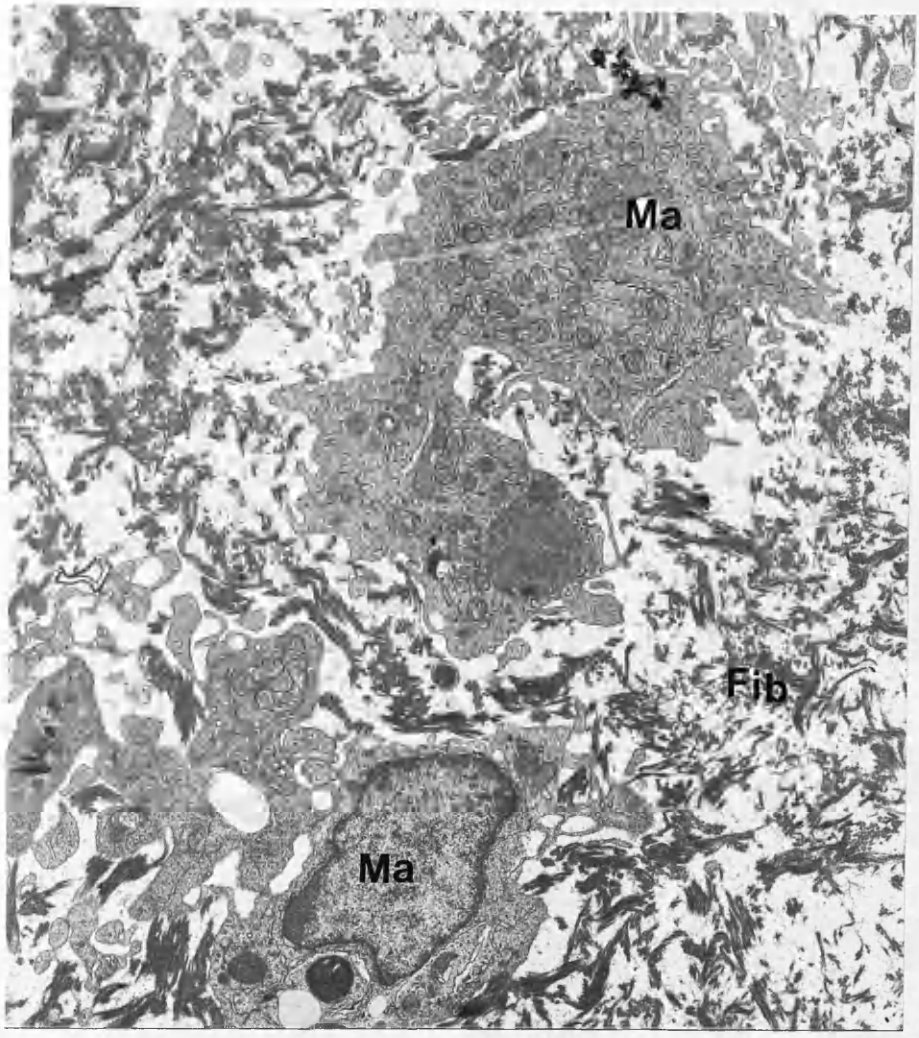

Fig. 40 Alveolar lumen containing two alveolar macrophages (Ma) and surrounded by masses of fibrin (Fib). Calf infected with lungworms.
(x 8,000)

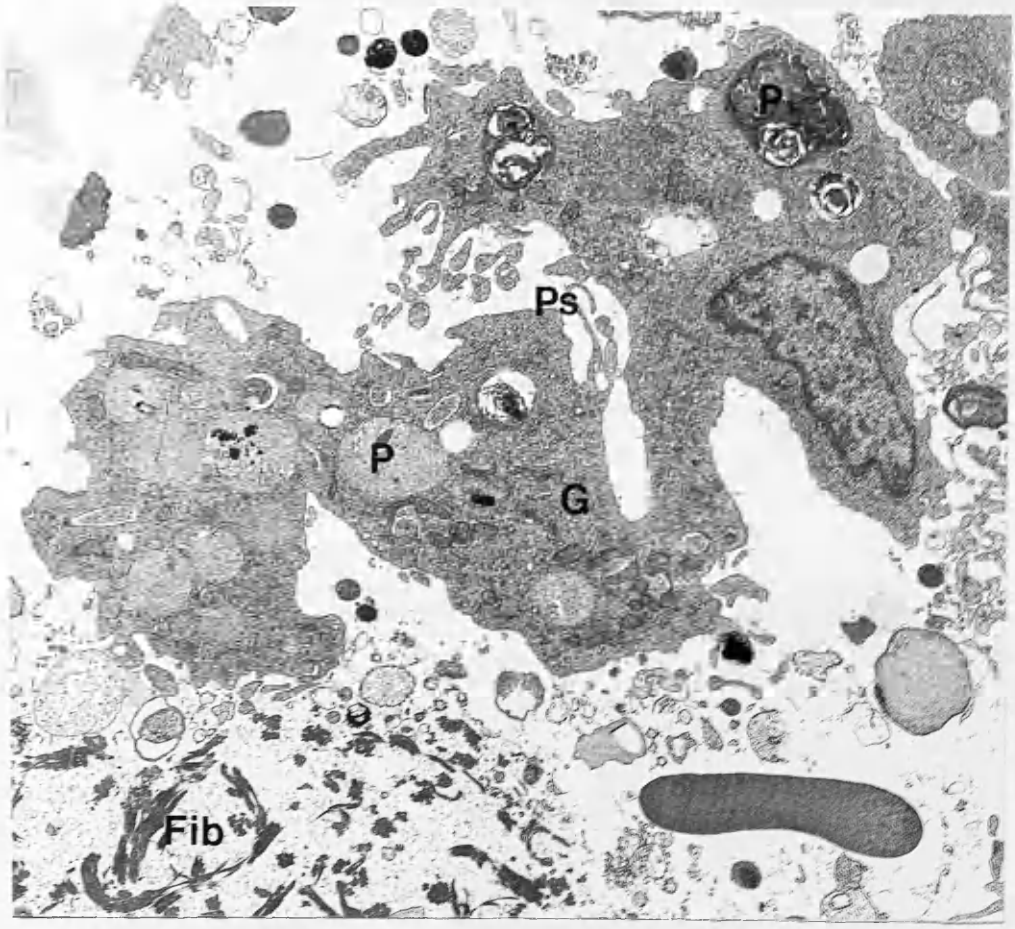

Fig. 41 Calf infected with lungworms. The alveolar macrophage is irregularly shaped and contains many phagosomes (P) and Golgi apparatus (G). Note also the relatively few pseudopodia (Ps) and adjacent fibrin (Fib).

(x 8,000)

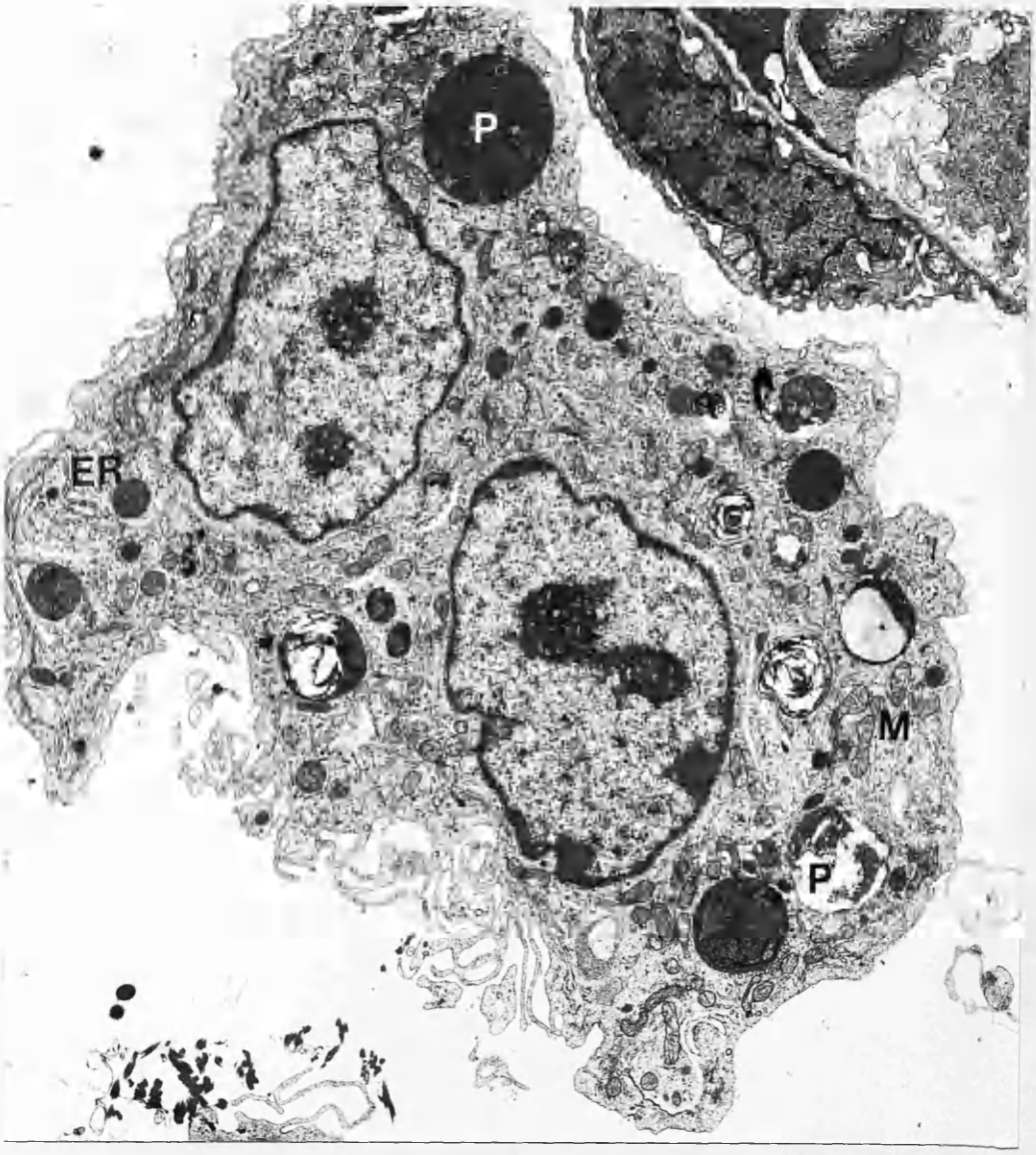

Fig. 42 Calf infected with lungworms. A large binucleate alveolar macrophage with many phagosomes (P), mitochondria (M) and rough surfaced endoplasmic reticulum (ER).
(x 8,000)

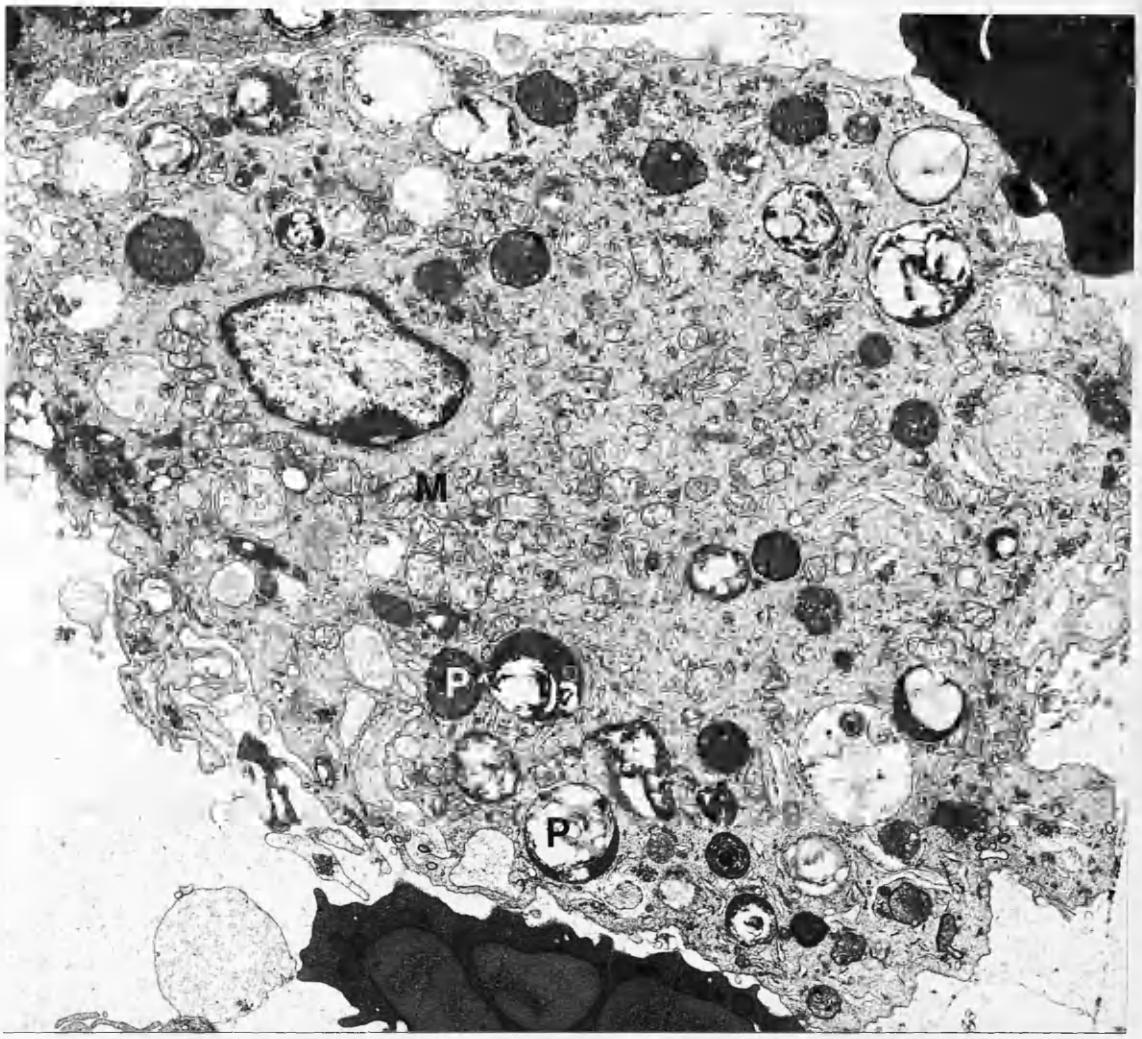

Fig. 43 Calf infected with lungworms. Alveolar macrophage with abundant phagosomes (P) and mitochondria (M). (x 8,000)

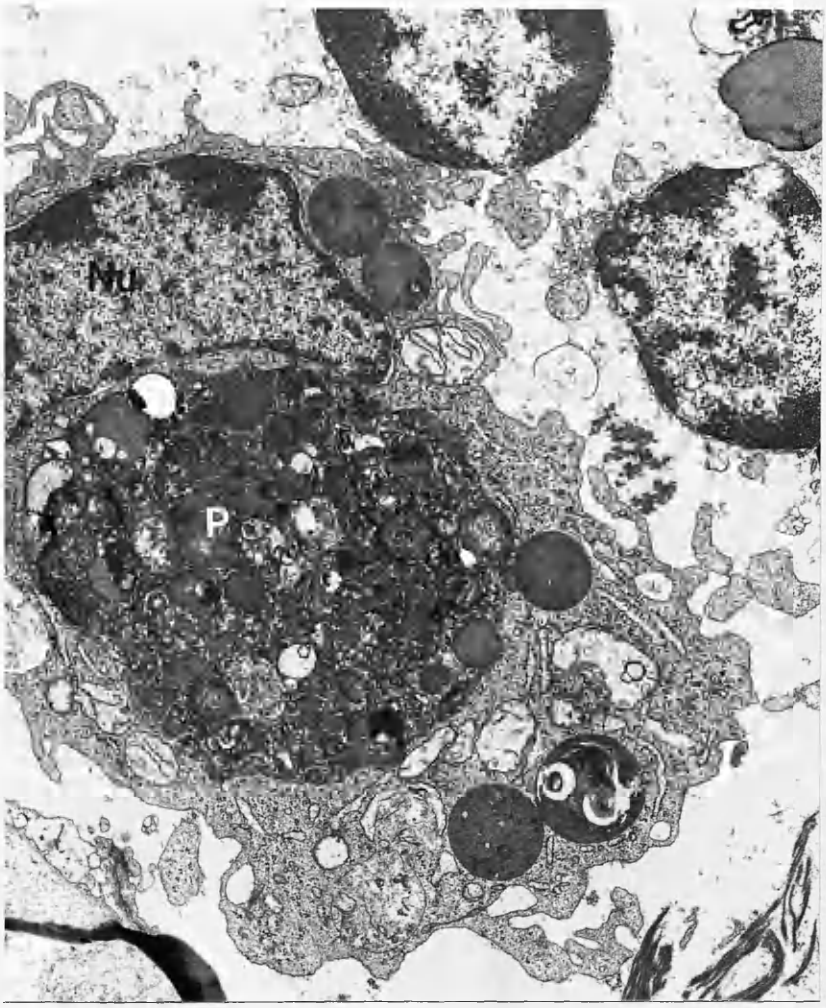

Fig. 44 Calf infected with lungworms. Alveolar macrophage containing a large phagosome (P). Note the size of the mitochondria for comparison. Nu = nucleus. (x 8,000)

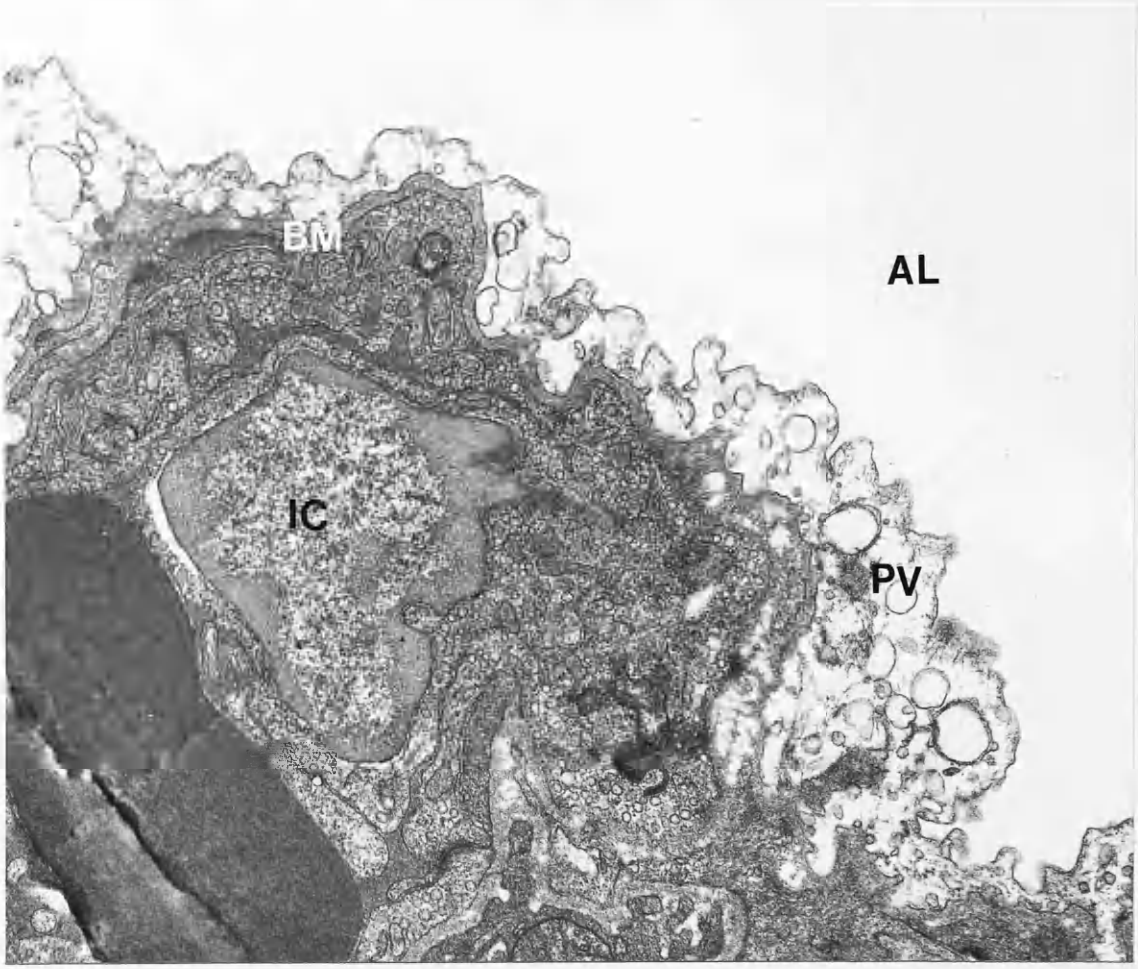

Fig. 45 Calf infected with lungworms. Alveolar wall with extensive swelling of the cytoplasmic extensions of the type I pneumonocyte. BM = basement membrane; PV = pinocytic vacuole; IC = interstitial cell, AL = alveolar lumen.
(x 20,000)

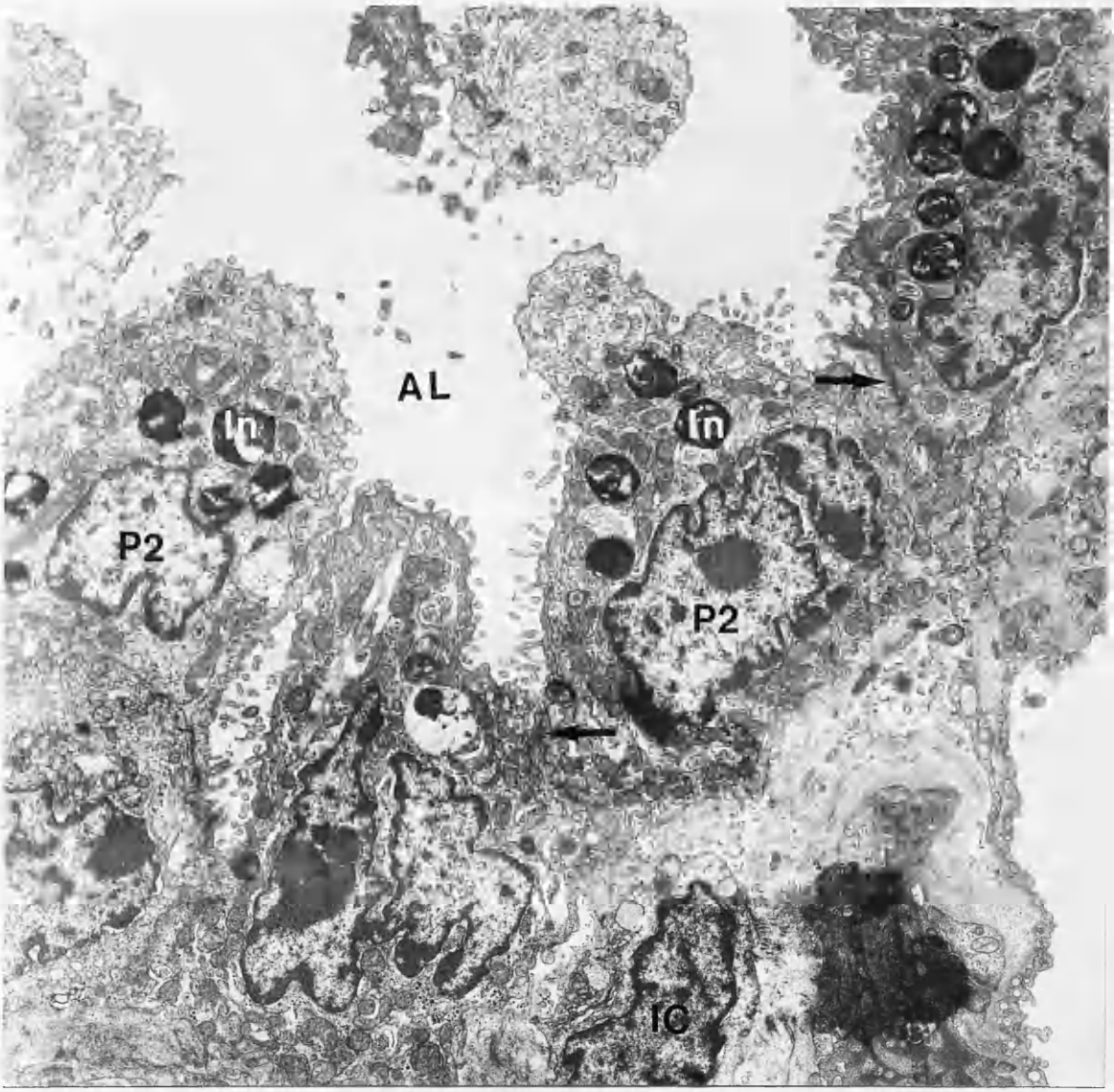

Fig. 46 Type 2 pneumonocytes (P2) projecting into the alveolar lumen (AL) from a calf infected with lungworms. The P2 have numerous inclusions (In) and microvilli. An interstitial cell (IC) is found in the interstitial connective tissue of the alveolar wall. Note tight junction (arrows) between adjacent cells.

(x 8,000)

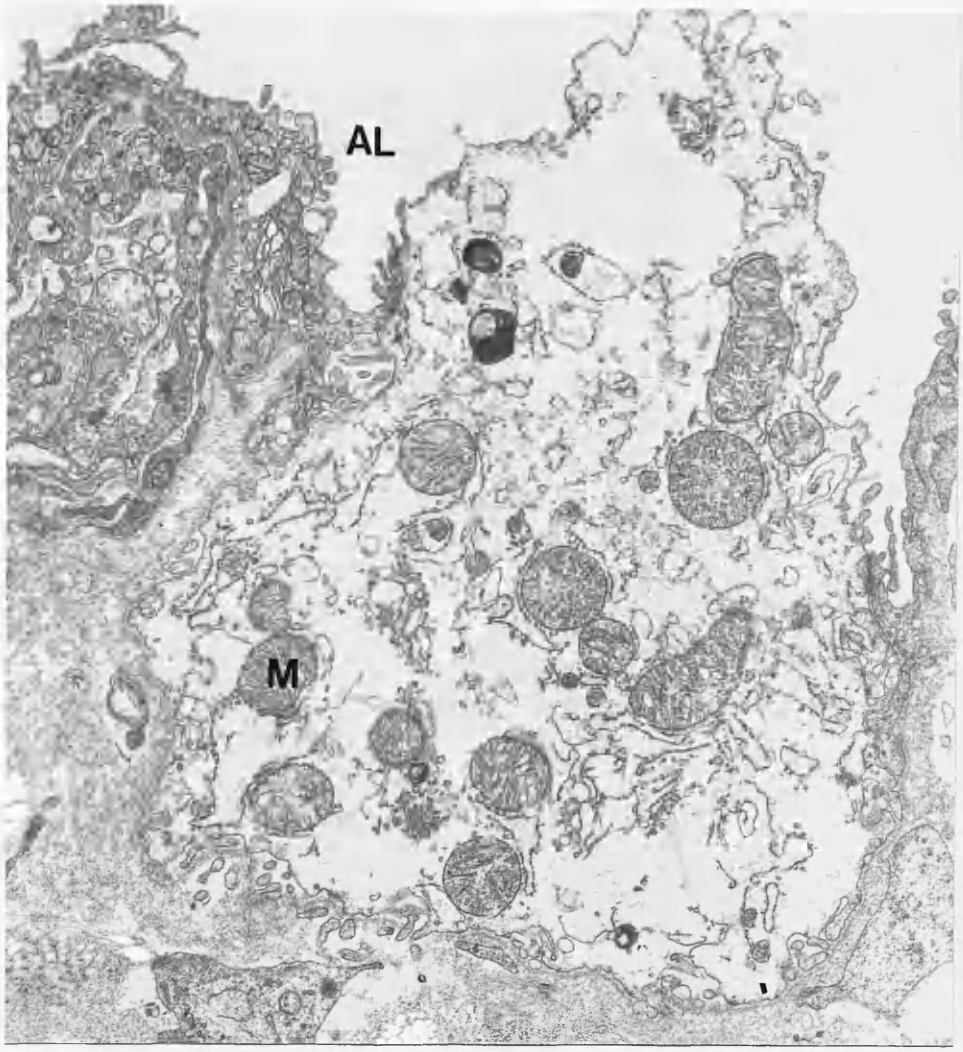

Fig. 47 Severe degeneration of a type II pneumonocyte from a calf infected with lungworms. The cell has few microvilli and a general loss of cytoplasmic organelles. M = mitochondria; AL = alveolar lumen. (x 12,500)

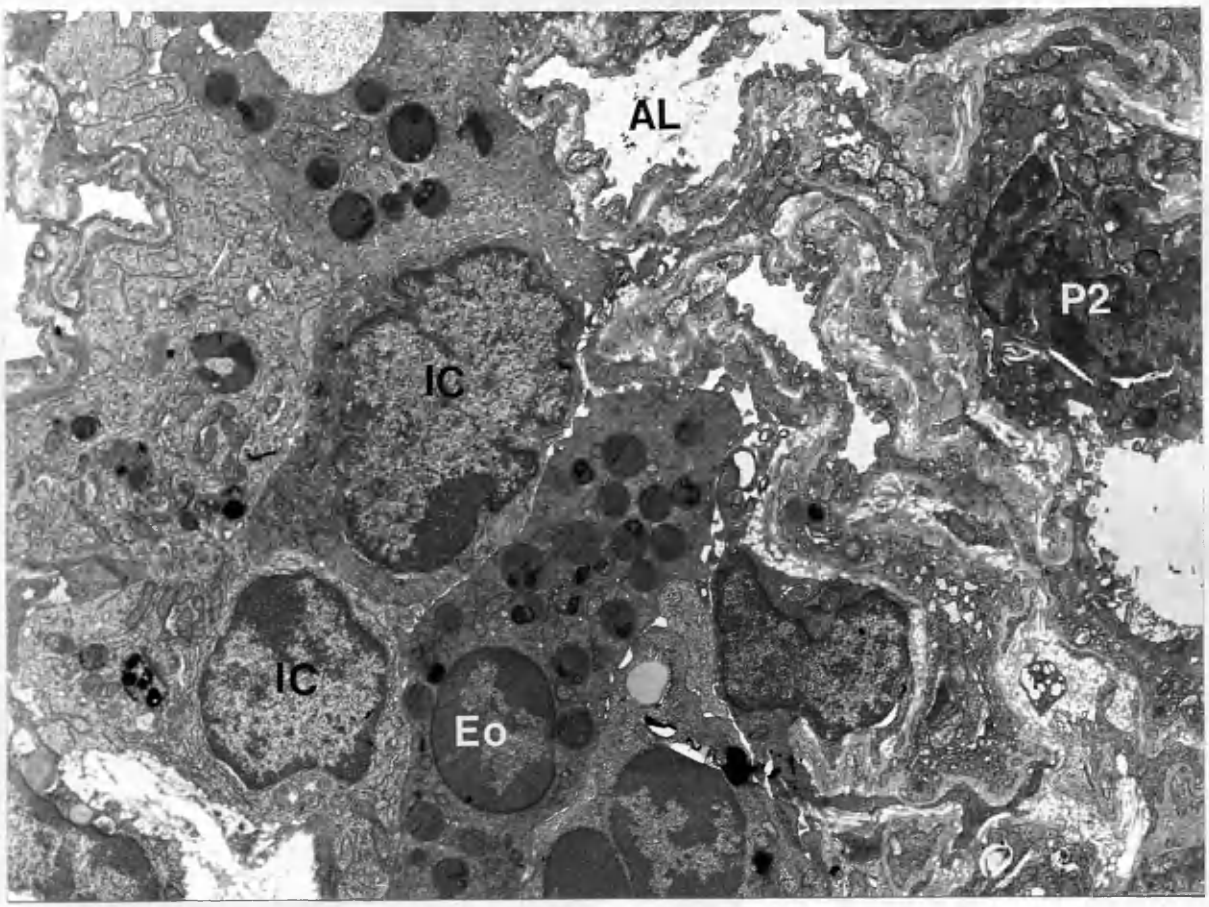

Fig. 48 Thickened interalveolar septa from a calf infected with lungworms. Note the numerous interstitial cells (IC) and an eosinophil (Eo). Portion of a type II pneumonocyte (P2) is seen. AL = alveolar lumen.

(x 12,500)

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION AND CONCLUSIONS

The structure of the lung alveoli and its components in the mammalian respiratory tissue has been extensively reviewed by Bertalanffy (1964). The epithelium lining the walls of the alveoli is composed primarily of the extensive type I pneumonocyte which covered about 93-97% of the alveolar surface (Evans and Shami, 1989). Interspersed throughout this area between the type I pneumonocytes are the granular type II pneumonocytes which covered the remaining 3-5% of the surface area.

Alveolar epithelial hyperplasia, or alveolar epithelialisation, is a lesion in which cuboidal cells come to line the alveoli and replace the previously largely squamous layer (Breeze and others, 1975). Epithelialisation is a common feature of many mammalian pneumonias (Jarrett, 1954). It occurs as a non-specific reaction of the lung to the destruction of the previous alveolar epithelium by a wide variety of unrelated toxic and infectious agents (Omar, 1964) or a loss of pulmonary surfactant (Manktelow, 1967).

Alveolar macrophages are an integral component of the primary lung defence mechanisms and are capable of a variety of activities related to their role as the principal phagocyte of the distal airways (Laegreid and others, 1988).

The origin, structure and function of the AM's has been extensively reviewed (Bowden, 1971; Hocking and Golde, 1979; Khadom and others, 1985; Gordon, 1986).

The means by which the total population of the AM's are maintained and replaced has been a subject of interest for many years (Bertalanffy, 1964). Early investigators proposed that the type II pneumonocytes or the interstitial macrophages found around large blood vessels and airways were the progenitor cells of AM's (Bowden and Adamson, 1972). Later investigations demonstrated that blood monocytes can enter the lung and transform into AM's (Blusse Van Oud Albus and others, 1983). It is well established now that the type II pneumonocytes are not involved in the formation of the AM's and presently there is no evidence to support the concept of an interstitial precursor cell of AM's (Evans and Shami, 1989).

Currently, there are two clearly defined mechanisms by which AM's develop. Firstly, blood monocytes migrate into the alveolar space and later differentiate into AM's. The second method is the division of existing AM's and maintaining the AM population. The migration of blood monocytes into the lung is known to be a source of AM's particularly during an inflammatory response (Blusse Van Oud Albus and others, 1983). The division of AM's was only recently demonstrated to be an important mechanism for

maintaining the AM population in the lung. The AM cellular population may be sustained by local division of cells and cellular proliferation in situ rather than by direct monocyte influx from the blood compartment (Sawyer, 1986; Shellito and others, 1987). However, the conditions and factors that stimulate division of existing AM's and migration of blood monocytes to the lung has not been clearly established (Evans and Shami, 1989).

Winkler (1988) reviewed the structural and functional properties of the PIM's in a number of domestic animals and reported that they are a pulmonary constituent of the mononuclear phagocyte system with respect to secretory, endocytic and functional properties. Warner and others (1986), using morphometric studies, found that the PIM's occupied 15.3% of the intravascular volume, had 15.9m^2 of free surface area available for contact with blood and were closely applied to 7.1% of the endothelial surface.

The general morphological appearances of the AM's in SPA and calves infected with lungworms were basically similar. The AM's in both diseases were activated and their cytoplasm were rich in organelles.

There were, however, some differences which could be readily identified. In SPA, the AM's possessed pseudopodia that were relatively longer and were more numerous. In

addition, many different sized vacuoles were present in the cytoplasm and smaller and lesser number of phagosomes were evident. In contrast, the AM's in calves with lungworms had pseudopodia that were relatively shorter and lesser in numbers, with the cytoplasmic vacuolation being relatively insignificant. The presence of larger and abundant phagosomes were however characteristic. This may be attributed to the fact that since the general ultrastructural changes in the calves with lungworm infection were more severe, the need to 'clean-up' more of the cellular debris becomes apparent. It can be concluded from this study that the AM's in SPA were more likely to be more actively mobile because they had pronounced pseudopodia. However, it is not possible from this study to draw any more specific conclusions as to the significance of the vacuolation in relation to the activation of the AM's in SPA.

Ciano and others (1986) from their investigations on AM migration in fibrin gel matrices have concluded that fibrin deposition in many inflammatory reactions can facilitate or inhibit AM migration. Fibrin was closely associated with many AM's in this study and since many AM's had few pseudopodia, it seems reasonable to conclude that the fibrin may actually have inhibited AM migration.

In conclusion, this TEM study has proved useful in the ultrastructural examination of the AM's in SPA and lungworm infection. The identification of different cell types and structures both in the normal and diseased lungs had been made with greater accuracy than is possible with LM and the examination of the intracytoplasmic changes in the various cell types has been possible.

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