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THE PATHOGENESIS OF PNEUMONIA IN SHEEP

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Massey University.

Maurice Rewi Alley
1975
ABSTRACT

The pathology of pneumonia in sheep in New Zealand is described in a study of over 400 naturally-occurring cases obtained from field and abattoir sources. The common forms of enzootic pneumonia consist of two distinct pathological and epidemiological entities; an acute pneumonia affecting sheep of all ages and a subacute or chronic, non-progressive pneumonia affecting lambs from approximately 3 to 10 months of age. Acute pneumonia is characterised by intense congestion, alveolar haemorrhage, fibrinous exudation and ventral consolidation of both lungs. Ultrastructurally the cellular exudate consists of a mixture of neutrophils, macrophages and detached alveolar epithelial cells with which bacteria are closely associated. Subacute and chronic pneumonia is characterised by varying degrees of dull red to grey consolidation of the anterior lobes. Ultrastructural studies reveal a variety of degenerative changes in the alveolar epithelium including several subcellular changes not previously recorded. Repair is by type II cell hyperplasia and this has been studied ultrastructurally and histochemically. Undifferentiated type II cells resembling those found in the foetal lamb and cells transitional between type II and type I have been observed. The significance of these findings in relation to the origin and dynamics of alveolar epithelial repair is discussed. The major factor underlying the pathological differences between acute and chronic pneumonia is considered to be the degree of damage to the alveolar epithelium which is universal in the former disease and less severe and localised in the latter.

Experimental injury to the ovine lung produced by the endobronchial instillation of dilute (1%) nitric acid with India ink as a marker was studied at periods from 2 hours to 10 days after administration. Alveolar collapse and neutrophil infiltration were the earliest changes seen but few neutrophils remained after 3 days. Large macrophages which were active from 3 hours were joined by smaller macrophages which migrated from interstitial tissues from 12 hours until 3 days after administration. The ultrastructural changes observed in the alveolar epithelium were similar to those encountered in naturally-occurring pneumonia. Proliferation of Clara cells and type II cells was detected
one day after administration and partial "epithelialization" of some alveoli at 5 days. There was complete loss of pulmonary surfactant from affected areas by 12 hours and return to normal activity was irregular.

Parentally administered Paraquat and oral dosing with busulphan were also tested for their value as agents for producing experimental pulmonary injury in sheep. Maximum pulmonary involvement occurred at between 6 to 10 mg/Kg of Paraquat but death appeared to result from liver and kidney toxicity. Paraquat pre-treatment did not affect pulmonary resistance to endobronchially inoculated bacteria in pure or mixed cultures, however lesions similar in nature to those of acute enzootic pneumonia were produced by *Staphylococcus aureus*. No significant pulmonary effects were produced with busulphan at high dose rates.

To investigate the bacterial flora of the respiratory tract of normal and pneumonic sheep, 184 normal sheep and 246 sheep aged 6 to 9 months with chronic or subacute pneumonia were examined at slaughter over a 2 year period. *Pasteurella haemolytica* was present in the nasal cavities of 73% of normal sheep and 78% of sheep with pneumonia, while *Neisseria catarrhalis* was also commonly isolated from both classes. Pneumonic lungs characterised by alveolar collapse yielded few bacteria whereas those in which cellular exudate predominated contained *P. haemolytica* in 75% of cases. In lungs with severe proliferative changes *P. haemolytica* was recovered in over 60% of cases and *N. catarrhalis* in 25 to 33%.

The prevalence of *Mycoplasma ovipneumoniae* and *Mycoplasma arginini* was also investigated in the respiratory tract of normal and pneumonic 6 to 9-month-old sheep. Both organisms were ubiquitous in the nasal cavity but *M. ovipneumoniae* was recovered more frequently than *M. arginini*. The recovery rate and titre of *M. ovipneumoniae* in pneumonic lungs were substantially higher than in normal lungs and several proliferative histological features were found to be associated with these titres. Cellular exudation and epithelial hyperplasia were associated with combined high titres of *M. ovipneumoniae* and bacteria. Lymphoid hyperplasia and mucus secretion were associated with low bacterial titres.
Transmission experiments with lung homogenate derived from cases of acute pneumonia succeeded in producing lesions similar to the natural disease when inoculated endobronchially into worm-free, housed lambs whereas cultures of *P. haemolytica*, *M. arginini* or pneumatic lung homogenised in medium containing antibiotic produced minimal or no effect. However, the excessive amount of inoculum and unnatural means of inoculation required suggested that host and environmental factors have a major role in the pathogenesis of the acute form of the natural disease.

Serial transmission of subacute and chronic pneumonia was achieved by intranasal aerosol inoculation of lung homogenate derived from abattoir cases. The clinical signs and pathological lesions were similar in most respects to the naturally-occurring disease. The pathological development of the lesions was studied in a further transmission experiment in which 12 lambs were slaughtered sequentially from 2 to 12 days after inoculation. In studying the effect of various chemotherapeutic agents on the development of chronic pneumonia it was found that both ronidazole at 100 mg/Kg and oxytetracycline suppressed the development of the disease while tylosin and penicillin suppressed the development of the lesions without completely inhibiting the growth of micro-organisms.

A controlled experiment to assess the effect of pneumonia transmission on weight gain produced a significant reduction in the weight gain of treated animals but there was no correlation between the weight gain of individuals and pneumatic lesions. It was presumed that the result was due to a transitory systemic effect immediately following inoculation.

Intranasal inoculation of *M. ovipneumoniae* cultures produced lesions in 2 caesarian-derived lambs but inoculation of 9 worm-free housed lambs was unsuccessful.

The balance of evidence indicates that pneumonia in sheep, as it occurs in this country, results from the interaction of host and environmental factors with infectious agents. In acute pneumonia, bacterial multiplication in alveoli, presumably damaged by systemic agents, is
responsible for the destructive changes which occur. In chronic pneumonia bacteria from the nasal cavity actively contribute to the severity of the lesions but it is unlikely that they initiate the disease process. *M. ovipneumoniae* is also closely associated with the lesions of chronic pneumonia but further inoculation experiments and epidemiological studies are needed to define this organism's role more closely.
ACKNOWLEDGEMENTS

I am particularly indebted to my chief supervisor Professor B.W. Manktelow for his constant encouragement, advice and helpful criticism during all phases of this work. Thanks are also due to Dr. H.G. Pearce for his interest throughout and his constructive criticism of the manuscript. I am also indebted to Dr. J.K. Clarke for many useful discussions as well as practical help with the microbiological aspects of this work and to Dr. R.B. Marshall who also provided helpful advice in this area. The interest and enthusiasm of Dr. R.D. Jolly which enabled this work to be commenced is acknowledged with gratitude.

This study could not have been fully undertaken without the technical assistance of a number of people whose help I gratefully acknowledge. Skilled assistance was provided by Mrs. P. Twentyman and Mr. P.N. Wildbore in the bacteriological studies; Mrs. J.R. Quinlan and Miss V.G. Brown in the mycoplasma studies; Mrs. J. Lang, Mr. C.G. Fletcher, Mr. R.P. Hanson and Mrs. P. Slack in the preparation of histological sections; Mr. D. Ward in the histochemical studies; Messrs. A.S. Craig, D.H. Hopcroft and C.G. Fletcher in the preparation of tissues for electron microscopy and Mrs. D. Lovelock in the transmission experiments.

My thanks are also extended to Mr. P.H. Whitehead for arranging the availability of the experimental animals; Dr. W.A.G. Charleston for his co-operation in the use of worm-free sheep and facilities; Mr. A.B. de Cleene for his assistance in animal handling operations and the veterinarians and meat inspectors at the Co-operative Wholesale Supplies Limited, Longburn for their co-operation and help in the collection of specimens.

The photographs in this thesis were printed by Mr. T. Law and Mr. I.D. Simpson and the final illustrations were drawn by Mrs. M. McComish. Their efforts are greatly appreciated.

Special thanks are also due to Mrs. G.P. Harper who typed the final copy of this manuscript.
I also wish to express my appreciation to my wife and family for their patience and help during the course of this study.

This work was financially supported by grants from the Vernon Wiley Trust, Gisborne Veterinary Club and Merck, Sharp and Dohme (N.Z.) Limited.
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Parallel elongation of pyknotic nuclei in necrotic cellular exudate. The "streaming" appearance is characteristic of acute enzootic pneumonia in sheep. HE x 250.

Alveolar spaces adjacent to those containing cellular exudate are distended with variable amounts of fibrin and proteinaceous fluid. Apart from leucocytes within the congested alveolar capillaries, few cells can be recognized in the alveolar septa. HE x 250.

Alveolar cellular exudate embedded in mucus (m). In this case elongated cells can be seen "streaming" through a pore of Kohn (arrow) into the adjacent alveolus. HE x 400.

Large alveolar macrophages with abundant cytoplasm are mixed with small numbers of neutrophils in areas away from the main lesions. Epoxy resin embedded. TbBf x 400.

Distended interlobular interstitial space containing strands of fibrin and infiltrations of equal numbers of mononuclear cells and neutrophils. The adjacent alveolar spaces are filled with a mixture of haemorrhagic, fibrinous and cellular exudate. x 100.

Area of necrosis surrounding a bronchiole. The necrotic area is demarcated by a margin of severe leucocytic infiltration. HE x 40.
The desquamating cell in the corner of the alveolar space \( (A) \) is probably a great alveolar type II cell as indicated by the remnants of microvilli \( (m) \) and lamellated bodies \( (b) \). The adjacent alveolar type I cell \( (E) \) is also sloughing leaving the underlying basement membrane \( (bm) \) exposed. x 9,000.

Fibrin and necrotic cellular debris appear to be passing from the alveolar capillary \( (C) \) into the adjacent interstitial space \( (T) \) through a rupture in the basement membrane \( (bm) \). The alveolar space \( (A) \) contains fibrin, clumps of bacteria and necrotic cellular debris. x 9,000.

A large rupture in the basement membrane \( (bm) \) of the capillary \( (C) \) has probably allowed the escape of erythrocytes \( (e) \) and neutrophil \( (N) \) into the surrounding interstitial space. The neighbouring alveolar epithelium is desquamating into the alveolus \( (A) \). x 10,000.

Cellular exudate in the central area of an alveolus. It consists of compressed amorphous nuclear debris \( (Nu) \) embedded in a coagulum of cytoplasmic contents \( (Cy) \) and amorphous exudate. x 9,000.

The cells contributing to the exudate rapidly lose their characteristic morphology but neutrophils \( (N) \) can sometimes be identified amongst them. x 9,000.

Large numbers of bacteria are present in the alveolar space \( (A) \). The adjacent interstitial space \( (T) \) is severely oedematous and a large amount of fibrin is accumulating within the congested alveolar capillary \( (C) \). x 9,000.

Bacteria are often closely associated with destructive changes in the alveolar wall. In this case a bacterium \( (B) \) appears to be embedded in the necrotic remnants of an alveolar type I cell \( (E) \). The alveolar space \( (A) \) contains acellular debris and strands of fibrin. x 16,000.

Dull red consolidation (Type 1 lesions) in chronic enzootic pneumonia. Affected areas have a homogeneous appearance and are sunken below the surface of the surrounding lung.

Red-grey consolidation (Type 2 lesions) in chronic enzootic pneumonia. Affected areas are usually more extensive and swollen and the pleural surface has a granular appearance in reflected light.

Grey-red consolidation (Type 3 lesions) in chronic enzootic pneumonia. The affected tissue has a finely granular mottled appearance, is firmer than normal and affected lobes are thickened.
Grey consolidation (Type 4 lesions) in chronic enzootic pneumonia. The affected lobes are thicker than normal and the tissue very firm in consistency. Fibrous adhesions are sometimes present between the consolidated areas and the parietal pleura.

Fibrous adhesions between the pleural surfaces of the apical, cardiac and diaphragmatic lobes of the right lung as well as between the apical and cardiac lobes and the parietal pleura. Lesions of this type are frequently seen in lambs during the April-June period.

An irregular area of dull grey consolidation merging dorsally with areas of compensatory emphysema. Lesions of this type were interpreted as representing recovery from earlier consolidation.

Mild emphysema and small bands of collapse are present in the right apical lobe and there is complete adhesion between the right diaphragmatic and cardiac lobes. Changes of this type may be the aftermath of earlier pneumonic lesions.

Peripheral areas of dull grey consolidation in the left apical and cardiac lobes surrounded by areas of severe compensatory emphysema. The left cardiac lobe is adherent to the left diaphragmatic. Lesions of this type probably represent the re-aeration of previously consolidated tissue.

Suppurative pleuropneumonia in the ventral areas of the right lung of a 9 month-old lamb with chronic sporodesmin poisoning. Abscesses of this type probably develop within areas of unresolved chronic pneumonia.

The Microscopic Appearance of Type I Lesions in Chronic Pneumonia

Extensive areas of alveolar collapse with the accumulation of mucus and moderate numbers of neutrophils in terminal bronchioles. HE x 40.

The collapsed alveoli present a featureless appearance with little evidence of inflammatory change at low magnification. HE x 100.

Small amounts of proteinaceous debris and occasional macrophages can be seen in alveolar spaces at high magnifications. Epoxy resin embedded. TbEf x 400.
Moderate numbers of neutrophils are present in alveolar ducts and alveolar spaces contain small numbers of neutrophils and macrophages. HE x 100.

An intense neutrophilic exudate has accumulated in many groups of alveoli disseminated throughout the lesion. HE x 100.

The epithelium of a terminal bronchiole shows moderate hyperplasia and large macrophages can be seen embedded in the neutrophilic exudate in surrounding alveolar ducts. Epoxy resin embedded. TbBf x 250.

The Microscopic Appearance of Type 3 Lesions in Chronic Pneumonia

Both exudative and proliferative changes are present. There is a prominent bronchiolar epithelial hyperplasia and an increase in the number of goblet cells. Lymphoid hyperplasia surrounds a bronchiole partially sectioned at bottom right and alveoli are filled with variable numbers of macrophages and neutrophils. HE x 100.

Proliferation of alveolar type II cells can be seen in many alveolar spaces resulting in partial alveolar epithelialization. HE x 400.

The alveolar exudate is a mixture of neutrophils and large macrophages and early proliferation of connective tissues can be seen in some alveolar septa. Epoxy resin embedded. TbBf x 400.

The Microscopic Appearance of Type 4 Lesions in Chronic Pneumonia.

Severe proliferative changes are the main feature including bronchiolar epithelial hyperplasia and para-bronchial nodular scars. HE x 100.

Macrophages predominate in the alveolar exudate and only occasional neutrophils can be found. There is severe peribronchiolar lymphoid hyperplasia adjacent to a bronchiole out of view at left. HE x 250.

Complete alveolar epithelialization of alveoli at the periphery of a lobule adjacent to interlobular connective tissue. HE x 250.

Knob-like swellings of smooth muscle tissue at the ends of interalveolar septa. These were found in some cases of chronic pneumonia but they were not a constant feature. HE x 250.
Partially aerated alveolar spaces in resolving chronic pneumonia. A few macrophages are present in alveoli and there is some residual peribronchiolar fibrosis together with severe lymphoid hyperplasia associated with a bronchiole out of view at right. HE x 100.

Edge of pulmonary abscess in a case of chronic pneumonia showing bronchiolar epithelium forming part of the margin of the abscess. HE x 100.

Various stages in the development of pulmonary corpora amylacea in chronic pneumonia.
(a) Sequestrated protein-rich exudate surrounded by macrophages in an alveolar duct.
(b) Necrosis of some of the macrophages in the space surrounding the sequestrum.
(c) Incorporation of necrotic cellular elements into the outer layers of the sequestrum.
(d) Well-developed sequestrum showing concentrically lamellated appearance. Remnants of cell nuclei are visible in some layers. PAS x 400.

Non-specific alkaline phosphatase reaction.
(a) In normal artificially collapsed lung the activity is concentrated in scattered great alveolar type II cells.
(b) In pneumonic lung the activity indicates aggregations of 2 or more type II cells.
Naphthol AS (NAS) method and haematoxylin.- x 250.

Non-specific acid phosphatase reaction.
(a) In normal artificially collapsed lung there is a mild diffuse activity in the alveolar septa.
(b) In pneumonic lung there is a marked increase in activity due to infiltration by macrophages and neutrophils.
NAS method and methyl green. x 250.

Early changes in an area of alveolar collapse in chronic pneumonia. There is oedema of the alveolar interstitium (T) and migration of a large mononuclear cell (M) through the interstitial space. The cytoplasmic extensions of the alveolar type I epithelial cell (E) are swollen and show loss of continuity of the outer plasma membrane. The alveolar type II cell (G) appears normal. x 9,000.

More advanced changes in an area of alveolar collapse. A large mononuclear cell (M) is migrating beneath a degenerating type II cell (G) which shows mitochondrial swelling and cytoplasmic vesiculation. The adjacent alveolar type I epithelium shows severe vesiculation (v). The alveolar space (A) contains part of a macrophage and necrotic debris. x 9,000.
Figure 2.57 An active alveolar macrophage in alveolar space (A). The cytoplasm contains numerous dense bodies (d) and the advancing edge (x) is taking proteinaceous debris into phagosomes (arrow). x 9,000.

Figure 2.58 Sloughing of the alveolar epithelium in chronic pneumonia. An alveolar type I cell (E) has become detached but shows little degenerative change. A degenerating neutrophil (N) and portion of a macrophage (M) have migrated beneath the epithelium and the alveolar space (A) contains necrotic cellular debris and occasional bacteria (arrow). x 9,000.

Figure 2.59 Early degenerative changes in an alveolar type II cell in an area of alveolar collapse. The mitochondria (m) are swollen and there are indentations in the nuclear membrane. Small dilatations (d) are present in the endoplasmic reticulum and there is loss of superficial microvilli. The adjacent alveolar type I epithelium (E) appears normal. x 12,000.

Figure 2.60 More advanced degenerative changes in an alveolar type II cell in chronic pneumonia. The mitochondria (m) are severely swollen and disrupted and there is loss of nuclear membrane. Several dilatations (d) are present in the endoplasmic reticulum and a cytoplasmic extension (c) is present at the surface of the cell. x 16,000.

Figure 2.61 Thickening of the superficial plasma membrane (t) is another common degenerative change in alveolar type II cells. The damaged cell also shows dilatation of the endoplasmic reticulum and loss of superficial microvilli. The adjacent type II cell is less severely affected but the alveolar type I epithelium (E) is extensively vacuolated. A macrophage (M) is present in the alveolar space and a plasma cell (P) can be seen in the underlying interstitium. x 9,000.

Figure 2.62 An intra-cytoplasmic inclusion (i) in an alveolar type II cell. These spherical bodies were occasionally found in damaged or proliferating type II cells. They contained evenly distributed dense granules and were often surrounded by semicircles of endoplasmic reticulum (r). A mast cell (H) is present in the underlying interstitium. x 9,000.

Figure 2.63 Early proliferation of alveolar type II cells in the corner of an alveolus. One cell shows a superficial cytoplasmic extension (c) as well as an intracytoplasmic inclusion (i). x 10,000.

Figure 2.64 Severely destructive changes in an alveolus in chronic pneumonia. There is loss of alveolar type I epithelial cells and type II cells (G) lie flattened against the basement membrane. The alveolar space (A) contains neutrophils (N), bacteria (B) and necrotic cellular debris. x 9,000.
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2.69 Clara cells (C) at the end of a terminal bronchiole which contains neutrophils (N). The end cell (D) appears to be differentiating into an alveolar type II cell as it contains a lamellated osmiophilic body (arrow). x 9,000.

2.70 Alveolar space (A) in an advanced case of chronic pneumonia. It is lined by a cell containing lamellated bodies (b) but with the flattened posture characteristic of an alveolar type I cell. The underlying interstitium (T) is severely thickened with connective tissue. x 9,000.

2.71 The alveolar interstitium in an advanced case of chronic pneumonia. It contains infiltrating plasma cells (P), a fibroblast (F) and a large mononuclear cell (M). x 9,000.

2.72 Aberrant cytoplasmic extension of alveolar type I cell (E) over the surface of an alveolar type II cell (G). The covered cell appears mildly damaged as indicated by loss of superficial microvilli and a small cytoplasmic extension (c). x 9,000.

2.73 An alveolar type II cell (G) trapped beneath the cytoplasmic extensions of a type I cell (E). The resultant build up of osmiophilic secretions (s) from the lamellated bodies (b) is protruding into the alveolar space (A). x 9,000.

2.74 Densely osmiophilic secretions (s) from an alveolar type II cell (G) have accumulated beneath the cytoplasmic extensions of a type I cell (E). x 12,600.
3.1 Twelve hours after instillation of dilute nitric acid and ink. Several irregular foci of dark red consolidation and patchy areas of congestion are present on the dorsal surfaces of the right apical and left diaphragmatic lobes.

3.2 Five days after instillation of dilute nitric acid and ink. The ventral areas of the right apical and left cardiac lobes show dull red consolidation and there is patchy congestion of the dorsal and ventral areas of the left diaphragmatic lobe.

3.3 Control animal 1 day after the administration of normal saline and ink. There is only very limited alveolar collapse despite the deposition of ink in bronchioles and alveolar ducts. HE x 100.

3.4 Alveolar collapse surrounding a terminal bronchiole 2 hours after instillation of dilute nitric acid and ink. Some of the alveoli also contain small amounts of proteinaceous exudate. HE x 100.

3.5 Neutrophil infiltration into alveolar spaces 6 hours after nitric acid instillation. Many alveoli contain a protein-rich exudate and strands of fibrin. Epoxy resin embedded. TbBF x 250.

3.6 At 12 hours after instillation of dilute nitric acid and ink neutrophil infiltration is still a prominent feature and alveolar collapse is severe. HE x 250.

3.7 Large carbon-laden macrophages are mixed with neutrophils in bronchiolar exudate 1 day after instillation of dilute nitric acid and ink. Macrophages outnumber neutrophils in the surrounding alveoli and occasional carbon-laden macrophages can be seen in the peribronchiolar interstitium. Epoxy resin embedded. TbBF x 400.

3.8 At 7 days after instillation, groups of carbon-laden macrophages remain sequestered within some alveolar spaces. The surrounding alveoli are collapsed and their septa are slightly thickened by mononuclear cells. Epoxy resin embedded. TbBF x 400.

3.9 Early epithelial proliferation in the most distal part of a terminal bronchiole 1 day after instillation of dilute nitric acid and ink. The cells involved are pale-staining cuboidal cells resembling Clara cells. Epoxy resin embedded. TbBF x 400.

3.10 Epithelial hyperplasia 2 days after instillation of dilute nitric acid and ink. Carbon-laden macrophages are present in surrounding alveoli. Epoxy resin embedded. TbBF x 400.
3.11 At 3 days after instillation, bronchiolar epithelial hyperplasia appears to be extending into adjacent alveoli in this severely affected area. Several carbon-laden macrophages are present in the peribronchiolar interstitium and there is early fibroblast proliferation. HE x 400.

3.12 Early alveolar type II cell hyperplasia in an area adjacent to a focus of cellular exudation at 2 days after instillation of dilute nitric acid and ink. Almost every alveolar space contains one or more type II cells. Epoxy resin embedded. TbBf x 400.

3.13 A severely collapsed area of lung, 3 days after instillation of dilute nitric acid and ink. Some alveolar spaces contain more than one finely vacuolated cell which were identified as type II cells by electron microscopy. Epoxy resin embedded. TbBf x 400.

3.14 At 5 days after instillation groups of proliferating type II cells (arrow) can be seen lining alveoli adjacent to a terminal bronchiole. Several large carbon-laden macrophages remain in surrounding alveolar spaces. Epoxy resin embedded. TbBf x 400.

3.15 The distribution of stable bubbles in air-dried, frozen sections of lung covered with air-saturated water at 6 hours after administration of dilute nitric acid and ink. (a) Unaffected area. (b) Affected area in which there is loss of bubbles from areas surrounding the instilled fluid. (Unstained x 100).

3.16 Similar sections to the above collected 3 days after the administration of dilute nitric acid and ink. (a) Unaffected area. (b) Affected area showing almost complete loss of stable bubbles. (Unstained x 100).

3.17 Degenerative changes in alveolar type I cells 2 hours after instillation of dilute nitric acid and ink. (a) Localised swelling of cytoplasmic extensions with the formation of a large bleb (b) which is ballooning into the alveolar space (A). (b) Diffuse swelling of the cytoplasmic extensions (E) lining alveolar space (A). A monocyte (M) and platelet (p) are present in the underlying capillary. x 14,000.

3.18 Six hours after instillation. The type I cell (E) lining alveolar space (A) is diffusely swollen and this swelling includes the nucleus which is enlarged to approximately twice normal size. A neutrophil (N) is present in the adjacent capillary. x 8,000.
Degenerative changes in an alveolar type II cell 2 hours after instillation of dilute nitric acid and ink. The cell (G) has swollen mitochondria, dilatations in the endoplasmic reticulum (d), loss of microvilli and a superficial cytoplasmic extension (c). x 10,000.

More advanced degeneration in a type II cell (G) 3 hours after instillation. There is loss of the nuclear membrane and aggregation of chromatin. At the cell surface there is loss of microvilli and early formation of an electron-dense plasma membrane. The alveolar space (A2) is lined by a type I cell with dense, shrunken cytoplasmic extension (E) and the underlying interstitium (T) is swollen and disrupted. x 9,000.

An alveolar space 3 hours after the instillation of dilute nitric acid and ink. Globules of protein (p) are present and the alveolar type I epithelium (E) is shrunken and dense. In one area (s) the epithelium has sloughed leaving the basement membrane exposed. Neutrophils (N) are closely adherent to the underlying capillary endothelium. x 13,000.

A neutrophil (N) can be seen migrating through an interstitial space 6 hours after instillation. The overlying alveolar type II cell shows early degenerative changes including dilatation of the endoplasmic reticulum (d) and the formation of electron-dense plasma membranes in some areas (arrows). x 12,000.

Early proliferation of alveolar type II cells in the corner of an alveolar space (A) 1 day after the instillation of dilute nitric acid and ink. One of the cells involved has lamellated bodies (b) which are larger and more numerous than usual. x 10,000.

At 5 days after instillation, groups of 4 to 5 alveolar type II cells (G) can be seen lining some alveolar spaces (A) adjacent to terminal bronchioles. x 9,000.

A binucleate alveolar type I cell (E) protruding into alveolar space (A) at 12 hours after instillation of dilute nitric acid and ink. x 10,000.

A macrophage (M) migrating into alveolar space (A) at 12 hours after instillation. The cell has a moderate amount of cytoplasm but lacks lysosomes and residual bodies. x 10,000.

A large macrophage (M) free in alveolar space (A) at 12 hours after instillation of dilute nitric acid and ink. The cell has an abundant cytoplasm containing a variety of lysosomes and residual bodies. A lymphocyte (L) is also present in the alveolus. x 9,000.
3.28 Sequestration of a carbon-laden macrophage (M) in the remnants of an alveolar space 7 days after instillation. The cell is heavily laden with carbon granules and surrounded by a thickened interstitium (T) containing a large amount of collagen (C). x 9,000.

3.29 Vesicle formation (v) in the cytoplasmic extensions of an alveolar type I cell (E) 3 days after the administration of Paraquat. The surrounding interstitium (T) contains infiltrating mononuclear cells (M). x 13,000.

3.30 An alveolus (A) adjacent to the one above shows little evidence of damage to the type I epithelium (E) although a macrophage (M) and erythrocyte (e) are present in the alveolar space. x 9,000.

3.31 Degenerative changes in an alveolar type II cell 1 day after the administration of Paraquat. There is dilatation of both lamellated bodies (b) and endoplasmic reticulum (d) and the cell surface shows loss of microvilli. The adjacent type I epithelium (E) shows mild vacuolation.

3.32 Two alveolar type II cells are present in the corner of an alveolar space (A) 5 days after the administration of Paraquat. The underlying interstitial space (T) is swollen and contains excessive amounts of collagen (C).

3.33 Gross lung lesions following systemic paraquat administration and endobronchial bacterial inoculation.

3.34 The margin of a necrotic zone from the lung of animal E89 (3 days after the inoculation of bacteria and 5 days after Paraquat administration). There is a dense infiltration of leucocytes many of which are necrotic. HE x 40.

3.35 A higher magnification of a non-necrotic area from the same lung as above. The alveoli are filled with fibrinous exudate in which are embedded variable numbers of neutrophils and mononuclear cells. HE x 250.

3.36 Severe epithelial hyperplasia with alveolar epithelialization at the margin of a necrotic area induced by the inoculation of *P. haemolytica* culture 6 days previously (no Paraquat administered). HE x 100.

4.1 Colonies of *Mycoplasma arginini* (centred) and *Mycoplasma ovipneumoniae* (centreless) in oblique transmitted light. x 45.
Figure

4.2 Distribution of titres of *M. ovipneumoniae* in normal and pneumonic lungs.

4.3 Correlation of *M. ovipneumoniae* and bacterial titres with severity of histological lesions.

4.4 The distribution of *M. ovipneumoniae* and bacterial titres in 40 cases of subacute and chronic pneumonia.

5.1 Gross lung lesions following endobronchial inoculation of pneumonic lung homogenate, *P. haemolytica* or *M. arginini*.

5.2 Dark red consolidation of the intermediate and ventral diaphragmatic lobe of the right lung after endobronchial inoculation with lung homogenate derived from a case of acute pneumonia. The surface of the affected area is covered with a fibrinous exudate.

5.3 Extensive red consolidation of the right apical and cardiac lobes and congestion of the remainder of the lung after endobronchial inoculation with lung homogenate derived from a case of acute pneumonia. The anterior part of the right apical lobe is necrotic and the pleural space is filled with cloudy fluid containing thick strands of fibrin.

5.4 A well-encapsulated necrotic area of lung containing brown, haemorrhagic contents in the diaphragmatic lobe of the left lung after endobronchial inoculation with a broth culture of *P. haemolytica*.

5.5 Nozzle of nebulizing gun used for intranasal inoculation

5.6 Method of restraint during inoculation. The forefingers were removed and the spray stopped every 3 to 5 seconds to allow exhalation.

5.7 The deposition of small quantities of ink in the anteroventral parts of the lungs of a lamb following the trial of the intranasal aerosol inoculation technique.

5.8 Lateral radiographs of the thorax of sheep No E65 - (a) Before inoculation (b) Fourteen days after inoculation. There is an increase in opacity in the thoracic cavity between the heart shadow and the thoracic inlet.

5.9 Antero-posterior radiographs of the thorax of sheep No E 66 - (a) Before inoculation (b) Fourteen days after inoculation. There is an increase in opacity between the heart shadow and the thoracic inlet.
5.10 Gross lung lesions obtained at sequential slaughter following the intanasal transmission of chronic pneumonia.

5.11 Two days after inoculation with lung homogenate derived from chronic pneumonia. Small dull red depressed foci are visible on the surface of the right apical and cardiac lobes.

5.12 Eight days after inoculation with lung homogenate derived from chronic pneumonia. Red-grey areas of consolidation are present in the apical lobe of the right lung.

5.13 Twelve days after inoculation with lung homogenate derived from chronic pneumonia. Extensive grey-red consolidation is present in the anterior lobes of both lungs.

5.14 Small numbers of neutrophils mixed with necrotic debris in a terminal bronchiole 4 days after inoculation with homogenate derived from chronic pneumonia. Macrophages are accumulating in surrounding alveolar spaces. HE x 250.

5.15 Amorphous fibrinoid material adherent to the wall of a terminal bronchiole 6 days after inoculation with lung homogenate derived from chronic pneumonia. The material is partially covered by epithelium and infiltrated with macrophages. HE x 250.

5.16 Focal neutrophil and extensive macrophage infiltration 12 days after inoculation with lung homogenate derived from chronic pneumonia. Proliferative changes such as epithelial hyperplasia are becoming prominent. HE x 100.

5.17 Gross lung lesions following the intranasal transmission of chronic pneumonia and administration of various chemotherapeutic agents.

5.18 Correlation between weight gain and severity of pneumonic lesions.

5.19 Dull red consolidation of the right apical and left cardiac lobes of a caesarian-derived lamb 12 days after inoculation with a broth culture of *M. ovipneumoniae*.

5.20 Diffuse discoloration of the ventral areas of both lungs of a caesarian-derived lamb 12 days after inoculation with a broth culture of *M. ovipneumoniae*.
"The seat of Pneumonia has been a matter of dispute for some time past, some say it exists in the Interlobular Texture others again affirm that it exists in the Capillary Walls of the air cells, this dispute is something similar to the one as regards the colour of the Chameleon for they are both right and wrong."

Professor William Dick, 1794-1866
(From the lecture notes of John Gillispie, Veterinary Surgeon, who qualified from Dick's Veterinary College, Edinburgh in 1865).