

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

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 pneumonic 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 pneumonic 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.

TABLE OF CONTENTS

	Page
LIST OF FIGURES	
INTRODUCTION	1
Clinical and Epidemiological Features of Pneumonia in sheep in New Zealand	1
The Economic Importance of the Disease in New Zealand	2
Approach to the Problem	3
CHAPTER 1 : GENERAL REVIEW OF LITERATURE	5
SECTION I : THE PATHOLOGY OF NATURALLY-OCCURRING PNEUMONIAS	5
Enzootic Pneumonia	6
Atypical Pneumonia	8
Montana Progressive Pneumonia	10
Maedi	11
Pulmonary Adenomatosis (Jaagsiekte)	11
Miscellaneous Sheep Pneumonias	13
SECTION II : INFECTIOUS AGENTS ASSOCIATED WITH PNEUMONIA IN SHEEP	15
<u>Pasteurella haemolytica</u>	15
<u>Pasteurella multocida</u>	23
Mycobacteria	23
<u>Pseudomonas pseudomallei</u> (Meliodiosis)	24
Miscellaneous Bacteria	24
Mycoplasma Species	25
Chlamydia (Bedsonia)	27
Parainfluenza-3 Virus	29
Miscellaneous Viruses	31
Combination of Agents	32
SECTION III : INJURY TO THE PULMONARY ALVEOLUS	34
Systemic Agents which may Injure the Alveolus	35
Inhaled Agents which may Injure the Alveolus	43
Gases	43
Fluids	46
Particles	50
SECTION IV : ALVEOLAR DEFENCE MECHANISMS	55
PART ONE : <u>THE REACTION OF THE OVINE PULMONARY ALVEOLUS</u> <u>TO INJURY</u>	

	Page
CHAPTER 2 : STUDIES OF SHEEP PNEUMONIA (THE NATURALLY- OCCURRING DISEASE)	63
INTRODUCTION	63
MATERIAL AND METHODS	64
Animals	64
Microscopy	64
Histochemistry	65
RESULTS	66
Normal Lung	66
Acute Pneumonia	68
Gross Pathology	68
Light Microscopy	69
Electron Microscopy	70
Subacute and Chronic Pneumonia	71
Gross Pathology	71
Light Microscopy	75
Histochemistry	78
Electron Microscopy	78
DISCUSSION	83
SUMMARY	92
CHAPTER 3 : EXPERIMENTAL INJURY TO THE OVINE LUNG	94
INTRODUCTION	94
EXOGENOUS AGENTS : DILUTE NITRIC ACID PLUS CARBON BLACK	94
Material and Methods	95
Results	97
Gross Pathology	97
Light Microscopy	98
Pulmonary Surfactant Studies	101
Electron Microscopy	102
ENDOGENOUS AGENTS : (i) PARAQUAT	104
Material and Methods	104
Results	105
The Toxicity of Paraquat to Sheep	105
The Effect of Paraquat Administration on Pulmonary Resistance to the Endobronchial Inoculation of Bacteria	109

	Page
(ii) BUSULPHAN	111
Material and Methods	112
Results	112
DISCUSSION	113
SUMMARY	119
 <u>PART TWO : THE AETIOLOGY AND TRANSMISSION OF SHEEP PNEUMONIA</u>	
 CHAPTER 4 : THE MICROBIAL FLORA OF THE NORMAL AND PNEUMONIC	
OVINE RESPIRATORY TRACT	122
INTRODUCTION	122
A SURVEY OF THE BACTERIAL FLORA OF THE NORMAL AND	
PNEUMONIC OVINE RESPIRATORY TRACT	123
Material and Methods	123
Collection of Specimens	123
Laboratory Procedures	124
Results	124
The Prevalence of Bacterial Species in	
the Normal and Pneumonic Respiratory	
Tract	124
The Prevalence of Bacteria in Different	
Types of Pneumonic Lesion	126
BACTERIAL ISOLATIONS FROM ACUTE PNEUMONIA AND	
PULMONARY OR PLEURAL ABSCESES	129
Material and Methods	129
Results	130
A SURVEY OF THE PREVALENCE OF <u>MYCOPLASMA OVIPNEUMONIAE</u>	
<u>MYCOPLASMA ARGININI</u> IN THE RESPIRATORY TRACT OF	
NORMAL AND PNEUMONIC SHEEP	130
Material and Methods	133
Collection of Specimens	133
Media	133
Processing of Specimens	134
Fluorescent Antibody Methods	134
Results	135
Identification of Isolates by Fluorescent	
Antibody Methods	135
Prevalence of Mycoplasmas in the Nasal	
Cavity	135

	Page
Preliminary Investigation of Mycoplasmas in the Lungs	135
Quantitative Study of <u>M. ovipneumoniae</u> in Normal and Pneumonic Lungs	137
CORRELATIONS BETWEEN HISTOPATHOLOGICAL FEATURES AND NUMBERS OF <u>M. OVIPNEUMONIAE</u> AND BACTERIA PRESENT IN PNEUMONIC LUNGS	138
Material and Methods	138
Results	139
DISCUSSION	141
SUMMARY	146
CHAPTER 5 : THE EXPERIMENTAL TRANSMISSION OF SHEEP PNEUMONIA	148
INTRODUCTION	148
THE TRANSMISSION OF ACUTE PNEUMONIA BY ENDOBRONCHIAL INOCULATION	149
Material and Methods	149
Results	150
Clinical Response	150
Necropsy Findings	152
Light Microscopy	153
SERIAL TRANSMISSION OF CHRONIC PNEUMONIA BY INTRANASAL AEROSOL INOCULATION	154
Material and Methods	155
Animals	155
Preparation of Inoculum	155
Method of Inoculation	155
Results	157
THE DEVELOPMENT OF CHRONIC PNEUMONIA : SEQUENTIAL SLAUGHTER FOLLOWING INTRANASAL INOCULATION	159
Material and Methods	159
Results	161
Necropsy Findings	162
Light Microscopy	162

	Page
THE EFFECT OF CHEMOTHERAPEUTIC AGENTS ON THE	
TRANSMISSION OF CHRONIC PNEUMONIA	163
Material and Methods	164
Animals	164
Method of Inoculation	164
Drug Administration	164
Results	166
Clinical Response	166
Necropsy Findings	169
Light Microscopy	171
Microbiological Findings	171
THE EFFECT OF PNEUMONIA TRANSMISSION ON WEIGHT GAIN	173
Material and Methods	173
Results	173
<u>MYCOPLASMA OVIPNEUMONIAE</u> INOCULATION TRIALS	175
Material and Methods	175
Pilot Trial	175
Inoculation of Housed Lambs	176
Results	177
Pilot Trial	177
Inoculation of Housed Lambs	177
DISCUSSION	180
SUMMARY	186
CHAPTER 6 : GENERAL DISCUSSION	188
REFERENCES	195
APPENDICES	
PUBLICATIONS	

LIST OF FIGURES

Figure		following page
2.1	Normal ovine alveolar type I epithelial cells (E) lining alveolar spaces (A). The cells have a flattened nucleus and thin cytoplasmic extensions (X) which cover the interstitial connective tissue (T). x 12,600.	66
2.2	Normal ovine great alveolar type II cell (G) protruding into an alveolar space (A). The cells have numerous short microvilli (m), vacuolated lamellated bodies (b) in the cytoplasm and are firmly attached to the underlying basement membrane. x 12,600.	66
2.3	Discharge of concentrically lamellated contents from vacuole (V) of an alveolar type II cell into alveolar space (A). The adjacent lamellated body (b) in the cytoplasm contains characteristic coarsely lamellated material seen most often in immature cells. Nucleus (N). x 27,700.	66
2.4	Pulmonary alveolar macrophage (M) projecting through a pore of Kohn in the alveolar septum (S) of normal lung. The cell has a moderate amount of cytoplasm and several lysosomes (r) of various types. x 12,600.	66
2.5	Mast cell (H) containing dense cytoplasmic granules in alveolar interstitial space of normal ovine lung. Several capillaries (C) contain erythrocytes and a fragment of an alveolar type II cell (G) can also be identified. x 10,000.	68
2.6	Alveolar capillary in a normal lung containing an erythrocyte (e) and lined by capillary endothelial cell (C). The thin blood-air barrier (p) separates the erythrocyte from the alveolar space (A). x 12,600.	68
2.7	Acute enzootic pneumonia. The right apical lobes show dark red consolidation and are swollen above the surface of the remaining lung.	68
2.8	A more advanced case of acute enzootic pneumonia. The right apical lobe (a) is necrotic and the remaining lung is severely congested. Thick deposits of fibrin (f) are visible between the apical and cardiac lobes and the pericardial sac.	68
2.9	Early accumulation of cellular exudate in acute pneumonia. Neutrophils and small mononuclear cells migrating into alveolar spaces which are filled with protein-rich exudate. HE x 250.	69

Figure		following page
2.10	Necrosis of cellular exudate. Alveolar spaces are distended with randomly distributed necrotic debris embedded in an amorphous exudate. Alveolar capillaries are severely congested. HE x 250.	69
2.11	Parallel elongation of pyknotic nuclei in necrotic cellular exudate. The "streaming" appearance is characteristic of acute enzootic pneumonia in sheep. HE x 250.	69
2.12	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.	69
2.13	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.	69
2.14	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.	69
2.15	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. HE x 100.	69
2.16	Area of necrosis surrounding a bronchiole. The necrotic area is demarcated by a margin of severe leucocytic infiltration. HE x 40.	69
2.17	Colonies of Gram-negative bacteria located within alveoli at the periphery of a necrotic lobule. Humberstone's Gram. x 400.	69
2.18	Disintegration and sloughing of alveolar epithelial type I cell (E) into alveolar space (A). Between the desquamating cell and the underlying basement membrane (bm) is an erythrocyte (e) and bacteria (B). x 9,000.	70
2.19	Necrotic alveolar macrophages (M) are present beneath the alveolar epithelial type I cell (E) which is desquamating into the alveolar space (A). Within the alveolus a degenerating neutrophil (N) and other cellular debris are already present. The endothelial lining of the capillary (C) appears relatively undamaged. x 9,000.	70
2.20	Clumps of bacteria (B), neutrophils (N) and possible remnants of alveolar epithelium (E) are present in the necrotic alveolar exudate. The basement membrane (bm) of the surrounding alveolar wall is exposed and the underlying interstitial tissues are oedematous and contain strands of fibrin. x 9,000.	70

Figure		following page
2.21	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.	70
2.22	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.	70
2.23	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.	70
2.24	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.	71
2.25	The cells contributing to the exudate rapidly lose their characteristic morphology but neutrophils (N) can sometimes be identified amongst them. x 9,000.	71
2.26	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.	71
2.27	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.	71
2.28	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.	73
2.29	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.	73
2.30	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.	73

Figure		following page
2.31	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.	73
2.32	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.	74
2.33	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.	74
2.34	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.	74
2.35	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.	74
2.36	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.	74
	The Microscopic Appearance of Type I Lesions in Chronic Pneumonia	75
2.37	Extensive areas of alveolar collapse with the accumulation of mucus and moderate numbers of neutrophils in terminal bronchioles. HE x 40.	75
2.38	The collapsed alveoli present a featureless appearance with little evidence of inflammatory change at low magnification. HE x 100.	75
2.39	Small amounts of proteinaceous debris and occasional macrophages can be seen in alveolar spaces at high magnifications. Epoxy resin embedded. TbBf x 400.	75
	The Microscopic Appearance of Type 2 Lesions in Chronic Pneumonia	76

Figure		following page
2.40	Moderate numbers of neutrophils are present in alveolar ducts and alveolar spaces contain small numbers of neutrophils and macrophages. HE x 100.	76
2.41	An intense neutrophilic exudate has accumulated in many groups of alveoli disseminated throughout the lesion. HE x 100.	76
2.42	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.	76
	The Microscopic Appearance of Type 3 Lesions in Chronic Pneumonia	76
2.43	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.	76
2.44	Proliferation of alveolar type II cells can be seen in many alveolar spaces resulting in partial alveolar epithelialization. HE x 400.	76
2.45	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.	76
	The Microscopic Appearance of Type 4 Lesions in Chronic Pneumonia.	76
2.46	Severe proliferative changes are the main feature including bronchiolar epithelial hyperplasia and para-bronchial nodular scars. HE x 100.	76
2.47	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.	76
2.48	Complete alveolar epithelialization of alveoli at the periphery of a lobule adjacent to interlobular connective tissue. HE x 250.	76
2.49	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.	76

Figure		following page
2.50	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.	76
2.51	Edge of pulmonary abscess in a case of chronic pneumonia showing bronchiolar epithelium forming part of the margin of the abscess. HE x 100.	76
2.52	Various stages in the development of pulmonary corpora amylacea in chronic pneumonia. (a) Sequestered 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	78
2.53	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.	78
2.54	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	78
2.55	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.	78
2.56	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.	78

Figure		following page
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.	79
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.	79
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	79
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.	79
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.	80
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 12,600.	80
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.	80
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.	80

Figure		following page
2.65	Alveolar epithelialization in chronic pneumonia showing electron-dense mature type II cells (G) and proliferating immature type II cells (F) containing coarsely lamellated osmiophilic bodies. The alveolar space (A) contains necrotic macrophages. x 9,000	81
2.66	A pocket of proliferating alveolar type II cells in an advanced case of chronic pneumonia. The cells have many coarsely lamellated inclusions and numerous other organelles. x 9,000.	81
2.67	A group of proliferating alveolar type II cells in the corner of an alveolar space (A). Their cytoplasm contains a variety of osmiophilic bodies ranging from large vacuolated types (m) typical of mature cells, to small irregular bodies (S) with a dense central core. x 9,000.	82
2.68	Partially aerated alveoli (A) from an area of resolving chronic pneumonia. A undifferentiated cuboidal epithelial cell (U) is separated by desmosomes (d) from neighbouring alveolar type I cells but lacks the features characteristic of a fully differentiated type II cell. x 9,000.	82
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.	82
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.	82
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	82
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	82
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.	82
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.	82

Figure		following page
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.	98
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.	98
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.	99
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.	99
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. Tb3F x 250.	99
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.	99
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.	99
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.	99
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.	100
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.	100

Figure		following page
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.	100
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.	100
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.	100
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.	100
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).	101
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).	101
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.	102
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.	102

Figure		following page
3.19	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.	102
3.20	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.	102
3.21	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. 13,000.	102
3.22	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). 12,000.	102
3.23	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.	103
3.24	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.	103
3.25	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.	103
3.26	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.	103
3.27	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.	104

Figure		following page
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.	104
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.	109
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.	109
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.	109
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).	109
3.33	Gross lung lesions following systemic paraquat administration and endobronchial bacterial inoculation.	110
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.	110
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	110
3.36	Severe epithelial hyperplasia with alveolar epithelialization at the margin of a necrotic area induced by the inoculation of <u>P. haemolytica</u> culture 6 days previously (no Paraquat administered). HE x 100.	110
4.1	Colonies of <u>Mycoplasma arginini</u> (centred) and <u>Mycoplasma ovipneumoniae</u> (centreless) in oblique transmitted light. x 45.	135

Figure		following page
4.2	Distribution of titres of <u>M. ovipneumoniae</u> in normal and pneumonic lungs.	137
4.3	Correlation of <u>M. ovipneumoniae</u> and bacterial titres with severity of histological lesions.	139
4.4	The distribution of <u>M. ovipneumoniae</u> and bacterial titres in 40 cases of subacute and chronic pneumonia.	140
5.1	Gross lung lesions following endobronchial inoculation of pneumonic lung homogenate, <u>P. haemolytica</u> or <u>M. arginini</u> .	152
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.	152
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.	152
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 <u>P. haemolytica</u> .	152
5.5	Nozzle of nebulizing gun used for intranasal inoculation	156
5.6	Method of restraint during inoculation. The forefingers were removed and the spray stopped every 3 to 5 seconds to allow exhalation.	156
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.	156
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.	157
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.	157

Figure		following page
5.10	Gross lung lesions obtained at sequential slaughter following the intranasal transmission of chronic pneumonia.	161
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.	161
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.	161
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.	161
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.	162
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.	162
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.	162
5.17	Gross lung lesions following the intranasal transmission of chronic pneumonia and administration of various chemotherapeutic agents.	168
5.18	Correlation between weight gain and severity of pneumonic lesions.	175
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 <u>M. ovipneumoniae</u> .	178
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 <u>M. ovipneumoniae</u> .	178

"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 Veter-
inary College, Edinburgh in 1865).

INTRODUCTION

Because of its geographic isolation New Zealand has been fortunate in escaping two of the world's major respiratory diseases of sheep; pulmonary adenomatosis (Jaagsiekte) and progressive interstitial pneumonia (Maedi). Perhaps it is because of the absence of these serious diseases that the respiratory diseases which are endemic in sheep in New Zealand have not in the past received the attention from veterinary scientists which they undoubtedly deserve. The first record of pneumonia in sheep in this country was in 1900 when J. A. Gilruth noted the occurrence of suppurative pneumonia and pleurisy in sheep slaughtered at freezing works. He also recorded outbreaks of a "virulent" form of the disease which occurred in lambs soon after dipping and was able to isolate a Gram-negative, aerobic bacillus from pus collected from these outbreaks. Although occasional references to the disease were made in the Annual Reports of the New Zealand Department of Agriculture thereafter it was more than 50 years before further significant information was added to Gilruth's early observations.

Clinical and Epidemiological Features of Pneumonia in Sheep in New Zealand

Pneumonia in sheep in this country has two distinct clinical and epidemiological forms. The first form will be referred to here simply as acute pneumonia because of its rapid onset and short duration usually resulting in death within 12 hours following the appearance of symptoms. Outbreaks of acute pneumonia in New Zealand have been investigated by Salisbury (1957) who found some flocks in Otago and Southland with a mortality rate of up to 8%. Sheep of all ages were affected although Salisbury believed that the disease occurred mainly in adult sheep that were 3 years and older. He noted that the first obvious indication that something was wrong was that a ewe would separate out from the mob and seek shelter. On examination of the sheep there was usually a mucoid nasal discharge, increased body temperature and respiratory rate together with very shallow respirations. This form of the disease is often associated with predisposing environmental stress factors such as bad weather, shearing, dipping or transportation.

The second common form of pneumonia in sheep in New Zealand is subacute and chronic. It is confined to lambs aged 3 to 10 months and for this reason it is often referred to as "hogget pneumonia". Although usually proliferative in nature the disease differs from maedi, Montana progressive pneumonia and other exotic proliferative pneumonias in being non-progressive since resolution invariably occurs during the late autumn months. Affected animals usually exhibit few clinical signs although in severe cases there may be loss of condition or coughing especially following exercise. Little factual information is available on the prevalence of the disease but reports from Animal Health Division veterinarians indicate that it is widespread throughout New Zealand and may sometimes affect up to 70 to 80% of some groups of lambs being slaughtered during the March-April-May period (Davis, 1970; Smith, 1970). Despite this high morbidity, mortalities attributable directly to the disease are low although on some severely affected properties they have been estimated at up to 15% in bad seasons (Smith, 1970).

The Economic Importance of the Disease in New Zealand

Economic loss due to acute pneumonia is thought to be high because the disease is nearly always fatal. Treatment of individual animals is often ineffective because the disease has a short duration and is not usually diagnosed early enough for effective treatment to be implemented. Treatment on a whole flock basis is usually too costly to be economically acceptable. In a survey of 9 properties in Hawke's Bay, Davis (1974) ranked pneumonia as the second most common cause of death being responsible for 9.5% of all mortalities. However, this included cases of chronic pneumonia in hoggets which occurred from January to April and caused death through complication by secondary bacterial infection. Similar records of sheep mortalities from a King Country farm rank pneumonia as the third most common cause of death being responsible for 10% of all mortalities (Pyke, 1974). While these figures should be viewed with caution because of lack of full laboratory corroboration in these surveys it is apparent nevertheless that acute pneumonia is a serious cause of economic loss to the farming industry in New Zealand.

The importance of subacute and chronic pneumonia as a cause of economic loss is more difficult to assess. Many field veterinarians are of the opinion that the disease is associated with poor growth rates but

the presence of concurrent disease problems during the late summer and autumn months such as facial eczema (sporodesmin toxicity), internal parasites and malnutrition due to drought conditions make the effect of chronic pneumonia on weight gain difficult to measure on a flock basis. It is not uncommon for veterinarians to necropsy individual lambs during the autumn months which have more than 50% of their lung capacity involved in a pneumonic process but lack other obvious pathological findings. This experience has been responsible for the commonly held belief that extensive pneumonias of this type result in inefficient food conversion and slow growth rate.

The effect of residual pneumonic lesions such as chronic pleuritis and pulmonary or pleural abscesses on meat production can be more accurately evaluated. During the 1973 killing season the total condemnation rates for pleurisy in New Zealand meatworks were 0.02% for lambs and 0.4% for sheep (McNab, 1974). At the scheduled prices operating in that year the estimated annual loss to the farming industry would be between 0.5 and 1 million dollars.

Approach to the Problem

Conventional methods of investigation into the problem of sheep pneumonia have usually involved the isolation and characterization of an infectious agent or agents from pneumonic lungs followed by inoculation of these agents into the respiratory tract of susceptible sheep by various routes. In these investigations the emphasis has been placed heavily on the suspected aetiological micro-organisms. Some authors have conceded the possibility of multiple aetiology by using combinations of micro-organisms such as viruses plus bacteria, chlamydia plus bacteria or mycoplasmas plus bacteria but their attempts at reproducing exactly the lesions of ovine pneumonia have been largely disappointing.

The aim of the present work was to obtain information on the sequence of events and processes which give rise to the characteristic lesions of pneumonia in sheep. In order to do this a two-pronged approach to the problem was necessary, one series of investigations concentrating on the morphological aspects of pulmonary injury and the

other on the microbiological aspects. Accordingly, this thesis is divided into two parts. The first deals with the reaction of the ovine alveolus to injury both in the naturally-occurring disease and in experimentally-induced pulmonary damage. Observations were confined mainly to the pulmonary alveolus because it was considered to be in this area that the basis of the events which gave rise to the pneumonic process occurred. At the same time, the importance of the bronchial tree, vascular and lymphatic systems in influencing the course of the disease were realised but detailed examinations of these regions were considered to be beyond the range of the present investigations.

The second section deals with the infectious agents with which the disease in New Zealand is associated and seeks to examine their distribution in the respiratory tract and relate them to the pathological changes which occur in the lung. Attempts to transmit the disease using lung extracts and microbial agents were also carried out and are reported in this section.

Also included in the thesis are reviews of the literature relevant to both ovine pneumonia and experimental alveolar injury in general. The coverage of this latter topic has of necessity been superficial because of the wide range of information available. However, its inclusion was considered useful as a background against which the current investigations can be viewed.

CHAPTER 1

GENERAL REVIEW OF LITERATURE

SECTION I: THE PATHOLOGY OF NATURALLY-OCCURRING SHEEP PNEUMONIAS

The descriptions of pneumonia in sheep which are present in the literature show a confusion in terminology which makes their understanding and pathological classification difficult. Part of the problem is due to the use of a variety of names for pneumonias which in many cases have similar gross and histological features. This often appears to have arisen when parochial and geographic names have been applied to outbreaks of respiratory disease which have caused spectacular economic losses in certain areas (e.g. Southland pneumonia in New Zealand). In other cases collective terms such as "Laikipia lung disease", have been used to describe respiratory disease in an area where a number of conditions are endemic and occur simultaneously (Shirlaw, 1956; Wandera, 1967).

The lack of understanding of the complex aetiology and pathogenesis of pneumonia is another important factor at the root of the problem of pathological classification. Thus many pneumonias recorded as caused by certain epidemiological or aetiological events may in fact have had a more complex cause involving several inter-relating factors. Hore (1970) has reviewed pneumonia in sheep with the emphasis on aetiology and Stevenson (1969) in a more comprehensive review, has listed a large number of aetiological agents and described the main pathological changes with which they have been associated. On this simple basis neither of these authors has been able to convincingly classify a noteworthy proportion of the pneumonias which have been recorded in the literature.

A further complication in attempting to classify sheep pneumonias is the possibility that some of the pneumonic conditions reported as separate diseases may merely be different stages of development of the same pathological and aetiological process.

Despite these shortcomings the conventional systems of classification and terminology remain the best for the purposes of this discussion.

Enzootic Pneumonia

Montgomerie et al. (1938) were the first to use this term to describe an outbreak of acute pneumonia in adult sheep in Wales and since then it has been used by various authors (Salisbury, 1957; Downey, 1957; Stevens, 1957; Van der Veen and Zumpt, 1967) to describe outbreaks of pneumonia in sheep of all ages. Before 1938, several outbreaks of sufficiently similar nature to be included in this category had been described by Jowett (1930), Dungal (1931) and Leyshon (1932).

The gross pathological lesions described as characteristic of enzootic pneumonia are varying degrees of red or grey consolidation of the apical, cardiac and diaphragmatic lobes of one or both lungs. Fibrinous exudate may cover the pleura and fibrous adhesions to the thoracic wall may occasionally be present (Montgomerie et al., 1938; Salisbury, 1957; Shirlaw, 1959).

The histological appearance of affected lungs is one of severe hyperaemia of the capillaries with serous effusion and haemorrhages into many alveoli. In some areas alveoli are densely packed with elongated cells situated within a structureless exudate while other areas show extensive haemorrhagic necrosis. Immediately surrounding these areas are alveoli filled with large macrophages. Small focal areas of neutrophil infiltration are occasionally present, but such areas of suppuration are never large (Stamp and Nisbet, 1963).

Pasteurella haemolytica has been associated with the majority of reported outbreaks of the disease and numerous gram-negative cocco-bacillary bacteria have been demonstrated within the affected areas of lung (Salisbury, 1957; Stamp and Nisbet, 1963). However, both Montgomerie et al. (1938) and Salisbury (1957) have suggested that the disease is primarily a viral infection with P. haemolytica as a secondary invader although neither Montgomerie et al. (1938) nor Downey (1957) were able to demonstrate viral aetiology.

A further example of a pneumonia of this same pathological type has been reported by McGowan et al. (1957). These authors investigated an outbreak in lambs in California in which 500 were considered to have died

from pneumonia and 96 were studied pathologically. Severe congestion of the lungs and fibrinous pleuritis were common gross findings at necropsy. There was inflammatory oedema of alveoli in which macrophages and neutrophils were present. A variety of bacteria including Pastuerella multocida, Corynebacterium pyogenes, Pseudomonas sp., Staphylococcus sp., and Streptococcus sp. were cultured. Stevenson (1969) has disregarded this reported outbreak of pneumonia when discussing enzootic pneumonia in his review of sheep respiratory diseases, presumably because of the authors' failure to isolate P. haemolytica. Nevertheless, striking similarities exist between the pathological descriptions of McGowan et al. and those of authors such as Montgomerie et al. (1938), Salisbury (1957) and Shirlaw (1959).

Included in McGowan et al.'s paper are observations by these authors on the incidence of pneumonia in fat lambs at slaughter. Depressed rhomboid and linear areas of red consolidation characterised microscopically by collapse and peribronchial lymphoid hyperplasia are described. Stevenson (1969) considered that the lesions seen by McGowan et al. in abattoir lambs were similar to those of atypical pneumonia described subsequently by Stamp and Nisbet (1963).

Apart from some very early reports (Bennet, 1836; Bruce, 1868; Sjogren, 1933) there has been surprisingly little written on pneumonia in sheep in Australia until quite recently. Seddon (1967) carried out surveys on the incidence of pneumonia at a Melbourne abattoir and reported a prevalence of 16.7% in lambs and 11.2% in other sheep but attributed all the lesions observed to lungworm infestation. The first authentic report of enzootic pneumonia is that of St. George (1972) who examined material from a diagnostic laboratory as well as observing the incidence of the disease on 4 farms in south-eastern Australia. A high morbidity was found in sheep aged 6-12 months but the disease was not restricted to sheep of this age group. The lesions consisted of consolidation of whole or part of the apical, cardiac and diaphragmatic lobes which varied in colour from reddish purple to pale grey. The microscopic changes were also variable ranging from haemorrhage and oedema to catarrhal or "necrotic" pneumonia. Areas of collapse, proliferation of the alveolar epithelium and lymphoid hyperplasia were seen but found to vary considerably within and between lungs. Severe proliferative and interstitial changes of the type described in atypical pneumonia (Stamp and Nisbet, 1963), Montana progressive pneumonia (Marsh, 1923) or Graaf-Reinet disease (de Kock, 1929)

were observed.

The situation in Australia has been clarified by the recent review by St. George and Sullivan (1973). These authors have listed 3 primary non-bacterial agents; Mycoplasma ovipneumoniae, parainfluenza-3 virus and a Chlamydia sp. which they have suggested as causes of specific subclinical pneumonias of sheep in Australia. They have postulated that these organisms have a role in the aetiology of the common enzootic, sub-acute or chronic pneumonia ("Summer pneumonia") which may have a morbidity rate up to 100% in some Australian flocks. This disease is manifest grossly by variable red-grey consolidation of the ventral portions of the lungs particularly the right apical lobe. Microscopically there is a combination of both proliferative and exudative reactions. Interstitial proliferation of septal cells, desquamation of macrophages and peribronchial lymphoid hyperplasia all occur, together with proliferative changes in the epithelium of small bronchioles and alveoli. Suppurative changes are superimposed on this reaction and neutrophils are present in clumps in bronchioles and scattered throughout collapsed alveoli (Sullivan et al., 1973a). Although St. George (1972) previously found no resemblance between the pneumonia in South Australian or Victorian sheep and the atypical pneumonia described by Stamp and Nisbet (1963), Sullivan et al. (1973a) have commented that the pneumonia observed by them in Queensland sheep ("Summer pneumonia") is histologically similar to atypical pneumonia.

Within the enzootic pneumonia complex in Australia St. George and Sullivan (1973) have included an acute exudative pneumonia which occurs in outbreaks of sudden onset and high mortality. P. multocida and P. haemolytica are usually isolated and the gross and microscopic features closely resemble the original acute enzootic pneumonia described by Montgomerie (1938).

Atypical Pneumonia

However inappropriate this term may be as a name for a group of respiratory diseases its usage now appears to be firmly established in the veterinary literature. It was first applied to respiratory diseases in sheep by Stamp and Nisbet (1963) but presumably had its origins in human medicine. Here it was used originally as a clinical term in the

1930's (Scadding, 1937). It described an insidious pneumonia with variable symptomatology which subsequently proved resistant to both sulphonamides and penicillin (Stuart-Harris, 1950). More recently the human disease has been found to be associated with Mycoplasma pneumoniae infection. The lesions of human atypical pneumonia are non-specific, ranging from pulmonary oedema and hyaline membrane formation to focal interstitial infiltration by lymphocytes and plasma cells (Spencer, 1968).

The disease described in sheep in Scotland by Stamp and Nisbet (1963) also shows an extensive interstitial involvement in the inflammatory reaction. Four basic types of tissue reaction are seen in the disease described by these authors:- a) alveolar epithelialization, b) interstitial cellular infiltration, c) bronchial and vascular lymphoid cuffing, d) macrophage infiltration of the alveoli. In early cases infiltration of the alveolar septa with lymphocytes and macrophages and of the alveolar spaces with large macrophages are predominant changes. In more advanced cases peribronchial and peribronchiolar cuffing with lymphocytes, plasma cells and "reticulo-endothelial cells" may be present. Nodular scars of fibrous tissue which often have a hyaline or myxomatous nature are often found in the walls of chronically inflamed bronchi and bronchioles, and focal alveolar epithelialisation is present in the vicinity of chronically affected bronchioles. In a small percentage of cases lymphoid hyperplasia occurs alone without interstitial involvement.

The macroscopic appearance of the lesions seen in atypical pneumonia varies from dark red or grey areas of consolidation to narrow branching bands of collapse in the anterior lobes of both lungs. In an outbreak observed in Scotland by Gilmour and Brotherston (1963) high temperatures and increased respiratory rates were commonly recorded. However, other clinical signs were mild and easily missed and these authors were of the opinion that in the absence of secondary infection the disease caused no appreciable change in the general condition of the sheep.

Although the aetiology of atypical pneumonia of sheep is unknown, Stamp and Nisbet (1963) noted that the lesions they described resembled those found in sheep inoculated with an organism of the chlamydia group (Dungworth and Cordy, 1962). Stevenson (1969) and Stevenson and Robinson (1970) have shown that lesions resembling those of atypical pneumonia are present during the stage of resolution of pneumonia produced in lambs by

the inoculation of either parainfluenza-3 virus or chlamydia organisms. Stevenson (1969) has concluded therefore, that atypical pneumonia of sheep is not a specific aetiological entity.

Montana Progressive Pneumonia

The original description of this disease was by Marsh (1923) who observed that up to 10% of sheep in flocks of all ages in the State of Montana were affected with a progressive pneumonia characterised clinically by laboured breathing and nasal exudation. The disease showed no seasonal incidence and had a mortality rate of 2% in sheep older than 4 years.

At necropsy, Marsh found that the lungs usually failed to collapse on opening the thorax. There was dull red to grey consolidation of the apical and cardiac lobes often with involvement of parts of the diaphragmatic lobes. Some cases however, showed a patchy consolidation of the entire lungs and pleuritis often with adhesions was frequently present.

Marsh (1923) considered the disease to be primarily an interstitial pneumonia with peribronchial, perivascular and focal lymphoid infiltrations together with macrophage accumulations in many alveoli. Later, Cowdry and Marsh (1927) reported the presence of alveolar epithelialization in isolated foci in some, but not all of the lungs they examined. They felt that these adenomatous foci were secondary to the infiltrative and exudative changes and compared the adenomatous lesions with those seen in jaagsiekte (pulmonary adenomatosis) in which the proliferations were more severe and extensive.

In the literature various authors have likened Montana progressive pneumonia to pulmonary adenomatosis (Cowdry and Marsh, 1927), Graaf-Reinet disease, Laikipia lung disease and maedi (Duran-Reynals et al., 1958). Although the classification of the disease remains uncertain, Stevenson (1969) had the opportunity to examine four microscopic sections of lung from the disease and found histological evidence suggestive of maedi in at least two of the sections. His observations add support to the work of Kennedy et al. (1968) who reported the isolation of a maedi-like virus from the lungs of sheep affected with progressive pneumonia in Montana.

Maedi

The name "maedi" was derived from the Icelandic term meaning dyspnoea since this disease was prevalent in sheep in Iceland between 1939 and 1942 and caused heavy stock losses. In 1953, Sigurdsson et al. reported transmission of the disease and since then evidence has accumulated which indicates that the disease is caused by a virus related antigenically to the visna virus (Sigurdardóttir and Thormar, 1964; Thormar, 1965; Thormar and Helgadóttir, 1965).

The disease is a slow progressive pneumonia with a fatal termination. The clinical and pathological characteristics have been clearly defined by Sigurdsson et al. (1952, 1953) and Georgsson and Pálsson (1971). Grossly the lungs are grey-blue in colour, have a uniform rubbery consistency and are heavier than normal. The primary microscopic lesion appears to be an interstitial proliferation of mesenchymal cells resembling lymphocytes and reticulum cells. In addition large numbers of macrophages may fill the alveolar spaces and there is hypertrophy of the smooth muscle tissue of alveolar ducts. Sometimes the lymphoid accumulations may be present as nodules within the septal tissue or in peribronchial and perivascular locations. Intracytoplasmic inclusion bodies are sometimes seen within large mononuclear cells in Giemsa-stained smears of affected lung but their significance is unknown. Affected sheep may succumb to a superimposed acute pneumonia of the purulent type before the disease has run its full course.

A disease of very similar nature to maedi also exists in Holland where it is known as "zwoegerziekte" (Ressang et al., 1966) and as already mentioned there is some evidence that progressive pneumonia in Montana and Graaf-Reinet disease in South Africa are also related to this condition.

Pulmonary Adenomatosis (Jaagsiekte)

This respiratory disease is widespread in South Africa (Cowdry and Marsh, 1927; Tustin, 1969), India (Rajya and Singh, 1964), the Middle East (Nobel, 1958), Europe (Nieberle and Cohrs, 1967), Great Britain (Stamp and Nisbet, 1963; Markson and Terlecki, 1964) and some parts of South America (Cuba-Caparo, 1961). Tustin (1969) and Wandera (1971) have both written comprehensive reviews on its geographic distribution, epidemiology and pathology.

Confusion over the exact nature of pulmonary adenomatosis has persisted until recently. One of the earliest reports (Robertson, 1904) describes the disease as a chronic catarrhal pneumonia and Cowdry and Marsh (1927) considered it to be the same as Montana progressive pneumonia although they recognised that the alveolar epithelial proliferation in jaagsiekte was more marked. Much confusion has arisen because of the disease's long incubation period of up to 12 months (Dungal, 1946; Wandera, 1971) and its frequent complication by other respiratory diseases such as maedi and secondary bacterial pneumonia.

It is thought that Eber (1899) was the first to recognise the disease as a neoplastic condition when he found adenomatous foci originating from alveolar ducts and bronchioles in the lungs of sheep in Germany. Since then numerous authors have observed metastases in both pulmonary lymph nodes and extra-thoracic locations and these have been historically tabulated by Wandera (1971). The disease is now well established as a transmissible lung carcinoma and several workers have succeeded in reproducing typical lesions by intratracheal or intrapulmonary inoculation of adenomatous tissue (Dungal, 1938; Sigrurdsson, 1958; Shirlaw, 1959; Markson and Terlecki, 1964; Tustin, 1969; Wandera, 1970).

Although the aetiological agent has not yet been identified MacKay (1969) and Malmquist et al. (1972) have studied a herpes-like agent which has been associated with the disease and Tustin and Geyer (1971) have succeeded in transmitting the disease in South Africa using tissue cultures of neoplastic cells. Virus-like particles have also been observed by Wandera (1971) who thought it unlikely that they were of any significance. Both mycoplasmas (MacKay et al., 1963; MacKay and Nisbet, 1966) and chlamydia (Wandera, 1971) have been found in the lesions but it is not likely that these have any role in the aetiology of the disease.

Affected lungs initially contain nodules 2-3 cm in diameter which project slightly above the pleural surface of the lung. These nodules increase slowly in size by invading the lung tissue until they form extensive areas of greyish-white consolidation usually in the ventral two thirds of the lungs (Markson and Terlecki, 1964). The essential lesion consists histologically of focal papillary ingrowths of cuboidal epithelium into groups of alveoli. This gives rise to adenomatous nodules which may spread throughout the lung or appear in some cases to arise

multicentrically. Secondary infection may cause macrophage exudation or abscess formation and interstitial fibrous tissue may form between affected alveoli in older lesions (Stamp and Nisbet, 1963).

Wandera and Krauss (1971) and Nisbet et al. (1971) have recently studied the ultrastructure of pulmonary adenomatosis and both have observed cuboidal and columnar cells with a similar ultrastructural appearance to those seen in some carcinomas of the lung in man. Wandera and Krauss concluded that most tumour cells originate from the alveolar epithelium which is transformed as the result of infection with an aetiological agent. Their observation tended to be confirmed by Nisbet et al. (1971) who showed that the cell type forming the adenomatous tissue appeared to be derived from the type II alveolar cells whereas those involved in the less common intrabronchiolar proliferations resembled Clara cells of the normal terminal bronchiole.

Miscellaneous Sheep Pneumonias

Among the reports of pulmonary diseases in sheep are several non-specific terms that have been derived from place names or native languages. The term "Laikipia lung disease" has been applied to a pneumonic condition in sheep in the Laikipia district of Kenya (Whitworth, 1926; Mettam, 1927, 1929) but Wandera (1967) has subsequently shown that at least 4 distinct respiratory diseases, including maedi and jaagsiekte are prevalent in this area and therefore considered the term obsolete. In the same way, "bouhite" was a term used to describe a chronic respiratory condition occurring in sheep in the Landes province of France in which more than one disease, including jaagsiekte appears to be involved (Lucam, 1942). Graaf-Reinet disease was also a local term used by South African farmers for a progressive interstitial pneumonia described by de Kock (1929) but since found to be pathologically similar to maedi (Wandera, 1970).

Two other pathological entities have been briefly reported in the literature. Wandera (1967) has used the term "peribronchial proliferative pneumonia" for a pneumonia of this histological type which occurs in sheep in Kenya. The aetiology is unknown although chlamydia have been suggested as a possible cause. Stevenson (1969) described a "moderate epithelializing pneumonia" (MEP) characterised by areas of dark red, firm

consolidation in the anterior lobes with a definite border between affected and normal tissue. The main histological lesions were hyperplasia of the alveolar and bronchiolar epithelium and surrounding reticulum network together with macrophage infiltration of alveoli. Although Stevenson has made a clear differentiation between MEP and atypical pneumonia the differences described are not great and appear to consist mainly of the absence of peribronchial lymphoid hyperplasia and the presence of a milder degree of proliferative change in the former condition.

Also present in the literature is a report of 4 outbreaks of atypical interstitial pneumonia (acute pulmonary emphysema) which occurred in sheep in Australia (Pascoe and McGavin, 1969). The disease occurred in southeast Queensland in sheep grazing the stubble of wheat, barley and canary grass (Phalaris canariensis) and appeared to be clinically and pathologically identical to bovine atypical interstitial pneumonia (Bennell, 1966; Jubb and Kennedy, 1970). Oedema, alveolar emphysema, hyaline membrane formation, alveolar epithelialization and bronchiolar epithelial proliferation were prominent microscopic findings.

SECTION II: INFECTIOUS AGENTS ASSOCIATED WITH PNEUMONIA IN SHEEP

The most striking feature which emerges from the literature on the infectious agents associated with pneumonia in sheep is the paucity of agents which have been shown to be capable of producing pneumonia in this species experimentally. Although success has been claimed for certain agents by some authors, results have not been consistent and the lesions produced have often had little resemblance to the pneumonia from which the organism was originally isolated. Some of the attempts which have been made to produce pneumonia in sheep are listed in Table I.

Up until the present time pneumonia in sheep has not been as fully investigated as have the respiratory diseases in other more intensively reared species such as pigs and cattle. Nevertheless a wide range of micro-organisms has been isolated from pneumonic lungs in sheep. The most complete list of these agents which has been compiled to date is that of Stevenson (1969) who lists 11 species of bacteria, 6 viruses and 5 species of fungi. It is obvious that a large number of these agents are likely to be secondary invaders which are only present under certain as yet ill-defined conditions. This section of the literature review will therefore concentrate on those agents for which there is some evidence of a possible role in the pathogenesis of respiratory disease in sheep.

Pasteurella haemolytica

There are many reports in the earlier literature which incriminate Pasteurella-like organisms as the causative agent of pneumonia in sheep (Spray, 1923; Dungal, 1931; Leyshon, 1932; Creech and Gochenour, 1936; Campbell et al., 1949). Because of the poor characterization of the Pasteurella spp. these earlier workers experienced problems in differentiating members of the Pasteurella group and many reports refer simply to Pasteurella-like organisms or even to gram-negative, bipolar-staining, short rods.

An organism first described as Pasteurella haemolytica was isolated from cases of acute pneumonia by Newsom and Cross (1932). These workers distinguished two separate strains of Pasteurella on the basis of haemolysis of rabbit blood agar, indole production and pathogenicity to

TABLE I
ATTEMPTS AT THE PRODUCTION OF PNEUMONIA IN SHEEP WITH MICROBIAL
AGENTS

Author	Agent	Route Inoculated	Age	Result	Affected/ Unaffected
Dungal (1931)	<u>Pasteurella haemolytica</u>	i/t	2-6 yrs	Acute pneumonia	7/7
		i/pm	adult	Pneumonia & septicaemia	3/3
Henning & Brown (1936)	<u>Pasteurella sp.</u>	i/v	"	Septicaemia	4/6
		i/t	"	Negative	
Hopkirk (1936)	<u>Pasteurella- like organism</u>	i/v	-	Septicaemia	1/1
		i/t	-	Negative	
<u>Montgomerie et al.</u> (1938)	<u>P. haemolytica</u>	i/pm	adult	Negative	
		i/v	"	"	
		i/t	"	"	
		i/n	"	"	
Salisbury (1957)	<u>P. haemolytica</u>	i/pm	all ages	Negative	
		i/t	"	"	
		i/n	"	"	
Downey (1957)	<u>P. haemolytica</u>	i/v)	adult	Negative	
		i/t)			
Hamdy & Pounden (1959)	<u>P. haemolytica</u>	i/t)	6 wks	Negative	
		i/n)			
Smith (1960a)	<u>P. haemolytica</u> Type A	i/v	3 wks	Peritonitis & pneumonia	7/12
		i/t	"	Pneumonia & septicaemia	3/3
		i/v	1-2 yrs	Negative	
Smith (1960b)	<u>P. haemolytica</u> Type T	i/v	7 mths	Septicaemia	-
Smith (1964)	<u>P. haemolytica</u> Type A	i/t	adult	Negative	
		i/b	"	"	
		i/b	"	Acute pneumonia	10/12
<u>Biberstein et al.</u> (1967)	<u>P. haemolytica</u> Type A	i/b	2-3 yrs	Focal pneumonia	2/3
	Type T	i/b	"	Focal pneumonia	3/17

cont'd over ...

TABLE I (cont'd)

Author	Agent	Route Inoculated	Age	Result	Affected/ Unaffected
Hamdy & Pounden (1959)	<u>P. multocida</u>	i/v) i/p)	6 wks	Septicaemia	1/2
Biberstein et al. (1967)	<u>P. multocida</u>	i/b	2-3 yrs	Focal pneumonia	2/3
Biberstein et al. (1967)	<u>Staphylococcus aureus</u>	i/b	"	Focal pneumonia	2/2
Biberstein et al. (1967)	<u>E. coli</u>	i/b	"	Negative	
Biberstein et al. (1967)	<u>Streptococcus viridans</u>	i/b	"	"	
Grieg (1955)	<u>Mycoplasma</u> sp.	i/v i/p	4-5 mths "	Negative "	
Durusan & Doguer (1956)	<u>Mycoplasma</u> sp.	-	-	Negative	
Boidin et al. (1958)	<u>Mycoplasma</u> sp.	i/v i/t	4 mths "	Negative "	
Hamdy & Pounden (1959)	<u>Mycoplasma</u> sp.	i/v) i/t) i/n)	8 wks	Negative	
Farzaliev et al. (1962)	<u>Mycoplasma</u> sp.	i/n) i/p)	young lambs	Acute septicaemia	-
St. George et al. (1971)	<u>M. ovipneumoniae</u>	i/t) aerosol)	3-28 days	Pneumonia	4/5
Sullivan et al. (1973b)	<u>M. ovipneumoniae</u>	i/v) aerosol)	9-56 days	Interstitial pneumonia	4/4
McKercher (1952)	<u>Chlamydia</u> sp.	i/t) i/n)	9 mths	Pneumonia	1/1
Boidin et al. (1958)	<u>Chlamydia</u> sp.	i/t	6 mths	Pneumonia	12/12
Hamdy & Pounden (1959)	<u>Chlamydia</u> sp.	i/v) i/t) i/n)	4-10 wks	Pneumonia	1/6
Dungworth & Cordy (1962a)	<u>Chlamydia</u> sp. (sheep pneumonitis strain)	i/t aerosol	5-6 mths "	Pneumonia "	- 3/3
Dungworth & Cordy (1962b)	<u>Chlamydia</u> sp. (faecal strain)	i/t	5-6 mths	Pneumonia	6/6
	<u>Chlamydia</u> sp. (enzootic abortion strain)	i/t	"	"	4/4

cont'd over ...

TABLE I (cont'd)

Author	Agent	Route Inoculated	Age	Result	Affected/ Unaffected
Romváry <u>et al.</u> (1962)	<u>Chlamydia</u> sp. (sheep pneumonitis strain)	i/t i/t	6 wks lambs & ewes	Pneumonia "	1/1 -
Stevenson & Robinson (1970)	<u>Chlamydia</u> sp. (sheep pneumonitis strain)	i/t	1-8 days (colostrum deprived)	Pneumonia	4/4
Hore & Stevenson (1967)	Parainfluenza-3 virus	i/t i/n)	1 day (colostrum deprived) 1 day (colostrum fed)	Pneumonia Pneumonia	9/9 4/4
Gilmour <u>et al.</u> (1968)	Parainfluenza-3 virus	i/n	7-9 mths (vaccinated and unvaccinated)	Negative	37/37
Hore & Stevenson (1969)	Parainfluenza-3 virus	i/t	1 day (colostrum deprived)	Pneumonia	15/15
Stevenson & Hore (1970)	Parainfluenza-3 virus (cattle strain)	i/t	1 day (colostrum deprived)	Pneumonia	5/5

Key:- i/t = intratracheal i/n = intranasal
 i/v = intravenous i/p = intraperitoneal
 i/b = intrabronchial i/pm = intrapulmonary

rabbits (Table II). Despite the criteria outlined by these authors confusion regarding classification continued. Montgomerie et al. (1938) examined biochemically 24 slightly haemolytic strains and although acid production from a variety of sugars is recorded their growth on MacConkey's medium and the indole reaction are not stated. Stamp et al. (1955) also reported acid production from a variety of sugars on all but three of the strains they examined. The indole reaction was negative and there was fair growth on MacConkey but no mention was made of haemolysis. Rosenbusch and Merchant (1939) examined 13 strains of a Pasteurella-like organism isolated from sheep. All were haemolytic on blood agar but 3 were indole-negative and non-pathogenic for mice and 10 were indole-positive and pathogenic for mice. The situation at that time was fully reviewed by Shirlaw (1959) who elaborated on the variability of the pathogenicity of the organisms isolated by various workers.

In New Zealand, Hopkirk (1936) was first to report the organism in sheep lungs. He observed a large number of cases of ovine pneumonia during the summer months and recovered a haemolytic organism from almost every case. Salisbury (1957) later isolated a haemolytic Pasteurella-like organism from outbreaks of pneumonia in Southland and described numerous gram-negative cocco-bacilli within the alveoli in histological sections of affected lungs.

In 1953, Marsh reviewed the role of Pasteurella spp. in sheep diseases and concluded that the organisms were the cause of pneumonia in young lambs as well as being the principle cause of shipping fever in sheep. This view was upheld by Stevens (1957) who thought that it was unlikely that the primary cause of enzootic pneumonia was a virus or other organism because if this were the case the proportion of the isolations of P. haemolytica from this condition might be expected to be lower and the flora of the infected lung more varied.

There was however, a growing body of opinion which held that P. haemolytica was not a primary pathogen in the lung and that invasion was only able to occur following damage by some other agent or agents (Montgomerie, 1938; Miller, 1940; Morcos et al., 1946; Salisbury, 1957; Downey, 1957). Further evidence in support of this view came with the discovery by Bosworth and Lovell (1944) of a Pasteurella-like organism in the nasal cavity of normal sheep and in the lower portion of the trachea

of healthy sheep examined immediately after slaughter. These findings were however, contrary to the work of Newson and Cross (1932) who had found the organism in only one bronchial scraping in 92 examined from healthy sheep at slaughter, and to the work of Stevens (1957) who found the organism in the trachea of only one of 18 sheep which had died from diseases other than enzootic pneumonia.

Confirmation of Bosworth and Lovell's observations on the nasal carriage of P. haemolytica came from the work of Campbell et al. (1949) and later from Hamdy et al. (1959). This prompted a more extensive investigation by Biberstein and Thompson (1966) into the nasal carriage of P. haemolytica in normal sheep and those exposed to pneumonia. It was shown that normal flocks tended to have a relatively low incidence of P. haemolytica and a wide range of serotypes, whereas flocks with pneumonia had a higher incidence of the organism and a predominance of one or a very few serotypes. Subsequent work by Biberstein et al. (1970) has shown that the peak rates of nasal carriage in sheep in Scotland are in late autumn and late spring to early summer thus coinciding with the known seasonal pattern of enzootic pneumonia in the region.

Although it is now well established that P. haemolytica is often present in the upper respiratory tract of healthy sheep the reports of McGowan et al. (1957) and St. George (1972) appear to indicate that the organism is far from ubiquitous as a secondary invader of the ovine lung. McGowan et al. examined 50 lungs bacteriologically during a large outbreak of pneumonia in sheep in California. A variety of bacteria were cultured but P. haemolytica was not amongst those recognised by these authors. In Australia, St. George (1972) has recently examined pneumonic material from sheep which was submitted to a diagnostic laboratory in Victoria. Of the 66 lungs cultured, an organism resembling P. haemolytica was isolated from only 5 although a further 14 yielded an organism of the Pasteurella group which was not further identified.

The difficulty in producing respiratory disease experimentally with P. haemolytica is highlighted by the work of Smith (1960a, 1961a, 1964). He was able to reproduce pneumonia and septicaemia in 3 week old lambs by intra-tracheal inoculation of large doses (335×10^6 orgs/ml) of a type A (arabinose-fermenting) strain of the organism isolated from enzootic pneumonia in adult sheep. However, similar large doses administered to

adult sheep failed to have any effect. Smith (1959, 1961b) also differentiated a type T strain of P. haemolytica on the basis of colonial morphology and trehalose fermentation (Table II). This strain was found to be associated with a haemorrhagic septicaemia of older lambs described by Stamp et al. (1955) who had previously shown that a septicaemic disease could be reproduced by the intravenous inoculation of cultures. In confirming this finding, Smith (1960b) found that the lethal dose of heat-sterilized broth culture by intravenous inoculation was only a low multiple of the lethal dose of living culture.

In 1964, Smith developed a technique for culturing a high concentration of the organism by means of intraperitoneal inoculation of mice. After two preliminary experiments which yielded conflicting results he used a different strain of the organism and succeeded in producing a fatal pulmonary infection resembling an acute form of enzootic pneumonia by means of intrabronchial inoculation. The doses used however, were of the order of 6×10^{10} organisms or above and although lower doses produced some lesions these did not have the typical pathological appearance of enzootic pneumonia.

The work of Smith (1964) was later confirmed by Biberstein et al. (1967). These authors went on to study the gross and histopathological features produced by P. haemolytica type T and found them to be indistinguishable from those caused by Type A. In their experiments Biberstein et al. found that the pathological response to P. haemolytica was erratic and not clearly related to dosage, type or source of the organism. This finding was contrary to the opinions expressed earlier by both Stamp et al. (1955) and Smith (1959, 1961b) who had postulated that the two biotypes were responsible for specific disease entities. However, later work by Biberstein and Thompson (1966) revealed that 78% of the typeable strains of P. haemolytica isolated from cases of ovine pneumonia were type A and 22% were type T. Similarly, of those strains isolated from septicaemia in lambs under 3 months of age 70% were type A and 30% were type T. But in the case of septicaemia in lambs over the age of 3 months all the typeable strains isolated were T. types.

From their study of the lesions induced by the intra-tracheal inoculation of various bacteria, Biberstein et al. (1967) noted the emergence of a basic pattern. There was invariably a central area of necrosis bounded by granulation tissue and an intervening more or less

TABLE II
DIFFERENTIATION OF PASTURELLA SP.
ISOLATED FROM THE RESPIRATORY TRACT OF SHEEP (1)

	<u>P. haemolytica</u> Type A	<u>P. haemolytica</u> Type T	<u>P. multocida</u>
Colony size	Small	Large	
Colony colour	Grey	Brown centres	
Haemolysis	Narrow zone	Narrow zone	-
Indole Production	-	-	
Arabinose Fermentation		-	nt
Trehalose Fermentation	-		nt
Growth on MacConkey Agar	Slight	Slight	-
Pathogenicity to mice	-	-	
Penicillin Sensitivity		-	nt
Tetracycline Sensitivity		Sl. sensitive	

(1) After Stevens (1957) and Smith (1959)

nt Not tested

orderly stratification of inflammatory cells of varying depth. The inner zone of inflammatory cells were degenerate as shown by their elongated pyknotic nuclei, and were packed densely into alveolar spaces. There appeared to be no specificity of the lesions induced by P. haemolytica since both P. multocida and Staphylococcus aureus evoked lesions which were qualitatively identical although in some cases more extensive.

Although the experiments of Smith, and Biberstein et al. have clearly shown that P. haemolytica is capable of producing an acute pulmonary disease in sheep both authors are at a loss to explain how natural infection could be initiated as it is inconceivable that sheep in the field could encounter infecting doses of the magnitude used in these experiments.

Pasteurella multocida

Occasional reports of this organism associated with pneumonia in sheep are present in the literature (McGowan et al., 1957; Gourdon et al., 1957; Hamdy et al., 1959; Van der Veen and Zumpt, 1967). Marsh (1953) considered that P. multocida was a frequent cause of pneumonic lesions in sheep. He isolated Pasteurella sp. from 20 out of 27 pneumonic lungs in lambs and 49 out of 73 cases of progressive pneumonia but made no reference to P. haemolytica or the criteria used to identify P. multocida.

Attempts to produce pneumonia in lambs by the inoculation of P. multocida have been unsuccessful (Hamdy and Pounden, 1959). However, Biberstein et al. (1967) were able to produce a focal necrotic lesion in the lungs of two sheep with a strain of the organism which was isolated from calf pneumonia.

Mycobacteria

Bronchopneumonia is the commonest form of tuberculosis which occurs in sheep. The disease is nevertheless relatively rare although infection with M. bovis, M. avium and M. tuberculosis have all been reported (Francis, 1958). Luke (1958) reviewed the literature on the disease in sheep. He described the necropsy findings and experimental work which had up until that time been undertaken, to test the susceptibility of sheep to inoculation with the three types of organism.

Francis (1958) found a marked similarity histologically, between the lesions in sheep and cattle and the present writer was able to confirm this observation when he had the opportunity of examining a series of 42 cases of the disease in 1970. In the present writer's series, pulmonary lesions were found in 67% (28) of the cases and lesions in the alimentary tract in 64% (27). The lesions invariably took the form of heavily calcified granulomas although there was considerable variation in the degree of inflammatory response between individuals.

Pseudomonas pseudomallei (Meliodosis)

An outbreak of this disease in sheep in North Western Queensland has been described by Cottew (1952). Eighty sheep in a mob of 4,000 died and those examined showed numerous abscesses in the lung and spleen. Cottew (1955) has reviewed the literature on melioidosis in both man and animals and Laws and Hall (1964) have reported a possible case of sheep to sheep transmission in Australia.

Miscellaneous Bacteria

A variety of bacteria have been isolated from advanced cases of pneumonia in sheep but evidence for their having anything other than a secondary role is lacking. Stevenson (1969) has listed these bacteria in order of the frequency with which they have been reported in the literature.

Among the more commonly reported isolations are those of Corynebacterium pyogenes (Spray, 1923; Jowett, 1930; Marsh, 1953; Downey, 1957; Stevens, 1957; Smith, 1957; McGowan et al., 1957; Hamdy et al., 1959; Gilmour and Brotherston, 1963; Ministry of Agriculture, Fisheries and Food (M.A.F.F.) 1964; St. George, 1972). This organism is usually associated with abscessation of the lungs or pleural surface and in such cases may be recovered from sheep of all ages (Downey, 1957; Gilmour and Brotherston, 1963). Less frequently found associated with lung abscesses are Staphylococcus aureus (Smith, 1957; Hamdy et al., 1959; M.A.F.F., 1964) and Fusiformis necrophorus (Jowett, 1930; Smith, 1957; M.A.F.F., 1964).

Other bacteria occasionally reported as present in pneumonic ovine

lungs are streptococci (Jowett, 1930; Smith, 1957; Hamdy et al., 1959; M.A.F.F., 1964), Escherichia coli (M.A.F.F., 1964), Salmonella abortus-ovis (Jack, 1968), Actinobacillus lignieresii (Smith, 1957, 1960a), Haemophilus sp. (Cheema et al., 1965), Pseudomonas aeruginosa (Hamdy et al., 1959) and a Neisseria catarrhalis-like organism (Miller, 1940; Alley et al., 1970; St. George, 1972).

Mycoplasma Species

Since the earliest report of the isolation of mycoplasmas from the lungs of sheep by Grieg (1955) considerable refinements have been made in the techniques for culturing these organisms. This has resulted in an increasing frequency of their isolation from a variety of countries in the past few years. Isolations from sheep lungs have now been recorded in Turkey (Durusan and Doguer, 1955; Cottew et al., 1968), Israel (Nobel, 1958), U.S.A. (Boidin et al., 1958; Hamdy et al., 1959; Barber and Fabricant, 1962; Dungworth and Cordy, 1962), Russia (Farzarliev et al., 1962), Britain (Mackay et al., 1963; Mackay, 1966; Mackay and Nisbet, 1966), Italy (Deiana and Ceretto, 1967), Kenya (Krauss and Wandera, 1970) and Australia (Cottew, 1971; St. George et al., 1971; Carmichael et al., 1972; Sullivan et al., 1973a). Until recently however, attempts to produce pneumonia in sheep by the inoculation of Mycoplasma spp. have been largely unsuccessful (Table I).

In 1971, two different types of organism were identified in the respiratory tract of sheep in Australia (Cottew, 1971; St. George et al., 1971; Carmichael et al., 1972). The characteristics by which they have been differentiated are shown in Table III. Working in Victoria, Cottew (1971) found that the type 1 (arginine-fermenting) strain comprised 46 (68%) of the 68 isolates he examined whereas the type 2 (glucose-fermenting) strain comprised 22 (32%). In Queensland, Carmichael et al. (1972) examined swabs from nasal sinuses of sheep with and without pneumonia and found a marked difference in the isolation rates of the two types of organism. The isolation rate for the type 1 strain was relatively constant at between 42-47% of the 117 sheep examined whereas the type 2 strain was recovered from only 6% of 33 healthy sheep compared to 46-87% of 84 pneumonic sheep. At slaughter the type 2 strain was recovered from 82% of 17 bronchial washings and 15-25% of 40 samples of ground lung

TABLE III
DIFFERENTIATION OF MYCOPLASMA SPECIES ISOLATED FROM THE
RESPIRATORY TRACT OF SHEEP

	Colonial Morphology	Arginine Utilization	Glucose Fermentation	Tetrazolium Reduction	Ovine Red Cell Haemolysis
Type 1 (<u>M. arginini</u>)	Centred Large Smooth	+	-	-	-
Type 2 (<u>M. ovipneumoniae</u>)	Centreless Small Granular Surface	-	+	+	+

tissue obtained from the pneumonic flock. Further work on the type 2 strain by Furlong and Cottew (1973) has confirmed that the isolates of Cottew (1971) and St. George et al. (1971) are morphologically and serologically identical.

The consistent recovery of the type 2 strain of the organism from the pneumonic respiratory tract together with evidence of its pathogenicity supplied by St. George et al. (1971) has lead Carmicheal et al. to consider that this strain was a primary cause of sheep pneumonia and they have therefore proposed that it be named M. ovipneumoniae. The type 1 strain has been identified as M. arginini (Carmicheal et al., 1972).

A proliferative interstitial pneumonia has been found associated with M. ovipneumoniae infection in sheep in Queensland (Sullivan et al., 1973a). In the flock of sheep investigated, gross lesions consisting of small areas of collapse were detected in lambs as early as 2 weeks of age. In older lambs of 50 days of age there was red consolidation of the right apical and less often the right and left cardiac lobes. The remainder of the lung generally had a greyish appearance. The predominant microscopic lesions described were proliferation of "alveolar septal cells" and of the epithelium of the terminal bronchioles together with perivascular and peribronchial lymphoid hyperplasia. Sullivan et al. (1973b) also observed the incidence of pneumonia in a group of 22 lambs placed in contact with 4 lambs experimentally-infected with M. ovipneumoniae. The lambs were slaughtered at various intervals up to 6 weeks after exposure, and collapse or consolidation was found in the lungs of 11 of the 22 lambs. However, M. ovipneumoniae could not be recovered from the lungs of the experimentally exposed lambs.

The work of these Australian authors has therefore demonstrated a close association between M. ovipneumoniae infection and pneumonia in sheep in Australia, however conclusive evidence of the pathogenicity of the organism in the ovine lung still appears to be lacking.

Chlamydia (Bedsonia)

The agent originally isolated from the lungs of pneumonic sheep by McKercher (1952) was called a virus of the psittacosis-lymphogranuloma-pneumonitis group but organisms of this type have subsequently been

classified as bacteria or derived from bacteria and will be referred to here as chlamydia. Chlamydia sp. have now been isolated from pneumonic sheep lungs by various workers in both North America and Europe (Boidin et al., 1958; Hamdy et al., 1959; Hamdy and Sanger, 1959; Romváry, 1962; Sărateanu et al., 1965; Stevenson and Robinson, 1970). To date all reported isolations have been confined to the Northern Hemisphere and the organisms recovered appear to be closely related to the agent causing enzootic abortion in ewes which is also excreted in the faeces of normal sheep (Dungworth and Cordy, 1962b; Romváry, 1962). Dungworth and Cordy have suggested that the intestinal tract is a reservoir of the organisms and that the faeces provide a source of the organism which, under suitable conditions could be capable of causing pneumonia.

Experimental pneumonia has been produced by intra-tracheal inoculation of either the ovine faecal strain, the ovine pneumonitis strain or the ovine enzootic abortion strain and the lesions in each case were identical (Dungworth and Cordy, 1962b). A detailed description of these lesions has been recorded by Dungworth and Cordy (1962a, 1962b) working in California. Similar lesions have been described by Stevenson and Robinson (1970) using a British strain of the organism although the clinical illness in their experimental animals was more severe than that observed by the previous authors.

The gross lesions produced experimentally began as linear, dark red-brown areas of consolidation in the apical, cardiac and antero-ventral parts of the diaphragmatic lobes and reached maximum extent by the 4th or 5th day after inoculation (Dungworth and Cordy, 1962a, b; Stevenson and Robinson, 1970). One animal inoculated by Stevenson and Robinson (1970) died at this stage with severe lung lesions involving 80% of the lungs. Resolution began after the 9th day and was complete by the 30th day in most cases.

The histological lung lesions consisted of early serous and neutrophilic exudation which had usually subsided by the 9th day after infection. Epithelial hyperplasia and mesenchymal proliferation then became predominant and lymphocytes, plasma cells and macrophages began to accumulate in the peribronchial and perivascular tissues. Peribronchiolar lymphoid hyperplasia was well developed by the 20th day (Boidin et al.,

1958; Dungworth and Cordy, 1962a, b; Stevenson and Robinson, 1970). The demonstration of elementary bodies in the cytoplasm of macrophages and epithelial cells was achieved most readily in the early stages of infection (2nd-5th day) although they could also be found in the later stages by using frozen sections and the modified Ziehl-Neelsen staining technique (Stevenson and Robinson, 1970).

Despite the work of Boidin et al. (1958), Dungworth and Cordy (1962a, b) and Stevenson and Robinson (1970) the role of Chlamydia sp. in the aetiology of sheep pneumonia in the Northern Hemisphere remains uncertain. While Stevenson and Robinson have suggested that they are one of the causes of ovine atypical pneumonia further epidemiological investigations are obviously necessary in order to clarify the situation.

Parainfluenza-3 Virus

In the past few years there have been numerous reports of the isolation of Parainfluenza-3 (PI-3) virus from both man (Parrot, 1963; Zollar et al., 1973) and cattle (Jolly and Ditchfield, 1965; Omar, 1966; Burroughs, 1967; Cole, 1970). These reports together with serological and histological studies, have succeeded in establishing an active role for this organism in some outbreaks of respiratory disease in these species. The current literature on the role of the organism in sheep pneumonia however, is less illuminating.

Working independantly, Hore in Scotland and Ditchfield in Canada, made the first isolations of the organism from the ovine respiratory tract in 1966. Hore (1966) recovered his strain from the nasal cavities of lambs in an intensively managed flock but later found the organism in the lungs and nasal cavity of sheep during an outbreak of respiratory disease (Hore et al., 1968). Ditchfield (1966) isolated the organism from the nasal cavities of lambs affected with pneumonia of the "Pasteurella" type. Serological studies have now revealed that the organism may be widespread, as there is evidence of its presence in Australia (St. George and French, 1966; St. George, 1971, 1972; St. George and Liefman, 1972), North America (Fischman, 1965, 1967; Woods et al., 1965), Egypt (Singh and Ata, 1967) and France (Faye et al., 1967). The virus has also been found in the nasal cavity of Rocky Mountain bighorn sheep showing clinical

signs of respiratory disease (Parks et al., 1972) and results of haemagglutination-inhibition tests have shown that both bighorn sheep and bighorn-domestic hybrids carry antibodies against the virus (Howe et al., 1966). In New Zealand, Carter and Hunter (1970) have isolated PI-3 virus from nasal swabs of 9 out of 20, 6-month-old lambs which had a serous or purulent nasal discharge. Five of the 9 positive lambs had pneumonia at necropsy and 3 of the 11 negative lambs also had pneumonia.

Despite the widespread distribution of PI-3 virus in sheep very little information linking the virus directly with sheep pneumonia has been forthcoming. No correlation could be found between PI-3 infection as measured by a rise in serum-neutralising antibody and the appearance of microscopic and clinical pneumonia in a flock of sheep in Victoria studied by St. George and Liefman (1972).

Exposure of both colostrum-deprived and colostrum-fed lambs to experimental infections with PI-3 virus has resulted in only a mild respiratory infection. Hore and Stevenson (1967, 1969) were able to produce a slight mucoid nasal discharge with a rise in temperature and loss of appetite although some of the lambs showed coughing and respiratory distress from the 4th or 5th day following intranasal or intratracheal inoculation. At necropsy dull red areas of consolidation were found constantly in the right apical lobe and irregularly in other lobes with maximal involvement occurring between the 6th to the 8th day following inoculation.

Several outstanding histological features have been observed in both the experimental and natural infections (Hore and Stevenson, 1967, 1969; Hore et al., 1968; Stevenson and Hore, 1970). These are; interstitial pneumonia with alveolar epithelialization, necrosis and desquamation of a hyperplastic bronchiolar epithelium, acidophilic cytoplasmic inclusions in bronchial, bronchiolar and alveolar epithelial cells, and formation of epithelial giant cells in the bronchioles and alveoli.

As yet, it has not been possible to connect PI-3 virus with any of the common naturally-occurring pneumonias in sheep which have been differentiated on an anatomical basis. However, Hore and Stevenson (1969) have suggested that the propensity of the virus to damage epithelial surfaces

could allow it to be implicated, together with other agents, in some outbreaks of the presently recognised acute and subacute ovine pneumonias. Further investigation is therefore needed to give a better perspective of this organism and its possible role in respiratory disease in sheep.

Miscellaneous Viruses

Romváry et al. (1962) have made the only isolation of influenza virus (type A2) from a sheep with respiratory disease that has been reported up until the present time. These authors have also recorded the production of pneumonia of a viral character in lambs inoculated intratracheally with human and porcine strains of influenza virus. Conversely, McQueen and Davenport (1963) were unable to produce respiratory disease in lambs by the intratracheal inoculation of type A influenza virus and could not recover the virus from the respiratory tract at necropsy.

Poxviruses such as contagious ecthyma (contagious pustular dermatitis, scabby mouth) and sheep pox virus which normally inhabit the skin, have been reported as causing pulmonary lesions in sheep. Of the two viruses, sheep pox appears to invade the respiratory tract more frequently where it causes proliferative and desquamative lesions in the bronchial and alveolar epithelium of the peripheral areas of the lung (Jubb and Kennedy, 1970). Darbyshire (1961) has reported a haemorrhagic pneumonia with necrotic foci associated with the contagious ecthyma virus.

In France, Russo (1972) isolated a paramyxovirus from the lungs and liver of a lamb during an outbreak of neonatal respiratory infection. The virus was not further identified as its isolation was incidental to the study of sheep chromosomes in tissue culture.

There is also serological evidence of antibodies to reovirus types 1, 2 and 3 (Stanley, 1967), adenovirus (Darbyshire and Pereira, 1964) and respiratory syncytial virus (Berthiaume et al., 1973) occurring naturally in sheep but none of these viruses has yet been isolated.

As previously discussed, maedi virus is now recognised as the cause of a specific interstitial pneumonia which occurs in Iceland and certain parts of Europe. This virus was first isolated by Sigurdardóttir and Thormar (1964) who noted its slow growth in tissue culture and

neutralisation by serum from maedi-affected sheep. Subsequently Gudnadóttir and Pálsson (1967) were able to produce the disease in healthy sheep by inoculation of the virus grown in tissue culture. Its similarity to visna virus was noted originally by Thormar (1965) and has been discussed recently by Pálsson (1973). The intrapulmonary inoculation of visna virus has produced lung lesions resembling the early stages of maedi (Gudnadóttir and Pálsson, 1965). Wandera (1970) in Kenya, has reported significant titres of neutralising antibody against the Icelandic viruses in sera collected from sheep clinically affected with a maedi-like progressive pneumonia.

Combination of Agents

Several investigators have been impressed by the lack of a single definitive agent capable of producing pneumonia in sheep experimentally and have therefore endeavoured to produce the disease by using various combinations of some of the agents already discussed.

The first record of the combined inoculation of two or more agents is that of Boidin et al. (1958). These authors isolated a chlamydia and a mycoplasma from the lungs of pneumonic lambs in California. Intratracheal inoculation of a suspension of the chlamydia consistently produced irregular, patchy consolidated areas in the lungs 4 days after inoculation (Table I). But the addition of mycoplasma, P. haemolytica or both to the inoculum did not significantly increase the severity of the lung involvement.

Similarly, Hamdy and Pounden (1959) used a chlamydia, mycoplasma and Pasteurella spp. in various combinations inoculated by both intratracheal and intranasal routes, to infect 1 to 3-month-old lambs. The agents were inoculated simultaneously and in some cases stress was applied in the form of heating and chilling or the intramuscular administration of cortisone. In contrast to the work of Boidin et al. none of the agents used was capable of producing pneumonia when administered singly, but a combination of any 2 of the 3 infectious agents resulted in a febrile response without pneumonic lesions in 5 of the 7 lambs. Combinations of the 3 microbial agents produced pneumonic lesions in 2 out of 4 lambs. However, when stress was applied with a combination of 2 or 3 agents, all of 7 lambs developed both the clinical signs and lesions

of pneumonia.

Following on from the work of Boidin et al. in California, Dungworth and Cordy (1962a) produced pneumonia in lambs aged 5 to 6 months by the intratracheal inoculation of chlamydia (Table I). In these experiments P. haemolytica and environmental stress in the form of transportation, heating and cooling, administered singly or together, exacerbated the initial lesion in only 1 of 30 lambs.

Further studies on the effects of combined inoculations of chlamydia and P. haemolytica were carried out in Scotland by Biberstein et al. (1967) but their studies also failed to demonstrate any synergistic effect between these 2 agents. When given together both agents produced lesions characteristic of each other without any clear-cut influence of one infection on the course of the other, either clinically or pathologically.

The synergistic effect of viral and bacterial agents in the respiratory tract of mice has been clearly demonstrated by the excellent experiments by Degré and Glasgow (1968). These authors developed an experimental model in which combined infection by parainfluenza-1 virus (Sendai strain) and Haemophilus influenza produced pneumonia with a significantly enhanced mortality as compared with the mortality after infection with either agent alone. In cattle, the investigations of Baldwin et al. (1967) and Thomson et al. (1969) have shown a close interrelationship between PI-3 virus and Pasteurella spp. in the production of bovine respiratory disease. Work of this nature in other species is sufficiently encouraging to highlight the need for further investigations into the possible interaction of multiple agents in diseases of the ovine respiratory tract.

SECTION III: INJURY TO THE PULMONARY ALVEOLUS

In view of the absence of published work dealing specifically with the reaction of the ovine alveolus to injury this section of the literature review will deal with alveolar injury in general terms. Such an approach seems justified because current light and electron microscope studies have not revealed any fundamental differences in alveolar structure and function between mammals (Liebow, 1962; Meyrick and Reid, 1970). Although inter-species differences in the thickness of the various components of the blood-air barrier have been clearly defined (Schulz, 1962) it seems unlikely that these differences could radically alter the general pattern of injury which occurs in the mammalian lung. It is conceivable however, that the degree of injury and the rate at which changes occur may be influenced by these differences.

It is also likely that important differences in the pathological response to alveolar injury exist between species and that these will have a marked effect on the outcome of any reaction to injury. Differences in response may be due to variations in the rate of cell turnover in the alveolus or the speed and efficiency with which inflammatory cells can be mobilised to deal with damaged tissue. Apart from limited work in laboratory animals (Spencer and Shorter, 1962; Shorter et al., 1964) comparative information of this type is not yet available in the literature.

Injury to the alveolus may arise from the action of a wide variety of agents arriving by either endogenous or exogenous routes. In this area of study most attention has been centred on the effects of inhaled agents. Relatively few investigations have been made on the effects of oral and parentally administered chemicals or endogenous substances on the lung. It is convenient therefore, to begin by examining the kinds of systemic agents that may be involved in alveolar injury before discussing the effects produced by various gases, fluids and particles. Finally the review will examine the defence mechanisms that are available at the alveolar level to cope with these potentially harmful agents.

Systemic Agents which may Injure the Alveolus

The normal lung consists of a vast vascular bed of capillaries, arterioles and venules which carry the entire output of the right ventricle. Its function in gaseous exchange necessitates that the large surface area of the capillary endothelium and adjacent structures are constantly brought into close contact with the blood in the pulmonary circulation. Because of this, many toxic and inflammatory factors circulating throughout the body may often have more severe consequences for the lung than for any other organ. In a similar way other circulating agents such as hormones or metabolic products which are normally present in the blood in physiological amounts, may readily have pathological effects on the lung when they are present in greater than normal amounts.

Systemic hypoxia and acidosis are two of the most important physiological causes of pulmonary oedema and lung damage. Both have been shown to produce their effects by causing pulmonary vasoconstriction and hypertension which in turn results in capillary damage. Sellor and Spector (1964) observed capillary damage histologically in the lungs of rats exposed to short periods of anoxia. They also demonstrated increased capillary permeability by showing migration of intravenously administered iron dextran into alveolar spaces. Further evidence of the close relationship between hypoxia and pulmonary hypertension comes from measurements on normal subjects breathing a mixture of air low in oxygen (Fishman et al., 1960) and experimentally in cats exposed to hypoxia (von Euler and Liljestrand, 1946; Duke, 1954). Von Euler and Liljestrand suggested that hypoxia acts directly at the alveolar level on the pulmonary arterial vessels and the more recent histological studies by Hasleton et al. (1967) and Heath (1968) have supported this view. Berry et al. (1965) on the other hand, have contended that the action of hypoxia in the pulmonary circulation is predominantly at the post-capillary venular level.

The pulmonary ultrastructural changes following prolonged hypoxia have been investigated by Schulz (1959) and those following acute hypoxia induced by asphyxiation are described by Reidbord and Spitz (1966). Common to both descriptions is severe vacuolation of the capillary endothelium together with mitochondrial swelling, dilatation of the

endoplasmic reticulum and intracellular oedema. It has been suggested that rendering the pulmonary tissues anoxic does not in itself produce these severe ultrastructural changes (Reidbord and Spitz, 1966). It seems likely that the accumulation of lactate from anaerobic glycolysis and the release of histamine and other inflammatory factors from damaged tissues are more important mechanisms in the production of these structural abnormalities.

The vasoconstrictive effects of elevated hydrogen ion concentration on the pulmonary vascular bed have been described by Liljestrand (1958) and Bergofsky et al. (1962). Enson et al. (1964) were able to produce pulmonary hypertension by the vascular infusion of hydrochloric acid, however these results were not confirmed by Heath (1968) who discussed the origins of pulmonary hypertension in patients with chronic bronchitis and emphysema.

Exudation of protein-rich fluid into alveolar spaces is a common radiological and pathological finding in cases of advanced renal failure (Doniach, 1947). Clinical evidence has demonstrated the essential role of left ventricular heart failure in this form of pulmonary oedema. Lung changes frequently appear with the onset of heart failure and resolve with the relief of this failure while blood urea levels remain constant. Some authors however, feel that additional toxic factors may act on the alveolar capillaries during renal failure allowing the escape of macromolecular proteins and erythrocytes (Spencer, 1968).

Table IV lists some of those agents known to produce pulmonary damage when administered systemically. It can be seen that many humoral agents which are released in elevated quantities under the conditions of shock have the ability to produce lung damage. Some of these act by increasing the resistance in the pulmonary vascular bed while others act by directly increasing the permeability of the pulmonary capillary endothelium. It has also been shown by several workers (Eaton, 1947; Jenkins et al., 1950) that the pulmonary circulation is particularly sensitive to changes in the electrolyte composition of the blood during the shocked state and that alveolar collapse may follow the over zealous administration of fluids intravenously.

In clinical medicine, improvements in the management of shock have resulted in the emergence of pulmonary damage as a major determinant to survival regardless of whether the shock is of thermal, haemorrhagic or traumatic origin (Eiseman, 1968; Rapaport et al., 1973). A review of the lung in haemorrhagic shock has been written by Sealy (1968) and experimental investigations have been carried out in dogs and cats by Ratliff et al. (1970, 1971). These later authors have suggested that in haemorrhagic shock, circulating neutrophils become adherent to alveolar capillaries and arterioles causing obstruction and shunting of blood away from the alveoli. This in turn results in capillary endothelial degeneration, interstitial oedema and damage to the alveolar epithelium all of which have been observed ultrastructurally. Such a sequence of events may explain the apparent protection afforded the lung by isolating it from the circulation during haemorrhagic shock (Willwerth et al., 1967).

A limited number of studies have been made on the effects of certain bacterial toxins on the lung. These studies indicate that the action of these substances is directly on the capillary endothelium since the degree of damage produced is not altered by the use of histamine or serotonin inhibiting drugs (Beall and Dalldorf, 1966).

There is at present increasing interest in the field of the experimental production of pulmonary damage using systemically administered chemicals. The early studies by Hesse and Loosli (1949) on the lining of the alveoli in mice, rats, dogs and frogs following acute pulmonary oedema produced by ANTU (alpha-naphthyl thiourea) poisoning aroused sufficient interest to stimulate further work with this compound in recent years (Table IV). Ultrastructural investigations by Meyrick et al. (1972) have shown that blebbing and scalloping of capillary endothelial cells and interstitial oedema occurred 2 hours after intraperitoneal injection of ANTU. Although some epithelial damage was present at high dose rates this only occurred when alveolar exudation was well advanced. These findings confirmed earlier work by Teplitz (1968) who used the electron microscope to trace fluid leakage with ferritin and saccharated iron oxide but are in contradiction to the work of Bohm (1966) who considered that fluid leakage was primarily from venules.

TABLE IV

SOME SYSTEMIC AGENTS WHOSE LUNG TOXICITY HAS BEEN INVESTIGATED

Author	Agent	Species	Effect
Stone & Loew (1949)	Adrenaline	Rabbit	Pulmonary oedema and haemorrhage
Bohm (1966)	Adrenaline	Rat	Massive pulmonary oedema involving venules and capillaries
Sukhhandan & Thal (1965)	Adrenaline Plasmakinins Histamine Serotonin	Dog	Pulmonary oedema Severe pulmonary oedema Increased vascular resistance, congestion Increased vascular resistance and congestion
Wang <u>et al.</u> (1971)	Adrenaline	Mice	Pulmonary oedema, degeneration of type I & endothelial cells then type II & Clara cells
Young <u>et al.</u> (1963)	Serotonin	Dog	Passive congestion
Sackner <u>et al.</u> (1966)	Serotonin	Dog	Passive congestion
Sun & Saueressig (1965)	Cortisone	Rat	Degeneration of Type II cells thinning of basement membrane
Hesse & Loosli (1949)	ANTU	Mice) Rats) Dogs) Frogs)	Pulmonary oedema
Richter (1952)	ANTU	Rats	Pulmonary oedema
Bohm (1966)	ANTU	Rats	Increased permeability of venules and capillaries
Teplitz (1968)	ANTU	Rats	Increased capillary endothelial permeability
Meyrick <u>et al.</u> (1972)	ANTU	Rats	Blebbing & scalloping of capillary endothelial cells
Cotterell <u>et al.</u> (1967)	Alloxan	Dogs	Degeneration of both endothelium & epithelium
Valdivia & Sonnad (1966)	Carbon tetrachloride	Guinea Pigs	Lipid droplets in Type II epithelial cells
Valdivia <u>et al.</u> (1967)	Monocrotaline	Rats	Interstitial oedema, cell proliferation & elastolysis
Butler (1970)	Pyrrole derivative of pyrrolizidine alkaloids	Rats	Enlarged, bizarre endothelial and epithelial cells
Cameron & Sheikh (1951)	Ammonium sulphate	Rats	Pulmonary oedema

Cont'd...

TABLE IV (Cont'd)

Author	Agent	Species	Effect
Bohm (1966)	Ammonium Sulphate	Rats	Increased capillary permeability
Hayes & Shiga (1970)	Ammonium Sulphate	Rats	Blebbing of capillary endothelial cells
Gil & Thurnheer (1971)	Bromhexine	Rats	Proliferation of lamellated bodies in type II cells
Lullman-Rauch <u>et al.</u> (1972)	Chlor-phentermine	Rats	Enlarged, phospholipid filled alveolar macrophages
Smith <u>et al.</u> (1973)	Chlor-phentermine	Rats	Severe hyperplasia & hypersecretion of type II cells
Clark <u>et al.</u> (1966)	Paraquat	Mice Rats Guinea pigs	Congestion, oedema, hyaline membranes & inflammatory exudate
Manktelow (1967a) (1967b)	Paraquat Paraquat	Mice Mice	Reduced lung surfactant Damage to alveolar epithelium
Fisher & Clements (1969)	Paraquat	Rats	Reduced lung surfactant
Robertson <u>et al.</u> (1971)	Paraquat	Rats	Collapse, oedema, hyaline membranes & interstitial inflammation
Brooks (1971)	Paraquat	Mice	Endothelial degeneration, alveolar collapse and interstitial inflammation
Vijayaratham and Corrin (1971)	Paraquat	Rats	Degeneration and necrosis of alveolar epithelium
Smith & Heath (1974)	Paraquat	Rats	Degeneration and necrosis of alveolar epithelium plus interstitial fibrosis
Marino & Mitchell (1972)	Butylated hydroxtoluene	Mice	Congestion and thickening alveolar septa
Kuida <u>et al.</u> (1958)	<u>E. coli</u> endotoxin	Dogs Cats	Increased pulmonary artery pressure and lung weight
Sukhnandan and Thal (1965)	<u>E. coli</u> endotoxin	Dogs	Increased vascular resistance and congestion
Beall & Dalldorf (1966)	Anthrax toxin	Rats	Elevation and detachment of capillary endothelium
Finegold (1967)	Staph enterotoxin	Monkeys	Degeneration and necrosis of capillary endothelium
Read (1958)	Anti-lung serum	Rats	Haemorrhage & alveolar exudation of inflammatory cells
Kitamura (1972)	Anti-lung	Rats	Injury to capillary endothelium, interstitium and alveolar epithelium

TABLE IV (Cont'd)

Author	Agent	Species	Effect
Warren & Gates (1940)	X-irradiation	Rats Rabbits Dogs Pigs	Epithelial degeneration & hyaline membrane formation
Leroy <u>et al.</u> (1965)	X-irradiation	Dogs	Vacuolation of alveolar epithelial and endothelial cells
Oikawa (1970)	X-irradiation	Rats	Diminished pulmonary surfactant
Adamson <u>et al.</u> (1970a)	X-irradiation	Mice Rats	Vacuolation & destruction of endothelium with immediate stripping of type I cells

A widely used compound of special interest because of its lung toxicity is the dipyridylum herbicide Paraquat (1, 1-dimethyl-4, 4-bipyridylum dichloride). Initial investigations by Clark et al. (1966) in experimentally poisoned laboratory animals showed that severe pathological changes occurred in the lungs from 2-5 days after administration by a variety of routes. Microscopic examination revealed haemorrhage, congestion and oedema with some exudation of fibrin, and variable acute inflammatory exudate. Those animals surviving longer showed interstitial fibrosis, macrophage and leucocyte infiltration and proliferation of alveolar lining cells. Manktelow (1967b) subsequently observed the lungs of experimentally poisoned mice ultrastructurally and found that the principle site of injury was the alveolar epithelium. These findings have been confirmed by Kimbrough and Gaines (1970), Vijayaratnam and Corrin (1971) and Smith and Heath (1974).

Experimental indications of the vulnerability of the lung to Paraquat poisoning have been supported by the numerous cases of accidental and suicidal poisoning in man which are now on record. These have been reviewed by Conning et al. (1969), Hargreave et al. (1969) and Nienhaus and Ehrenfeld (1971). Several cases of poisoning have also occurred in domestic animals with both Paraquat (Rogers et al., 1973; Smith, 1969) and the related dipyridylum compound Diquat (Thomas and Amor, 1968). Most of these have shown respiratory distress or presented a pathological picture similar to that described in laboratory animals.

Strong evidence has accumulated indicating that the pathogenesis of lung damage in Paraquat poisoning involves a severe diminution in the production of pulmonary surfactant (Manktelow, 1967a; Fisher and Clements, 1969; Robertson, 1971). However, this has been disputed by Fletcher and Wyatt (1970, 1972) who found no change in the incorporation of phospholipid into the lungs of Paraquat-poisoned mice.

In man, pulmonary fibrosis has been observed following the long term administration of busulphan (Myleran) in the treatment of chronic myeloid leukaemia (Oliner et al., 1961). Heard and Cooke (1968) noted that several fatalities attributed to this drug are now on record and found histological evidence of fibrinous oedema in the lungs of 6 out of 14

cases of chronic myeloid leukaemia treated with busulphan. Similar changes were found in only one out of 7 cases of leukaemia not given busulphan therapy. Electron microscopic observations by Littler et al. (1969) revealed desquamation and proliferation of type II alveolar epithelial cells followed by fibrosis of alveolar walls and intra-alveolar contents.

The effect of radiation injury on the lungs has been comprehensively reviewed in the texts of Spencer (1968) and Berdjis (1971). The most common cause of damage in man is the very large doses of X-irradiation administered during the treatment of carcinoma of the breast, lung, oesophagus, thyroid and malignant lymphomas of the mediastinal structures. Warren and Spencer (1940) estimated that the incidence of radiation pneumonitis in man is about 12% of those exposed to this therapy. Extensive experimental studies have been carried out on a variety of animals (Table IV) and in general there is a basic similarity between the lesions produced in all species and those observed in man.

The degree of pulmonary injury following irradiation depends on the total dose received as well as the time interval over which it is administered. Cytoplasmic vacuolation and mitochondrial degeneration of both capillary endothelium and alveolar epithelium are the initial ultrastructural changes observed in dogs 36 hours after exposure to 1,000r (Leroy et al., 1965). Larger doses however, result in congestion, alveolar oedema and macrophage infiltration within 24 hours. Hyaline membrane formation may follow in the next few days together with leucocyte infiltration and fragmentation of elastic fibres. Desquamation of the bronchial and bronchiolar epithelium is seen on about the 5th day. Following this, reparative changes including interstitial fibrosis and alveolar epithelialization commence and may continue for several weeks after exposure (Warren and Gates, 1940; Smith, 1963).

It can be seen from the preceding discussion that the majority of systemic agents which have the ability to damage the lung primarily injure the alveolar capillary endothelium. Several noteworthy exceptions to this rule are on record and investigation of their mode of action although just beginning, holds the promise of providing valuable tools for future use in experimental pathology. In this regard it is important

to note that because of the tenuous nature of the blood-air barrier, alveolar epithelial damage may rapidly follow endothelial damage (Adamson et al., 1970b; Harrison, 1971; Meyrick et al., 1972). Careful ultrastructural studies will therefore be necessary before the precise site of injury of any systemic agent can be firmly established.

Inhaled Agents which may Injure the Alveolus

Gases

While it is unlikely that irritant gases have any role in the pathogenesis of the pneumonias of extensively reared domestic animals a variety of such gases have been used as experimental agents in the study of lung injury. As a result a considerable volume of literature has accumulated in this area. Among the factors responsible for this escalation of knowledge are the technical advances in inhalation anaesthesia, positive pressure respirators and aerospace medicine which have occurred in the last two decades. These have necessitated the acquisition of more basic knowledge on the reaction of the lung to high concentrations of oxygen, carbon dioxide and anaesthetic agents. A further important factor has been the increasing burden of air pollution in modern, industrialised societies which has prompted research into the effects of ozone, nitrogen dioxide, sulphur dioxide and carbon monoxide on the pulmonary airways. Although it is outside the range of this review to examine this research in depth, work with gases has greatly added to the understanding of alveolar injury so a brief mention of the more significant findings in this field seems pertinent.

Studies on oxygen toxicity have shown that animals vary greatly in their susceptibility according to both species and age. These variations have been attributed to quantitative differences in lung enzyme levels based on the level of evolutionary development (Haugaard, 1968). The primary cellular target in oxygen toxicity is once again, the alveolar capillary endothelium. Profuse pulmonary oedema with interstitial thickening and capillary endothelial destruction has been produced by exposure to 90% oxygen at 1 atmosphere (Bowden et al., 1968). In these studies the alveolar epithelial lining showed little, if any change but subsequent work by Adamson et al., (1970b) with mice and Harrison (1971) with young rats revealed that disruptive changes in the epithelium quickly followed endothelial changes. Survival of the exposed animals was found to be directly related to the structural integrity of the type 1

alveolar epithelial cells and their destruction was an irreversible event resulting in hyaline membrane formation and death.

Alveolar epithelial damage and proliferation has also been noted with sublethal exposure to pure oxygen at a reduced pressure of 700 mm Hg (Kistler et al., 1967; Schaffner et al., 1967). Coalson et al. (1971) confirmed that pure oxygen exerts a direct cellular toxic effect on both alveolar endothelial and epithelial cells of the lungs of dogs. In their experiments however, one lung was ventilated with oxygen while the other was collapsed or ventilated with room air. The morphological changes seen in the lungs not exposed to oxygen, whether ventilated or not, were comparable to those in the exposed lungs. This suggested that a blood-borne toxic factor, possibly associated with peroxidation of erythrocytes may be responsible for the lung damage.

Carbon dioxide exposure has been found useful by several workers in the study of the development of hyaline membrane disease in guinea pigs (Niemoeller and Schaefer, 1962; Schaefer et al., 1964). The membranes were formed on the first day after exposure following an initial period of respiratory acidosis and pulmonary oedema. They were found to be associated with the disappearance of lamellar bodies in great alveolar (type II) cells and an associated decrease in pulmonary surfactant (Schaefer et al., 1964).

More subtle ultrastructural alterations have been observed in both type 1 alveolar epithelial cells and the capillary endothelium following the inhalation of anaesthetic ether by rats and mice (Finlay-Jones et al., 1971). These changes took the form of cytoplasmic swelling and bleb formation which in some cases lead to cell rupture with exposure of the underlying basement membrane. Since ether is able to stimulate the sympathetic nervous system thus releasing adrenaline (Millar et al., 1970) Finlay-Jones et al. have postulated that this hormone may be involved as an intermediary in the pathogenesis of ether induced ultrastructural changes.

The interdependant toxic gases ozone and nitrogen dioxide are produced by reactions between oxygen and the oxides of nitrogen which occur under the influence of solar photochemical energy in smoggy

atmospheres. High concentrations of these gases are sometimes encountered in heavy industry. In addition, nitrogen dioxide may be liberated from cattle forage such as corn silage, prepared from immature corn containing a high nitrate content (i.e. Silo-filler's disease) (Spencer, 1968).

The pathological changes in animals exposed to high concentrations of ozone and nitrogen dioxide are similar and consist of pulmonary oedema and haemorrhage. Sublethal doses may at first cause pulmonary oedema but later bronchopneumonia and bronchiolitis obliterans develop (Darke and Warrack, 1958; Scheel et al., 1959; Werthamer et al., 1970). Similarities in response to ozone and nitrogen dioxide also exist at low levels of exposure over long periods. In chronic ozone toxicity there is deposition of connective tissue in the walls of the alveolar ducts and respiratory bronchioles, an increase in the number of alveolar macrophages and metaplastic changes in the epithelium of terminal bronchioles (Bils, 1970; Freeman et al., 1973). Although the changes in long term nitrogen dioxide exposure are of a similar nature, the focal point of the lesion appears to be more proximal than in ozone exposure and the fibroblastic activity less evident (Freeman et al., 1968; Stephens et al., 1972). Recent studies of morphological surface changes in nitrogen dioxide-exposed rat lung have shown the replacement of type 1 cells in the alveolar duct region by cells covered with microvilli (Parkinson and Stephens, 1973).

Despite these similarities in pulmonary response to ozone and nitrogen dioxide, available biochemical evidence does not favour identical peroxidative mechanisms of cell injury (Roehm et al., 1971). However, cytochemical studies on ozone toxicity by Dillard et al. (1972) and Castleman et al. (1973) have suggested that lysosomal membranes in the terminal bronchiolar epithelium and adjacent intra-alveolar septa are severely damaged resulting in the release of acid phosphatase. Although histochemical data have not confirmed that nitrogen dioxide also causes acid phosphatase release, biochemical observations on mouse lung slices have shown significantly higher levels of acid phosphatase in nitrogen dioxide-exposed animals than in normal mice (Buckley and Loosli, 1969).

Valuable work on the lung clearance of bacteria by Erhlich and Henry

(1968), Blair et al. (1969), Buckley and Loosli (1969) and Goldstein et al. (1973) has shown that reduced clearance rates occur in the lungs of mice following long term nitrogen dioxide exposure. Similarly, exposure to low levels of ozone has been shown to depress the activity of hydrolytic enzymes within alveolar macrophages (Hurst et al., 1970), increase the number of polymorphs in pulmonary lavage fluid of rabbits (Alpert et al., 1973) and inhibit the pulmonary bactericidal activity in vivo of exposed mice (Goldstein et al., 1971). While it is hazardous to extrapolate from data obtained in laboratory animals these findings have obvious implications for urban populations which are exposed to low levels of these gases.

In contrast, work with sulphur dioxide, another common atmospheric pollutant, has failed to demonstrate a reduction in the elimination of E. coli from the lungs of experimentally-exposed guinea pigs (Rylander, 1969). However when the gas was administered at the same time as a dust aerosol of carbon black particles a synergistic effect was produced which resulted in a decrease in mucus flow.

The effects of the severely irritant gases phosgene and chlorine are well documented through their use as war gases. The pulmonary oedema and hyaline membrane formation which occurs were thought to result from direct action of the bases on the pulmonary capillary endothelium (Spencer, 1968). By using ultrastructural techniques Diller et al. (1969) have shown instead that the earliest changes in phosgene-exposed dogs are in the alveolar epithelium at $1\frac{1}{2}$ hours after initial exposure. They consist of folding of the alveolar epithelium, oedema of the alveolar wall and filling of alveoli with cell-free exudate. Rupture of alveolar walls, exudation of fibrin and cellular debris and alveolar macrophage proliferation follow within the first few hours.

Fluids

The presence of fluid in the mammalian alveolus does not in itself constitute a threat to life. Since mammals have evolved from fishes and the foetal lung develops in an environment of amniotic fluid it is not surprising that the lung has a high tolerance to fluids that are physiologically balanced. This point has been strikingly demonstrated in experiments by Kylstra (1965). When adult mice were submerged in

balanced and buffered isotonic saline, at 20°C and equilibrated with oxygen at a pressure of 8 atmospheres they survived as long as 18 hrs. Under similar conditions they survived only 11 minutes in oxygenated seawater and 5 minutes in oxygenated tap water. These experiments confirmed earlier work by Halmagyi (1961) and Spitz and Blanke (1961) who demonstrated the importance of osmotic pressure of fluids in relation to alveolar injury and respiratory arrest in experimental drowning.

Ultrastructural studies by Reidbord and Spitz (1966) have succeeded in differentiating freshwater from saltwater drowning and further work by Reidbord (1967) has shown that changes produced in the alveoli of rats following the administration of saline and water were closely related to the concentration of saline used. Concentrations below 0.5% produced mitochondrial swelling and nuclear chromatolysis in the alveolar epithelium together with endothelial vesiculation. More severe changes including alveolar epithelial swelling and dilatation of the endoplasmic reticulum were seen at concentrations of saline below 0.25%. Hypertonic solutions on the other hand, caused focal vesiculation in both epithelium and endothelium which increased in severity with the concentration of saline administered. Reidbord postulates that these alterations resulted from the excess movement of fluid across cell membranes.

The delicate nature of the alveolar epithelium makes it vulnerable to damage by any fluids containing irritant substances. One of the earlier studies of this type of injury was by Ross (1939) who produced alveolar epithelial desquamation and epithelialization in rabbits by the intra-tracheal injection of staphylococcal toxin. Acute chemical irritation has been studied more recently by the intra-tracheal injection of kerosene (Barter et al., 1966; Manktelow, 1967b), sodium arsenite (Manktelow, 1967b), hydrochloric acid (Teabeaut, 1952; Moran, 1955) and autologous gastric juice (Moran, 1955). If food particles were present in the aspirated gastric juice there could be bronchial obstruction which allowed the multiplication of putrefactive organisms resulting in abscess formation and gangrene (Teabeaut, 1952). Kerosene and hydrochloric acid both produced an acute haemorrhagic pneumonia with hyaline membrane formation in 8 to 48 hours after administration. After this time an abnormal epithelial lining developed in place of the necrotic exudate.

At the ultrastructural level Manktelow (1967b) observed extensive destruction and detachment of alveolar epithelial (type 1) cells 18 hours after the endobronchial administration of sodium arsenite. Kerosene administration however, produced more severe and very rapid damage to great alveolar (type 11) cells and following on from this, vesiculation, 'U'body formation and fragmentation of type 1 cells occurred within 25 minutes.

The aspiration of irritant fluids is of particular importance in sheep because commonplace husbandry practices such as drenching and dipping are potential sources of irritant inocula. Fortunately modern anthelmintics and insecticides tend to be less irritant than earlier remedies and as a consequence post-dipping and post-drenching mortalities now appear to be less common than previously reported.

There is conflicting evidence on the effects of arsenical dipwash on the ovine lung. Filmer (1957) states that bronchopneumonia and lung abscesses were produced by the intratracheal injection of 8 cc or more of arsenical dipwash. On the other hand Cook (1966) was unable to produce lesions in lambs by the intratracheal injection of up to 25 cc of fouled arsenical dipwash obtained from a property where heavy losses had been experienced after dipping. On the basis of his experiments and observations in the field, Cook concluded that aspiration of dipwash was not the underlying cause of the severe outbreaks of post-dipping pneumonia in sheep which he investigated.

The pathological effects of carbon tetrachloride on the lung are dependant on whether the chemical is aspirated as a liquid or inhaled as a vapour (Gallagher, 1962, 1964). Inhalation of vapour was more dangerous and produced lesions of pulmonary congestion, oedema and collapse which were more severe than equivalent doses of liquid. In addition the lesions induced by vapour were more diffusely spread throughout the lung than those caused by liquid which were concentrated in the dependant lobes.

Sheep are considerably more resistant to the intratracheal administration of the majority of modern anthelmintics which are in current use. St. George and Sullivan (1973) found that standard doses of thiabendazole

and tetramisole produced no gross lung lesions in sheep 3 or 6 days after intratracheal administration. However, the administration of some organo-phosphates including Neguvon⁽¹⁾ by the same route produced death in several minutes. Pulmonary oedema and focal areas of necrosis were visible grossly in the lungs at necropsy.

The inhalation of lipids produces pulmonary lesions which are sufficiently characteristic to have become recognised as a pathological entity. Lipid pneumonia has been described following the aspiration of a variety of mineral, vegetable and animal oils (Spencer, 1968). Although it occurs more commonly in man than in domestic animals it has been observed in cats following the administration of liquid paraffin or cod liver oil (Jubb and Kennedy, 1970) and cattle following the administration of raw linseed oil in the treatment of bloat (Smith et al., 1969). The typical lesion consists of infiltrations of large numbers of foam-filled macrophages into alveolar spaces. The macrophages also accumulate in lymphatics, interstitial tissue and around blood vessels where they become embedded in fibrous tissue.

This type of inflammatory response was thought to be merely the result of attempted mechanical removal of an inert foreign substance (Robbins, 1964). However, Baskerville (1969) noted that certain features of the reaction such as acellular alveolar exudation and interstitial, alveolar fibrosis were inexplicable on the assumption that the inhaled oils were bland substances undergoing mechanical removal. Accordingly, he administered liquid paraffin intratracheally to mice and using the electron microscope found that alveolar epithelial destruction and degeneration of capillary endothelial cells and great alveolar cells occurred from 4 days after administration onwards.

(1) Bayer, Henry York and Co. Limited.

Particles

The pulmonary alveolar surface is constantly exposed to the threat of damage by particulate matter present in the external environment. The entry of particles into the alveolar space depends on both the concentration and size of particles that are present in the inhaled atmosphere. Size is also important in determining whether an inhaled particle will adhere to, or penetrate the alveolar epithelium thus directly influencing the potential toxicity of the dust (Gross and Westrick, 1954). Some particles such as those containing lead, magnesium, aluminium and cadmium are toxic because of their chemical nature. Others may produce their effects through the initiation of an allergic response, radioactivity or the carriage of virulent micro-organisms. A further group of particles which are sometimes termed "inert" can nevertheless exert a destructive influence on the lung by overloading defence mechanisms, penetrating alveolar walls and initiating a fibrogenic reaction (Spencer, 1968).

Foremost among investigators into particle deposition was Pattle (1961) who worked with a monodisperse cloud of methylene blue. He found that practically all non-filamentous particles larger than $10\ \mu$ were impacted in the nasopharynx whereas 90% of those less than $1\ \mu$ were carried into the lungs. His work was confirmed by Toigo *et al.* (1963) who used carbon particles labelled with I^{131} . These findings were similar to the conclusions reached earlier by Brown *et al.* (1950) and Albert and Arnett (1955). The latter authors studied bronchial clearance of radioactive iron particles in human subjects. They found those subjects receiving larger particles ($3.4 - 4.3\ \mu$) cleared 95% within 8 hours whereas those receiving smaller particles ($1.4 - 2.3\ \mu$) cleared only 68% in 36 hours. A full discussion of the pulmonary deposition and retention of inhaled aerosols is contained in the text of Hatch and Gross (1964). Although there are wide differences in experimental results reported by various investigators there is now general agreement that the optimum size for intrapulmonary retention is between 1 and $3\ \mu$.

Large particles of non-compact shape such as asbestos or glass fibres, can nevertheless be sufficiently aerodynamic to permit penetration into the pulmonary airspaces. Once deposited such fibres may be difficult

to remove. This has been well demonstrated in experiments with rats by Timbell and Skidmore (1971). They showed that the fibre length of glass particles can be a minor factor in penetration into the lung but is a major factor in the retention of fibres within the lungs.

Micro-organisms are often dispersed in the atmosphere within small aerodynamic particles 2 to 3 μ in size. Wells (1955) showed that even in atmospheres of 90% relative humidity, liquid droplets less than 80 μ diameter such as those formed by coughing and sneezing, will evaporate quickly to form residual particles. He called these particles droplet nuclei. Such particles have low settling velocities and are thus capable of remaining in atmospheric suspension over long periods of time. When inhaled, the probability of their deposition within the lung is higher than the chance of their capture by the upper respiratory tract. Dust-borne bacteria on the other hand, have an average particle size of greater than 10 μ and will therefore tend to be deposited in the nasopharynx and large airways of the lung (Hatch, 1961).

The accumulation of dust in the lungs and the tissue reaction to its presence is termed pneumoconiosis. While it was known from ancient times that certain dusty occupations often led to respiratory illness, it was Zenker who is given credit for first having coined the term in 1867. Despite a high incidence of the disease in miners and industrial workers research in this area was largely neglected until the mid 20th century. Over the past 10 years however, a growing awareness of the importance of occupational diseases has led to significant advances in the understanding of the pathogenesis of many different forms of pneumoconiosis.

Two dusts known to have a dangerous capacity for inducing progressive pulmonary fibrosis are silica and asbestos. The mode of action of silica is now fairly well understood. Silica particles coated with serum proteins and bronchial washings are ingested by macrophages and lie within secondary lysosomes. After digestion of the protein coat the surface of the silica particles can interact by a hydrogen bonding mechanism with phospholipids of the lysosomal membrane making it permeable. As a result silica particles and hydrolytic enzymes escape into the cytoplasm and the cell is attacked by its own defensive mechanisms (Allison, 1971). The materials released by dead or damaged macrophages are then said to

stimulate collagen synthesis by fibroblasts (Heppleston and Styles, 1967).

Less is currently known of the mode of action of different forms of asbestos. One of the confusing aspects of the problem is the inability to reconcile the apparently inert quality of fibres when given to macrophages in cell culture with their well known fibrogenic properties when given to animals or inhaled by man (Miller and Harrington, 1972). However the primary lesions are not an interstitial fibrosis as they develop in the lumen of respiratory bronchioles and adjacent alveoli following the entrapment of fibres and the accumulation and disintegration of macrophages (Wagner, 1965).

Work on the pathogenesis of coal workers' pneumoconiosis has been complicated by the variable and often complex nature of coal dust. The effects of dusts of bituminous and lignite coals have been studied in monkeys, ponies and a variety of laboratory animals and it has been shown that the reaction is similar in all species (Heppleston, 1954). It results essentially from the overloading of alveolar clearance mechanisms with carbon particles. Dust-filled macrophages become packed within alveolar ducts and alveoli which then collapse so that alveolar walls become closely apposed to the dust masses. Gross et al. (1972) have suggested that this collapse may be the result of a reduction in surfactant secretion following damage to great alveolar (type II) cells. The character of the tissue reaction to coalmine dust may readily be altered by the presence of silica or by changes in the immunological status of pulmonary tissue caused by tuberculosis, rheumatoid arthritis and other, as yet unidentified entities. With immunologically altered tissue the same dust may initiate a progressive massive fibrosis of the lungs (Gross et al., 1972).

Inhalation experiments designed to compare the fibrogenicity of various mineral dusts have been complicated by the occurrence of endogenous lipid pneumonia. This is currently thought to be the result of hyperactivity of great alveolar (type II) cells. The pulmonary foam cells which fill alveolar spaces in this disease are macrophages which have ingested a large amount of the excess lipid secretions of the over-active cells (Corrin and King, 1969; Heppleston and Young, 1972).

The majority of research on inhaled particles to date has been devoted

to inorganic substances. But domestic animals, whether they are reared intensively or extensively, are more likely to be exposed to organic dusts of vegetable or animal origin. In all the diseases in this group the dusts appear to be antigenic and most of the damage is considered to result from induced hypersensitivity states. The antigens involved may be either the organic protein of the dust itself or contaminating fungal spores (Spancer, 1968).

The lung may undergo several types of allergic response to inhaled agents (Parish and Pepys, 1968). An anaphylactic reaction may occur when an antigen reacts with cells passively sensitized by reaginic antibody (IgE). This reaction involves the release of factors such as histamine, slow-reacting-substance-A, and prostaglandins. An Arthus-type reaction is seen when an antigen and antibody reaction takes place in excess of antigen, forming complexes that are locally toxic to tissues.

One of the best studied examples of the Arthus-type reaction is the disease syndrome of farmer's lung. The nature and sources of organic dust which may initiate this syndrome have been listed by Parish and Pepys (1968). The disease most often occurs following exposure to mouldy hay contaminated by the thermophilic actinomycetes of which Micropolyspora faeni is the principle allergen and Thermoactinomyces vulgaris is of lesser importance (Pepys, 1966). Attempts at the reproduction of the disease in laboratory animals by the inhalation of M. faeni organisms have not as yet been fully successful. Zaidi et al. (1971) succeeded in producing lung lesions in guinea pigs with a mixture of the actinomycete and hay dust but the lesions differed little from those produced by hay dust alone although they were more severe in the early stages. The inflammatory reaction is primarily interstitial with oedema of the alveolar walls and infiltration by lymphocytes, plasma cells and neutrophils. Fibrosis and diffuse granulomatous lesions may develop in the walls of alveoli and small bronchioles with recurrent exposure (Fuller, 1953). Both acute and chronic forms of the disease are now known to occur in dairy cattle (Pirie et al., 1971; Wiseman et al., 1973). In the latter form 85% of affected cows showed precipitating antibodies to M. faeni.

In conclusion, it is important to note that the degree of injury produced by a given particle whether it is of an allergic, toxic or infective nature, will depend on the duration of its exposure to the underlying pulmonary epithelium. For this reason, systemic and gaseous agents which impair alveolar clearance may also increase particle exposure time thus having a cumulative or synergistic effect on the degree of damage produced. Synergism between gases and particles has been clearly demonstrated in the experiments of Boren (1964) with nitrogen dioxide and carbon particles and by Rylander (1969) using sulphur dioxide and coal dust.

SECTION IV: ALVEOLAR DEFENCE MECHANISMS

The evolutionary need for a tissue surface specialized towards gaseous exchange has resulted in the development of an alveolar epithelium with little of the structural defences normally possessed by other epithelial tissues. The defence mechanisms which do operate in the pulmonary alveolus are nevertheless highly effective under normal circumstances. This is demonstrated by the fact that, despite almost continuous exposure to foreign agents, the bronchopulmonary system is normally able to maintain its sterility (Laurenzi, 1964). Among the many discussions of alveolar defence mechanisms which have appeared in the literature one of the most impressive is that of Sorokin (1970) who has reviewed in general terms, the properties of alveolar cells and tissues that strengthen alveolar defences. In addition, Rylander (1968) has covered the literature dealing with pulmonary defence against inhaled bacteria and Green (1968, 1970a) has given a detailed account of the pulmonary clearance of infectious agents.

Alveolar defence mechanisms may be divided into two main categories:-

- (a) Mechanical clearance in which foreign material is physically removed.
- (b) Cellular and immunological factors which have the capacity to inactivate potentially harmful agents (usually micro-organisms) within the lung tissue.

In the first category most attention has been paid to studying the efficiency of clearance by the muco-ciliary apparatus of the bronchial tree. Little information is available on the movement of fluids over the alveolar surface because of obvious difficulties involved in the study of a dynamic, expanding and contracting alveolar duct system. However, the constant production of pulmonary surfactant by great alveolar (type II) cells (Bensch et al., 1964) together with the leakage of plasma from blood vessels suggests that some movement of the alveolar lining film over the epithelial surface must occur. The current state of knowledge of the surfactant system of the lung indicates that it consists of a surface layer of lipids or lipoproteins beneath which is a fluid hypophase (Scarpelli, 1968; Weibel and Gil, 1968; Kilburn, 1970; Untersee et al., 1971; Slama et al., 1973). Kilburn (1969) has suggested that

there may be drainage of the liquid hypophase by surface minimizing forces from areas of low surface tension to areas of high surface tension and from plane surfaces to angles of alveolar walls. Thus it seems likely that fluid and particles from distal alveoli drain to terminal bronchioles where they become part of the muco-ciliary clearance system. Visual evidence supporting this view has been provided recently from the electron microscopic studies of mouse lungs by Roth et al. (1973) using freeze etching techniques. The possibility that alveolar secretions are sufficiently cohesive to allow bronchial cilia from adjacent airways to provide an additional traction force has also been suggested by Green (1970a) but remains to be investigated.

The removal of alveolar fluid and particles by lymphatic drainage is recognised as a second method of mechanical clearance. Anatomical studies on rabbits (Lauweryns and Boussauw, 1969) have shown that lymphatic capillaries may often have a perialveolar location as they extend at least as far peripherally, as the respiratory bronchioles. The absorption of fluids and particles through the alveolar wall has been fully discussed by Morrow (1972). He concluded that although the alveolar epithelium is readily permeable to many substances it is relatively and selectively impermeable to others. The question of which epithelial cells are engaged in fluid and particle transport remains controversial but evidence is now forthcoming which indicates that the alveolar epithelial type I cell possesses limited phagocytic or perhaps more strictly pinocytic abilities. Sanders et al. (1971) have shown that 0.5 - 4.2% of metallic oxide particles (mass median diameter 0.26 μ) can be found within alveolar type I cells 10 days following aerosol exposure and similar results have been obtained by Heppleston and Young (1973) using carbon particles.

Particle clearance from the alveolus occurs in a distinct three-phase pattern. The initial and most efficient phase removes 50% of deposited material within 24 hours (Green, 1970a). The second, intermediate phase has a biological half time of 100 hours (La Belle and Brieger, 1961) and the third, long phase has a half time of 62-70 days (Gibb and Morrow, 1962). The reasons for this pattern are not clearly understood but probably reflect the differences between mechanical and cellular clearance. However, it seems likely that the third phase may involve perivascular lymphatic transport, cell breakdown and rephagocytosis as described by

Heppleston (1963).

There is abundant evidence that the alveolar macrophage plays a central role in the pulmonary defence against inhaled agents. The most comprehensive review of the role of the alveolar macrophage in pulmonary defence available to date is that of Voisin et al. (1971). In addition, Green (1969, 1970b) has written shorter reviews on the response of the alveolar macrophage system to host and environmental factors.

While controversy still exists concerning the origin of alveolar macrophages the weight of new information now favours the blood monocyte, derived from bone marrow, as the main progenitor. This view has been disputed by Omar (1964), Schiller (1971) and many others who believed that alveolar macrophages were derived from alveolar septal (corner) cells. However, Pinkett et al. (1966) initiated a sophisticated approach to the problem by using mouse chimeras and injecting bone marrow cells carrying chromosomal markers into recipients whose marrow had been destroyed by irradiation. Thus they established that the majority of alveolar macrophages are of bone marrow origin. Their work was soon supported by the results of Bowden et al. (1969) and has subsequently been confirmed by Brunsetter et al. (1971) using mouse chimeras and an enzyme marking technique. Although it is now generally accepted that alveolar macrophages are derived from monocytes of bone marrow origin, the work of Bowden et al. (1969) and Velo and Spector (1973) has indicated that in the case of experimental pneumonia, many of these cells undergo a 2-3 day period of division and maturation in the lung before becoming actively phagocytic.

The reduction in viable bacteria in the lung within the first few hours following inhalation, is thought to be due principally to phagocytosis by alveolar macrophages. No other bactericidal mechanism has been shown to account for this phenomenon (Green and Kass, 1964a; Rylander, 1970). A large number of host and environmental factors are capable of depressing the bactericidal capacity of the lung presumably through impairing the activity of alveolar macrophages. Among these factors can be listed host variables such as nutrition, metabolic state, immunologic experience and chronic disease, and environmental factors such as virus infection, air pollutant gases and oxygen and carbon dioxide levels (Table V).

TABLE V
SOME AGENTS WHOSE EFFECTS ON ALVEOLAR DEFENCE MECHANISMS
HAVE BEEN MEASURED

Author	Agent	Species	Effect
(a) Reduced Mechanical Elimination			
Carson <u>et al.</u> (1966)	Ammonium chloride aerosol	Cats	Slowed mucus transport of particles
Carson <u>et al.</u> (1966)	Cigarette smoke	Cats	Slowed mucus transport of particles
Rylander (1969)	Cigarette smoke	Guinea pigs	Reduced mechanical elimination of <u>E. coli</u>
Laurenzi and Guarneri (1966)	Ethanol	Kittens	Reduced mucus transport rate
Rylander (1968)	Ethanol	Guinea pigs	Reduced mechanical elimination of <u>E. coli</u>
Ferin (1971)	Papain induced emphysema	Rats	Reduced particle clearance
Creasia <u>et al.</u> (1973)	Influenza virus	Mice	Impaired particle clearance for 56 wks post-inoculation
(b) Reduced Bactericidal Activity			
Green & Kass (1964b)	Hypoxia Starvation Cortico-steroids Ethanol	Mice	Depressed removal of viable staphylococci
Green & Kass (1965)	Hypoxia	Mice	Depressed removal of viable staphylococci but not <u>Proteus sp.</u>
	Cold stress Ethanol	Mice	Depressed removal of both staphylococci & <u>Proteus sp.</u>
Goldstein & Green (1966)	Acute renal failure	Mice	Defective <u>in situ</u> bactericidal mechanisms
Gardner <u>et al.</u> (1968)	Sulfamethazine	Rabbits	Reduced macrophage phagocytic capacity for <u>Streptococcus pyogenes</u>
Green (1970b)	Surgical nephrectomy	Mice	Reduced bactericidal capacity for <u>E. coli</u>
LaForce and Huber (1970)	Extra-pulmonary disease (peripheral abscess)	Mice	Decreased bactericidal capacity for <u>S. aureus</u>

Cont'd...

TABLE V (Cont'd)

Author	Agent	Species	Effect
Hahn <u>et al.</u> (1971)	X-irradiation	Mice	Decreased bactericidal capacity for <u>S. aureus</u>
Huber <u>et al.</u> (1970)	Immuno-suppressives	Mice	Decreased bactericidal capacity for <u>S. aureus</u>
LaForce <u>et al.</u> (1973)	ANTU	Mice	Decreased bactericidal capacity for <u>S. aureus</u>
LaForce <u>et al.</u> (1973)	Surgical aortic constriction	Rats	Decreased bactericidal capacity for <u>S. aureus</u>
Green (1968)	CO ₂ (acidosis)		Reduced alveolar macrophage phagocytic activity
Huber & LaForce (1970)	Oxygen 100% Ozone	Mice	Decreased bactericidal capacity for <u>S. aureus</u>
Alpert <u>et al.</u> (1971)	Ozone	Rabbits	Decreased viability and enzyme activity of macrophages
Goldstein <u>et al.</u> (1973)	Nitrogen dioxide	Mice	Decreased bactericidal capacity for <u>S. aureus</u>
Green & Carolin (1967)	Cigarette smoke	Mice	Reduced macrophage bactericidal activity <u>in vitro</u>
Holt & Keast (1973)	Cigarette smoke	Mice	Initial decrease in macrophage numbers then long term increase
Rylander (1968)	Coal dust	Guinea pigs	Reduced bactericidal capacity for <u>E. coli</u>
Rylander (1969)	Carbon black	Guinea pigs	Reduced bactericidal capacity for <u>E. coli</u>
Kass <u>et al.</u> (1966)	Influenza virus	Mice	Inhibition of bactericidal capacity for staphylococci
Degré (1970)	Parainfluenza-1 virus	Mice	Reduced macrophage phagocytic capacity for <u>H. influenzae</u>

The role of the alveolar macrophage in virus infections has been studied by Roberts (1962), Voisin (1965) and Degré (1970). Influenza B virus particles are phagocytosed by macrophages within one hour of infection. They may be able to destroy the virus or they may become a site of virus multiplication and undergo progressive morphological change and death (Guillaume et al., 1965). However, electron microscopic observations on the lungs of mice following experimental Influenza A infection have shown marked destruction of bronchial Clara cells and ciliated epithelial cells as the main lesion (Plummer and Stone, 1964; Loosli et al., 1970). This presumably is the basis for the slowing of mucus transport observed by Creasia et al. (1973).

The effectiveness of alveolar macrophage activity is dependent to a large extent on both acquired cellular immunity and specific humoral immunity. This is dramatically illustrated by the high prevalence of pyogenic pulmonary infections in immunological deficiency diseases such as congenital hypogammaglobulinaemia (Gell and Coombs, 1968). Also of interest in this regard is the appearance of fungal, yeast and parasitic diseases in the lung of patients with debilitating or malignant diseases or on prolonged corticosteroid or immunosuppressive therapy (Spencer, 1968). Such opportunist infections are thought to be associated with the disappearance of naturally-occurring antibodies from the blood stream of affected patients (Roth and Goldstein, 1962).

The immunologic competence of cells in alveolar exudates has been investigated by Holub and Hauser (1969) who studied rabbit alveolar exudates consisting mainly of macrophages. They found that cultured cells formed antibody after 7-13 days. This was confirmed by Ford and Kuhn (1973) who found that immunoglobulin G (IgG) and immunoglobulin M (IgM) were formed by cells which they classed as mainly lymphocytes. This supports the concept of Fishman (1969) who suggested that alveolar macrophages are a heterogeneous group of cells formed by local division as well as from bone marrow-derived lymphoid cells some of which are capable of antibody production.

During the past few years there has been increasing interest in the significance of secretory immunoglobulin A (IgA) in relation to respiratory

disease. This has been highlighted by the report of South et al. (1968) that people low or lacking in secretory IgA are predisposed to chronic respiratory infections. The majority of immunoglobulins in respiratory secretions are now known to be of the IgA class (Tomasi, 1970). There is evidence that these are formed by plasma cells in the bronchial mucosa and pass into the bronchial lumen via the apical cytoplasm of bronchial epithelial cells (Tourville et al., 1969). No information is available as yet, on the presence or absence of IgA in alveolar secretions.

The relative importance of bactericidal activity compared to mechanical elimination via mucus transport is difficult to estimate because environmental influences may necessitate a shift in emphasis from one system to another. Particle size and the distribution of deposition within the lung are important factors in determining the load on various clearance mechanisms (Hatch and Gross, 1964) as obviously, are the nature and toxicity of the agents which are inhaled. Failure of any one of the alveolar defence mechanisms outlined in the preceding discussion must ultimately lead to alveolar injury and the production of an inflammatory reaction.

PART ONE

THE REACTION OF THE OVINE
PULMONARY ALVEOLUS TO INJURY

CHAPTER 2

STUDIES OF SHEEP PNEUMONIA (THE NATURALLY-OCCURRING DISEASE)

INTRODUCTION

No reports on the pathology of pneumonia in sheep in New Zealand have been published since the first descriptions of acute enzootic pneumonia by Salisbury (1957) and Downey (1957). In some overseas countries however, there has been an upsurge of interest in the disease in recent years leading to several reports which have sought to define local disease situations in clinical and pathological terms. These include reports from the United Kingdom (Stamp and Nisbet, 1963; Stevenson, 1969), Kenya (Wandera, 1967) and Australia (Hore, 1970; St. George and Sullivan, 1973). Most of these reports contain descriptions of chronic or "atypical" pneumonias which have not been reported previously in New Zealand. It is nevertheless, common knowledge among field veterinarians, meat hygienists and veterinary diagnostic pathologists that pneumonias of this type are widespread in lambs throughout New Zealand during the late summer and autumn months (Alley, 1969; Smith, 1970; Davis, 1970).

It is necessary therefore, to begin this study of sheep pneumonia with a description in pathological terms of the main varieties of pneumonia which are encountered in this country. The material from which this description has been derived is biased in a geographical manner in that it was obtained mainly from areas in the lower half of the North Island. While it is likely that climatic factors influence the seasonal occurrence and severity of the disease there is no indication at present of the occurrence of different pathological entities in definable geographical localities within New Zealand.

This chapter includes ultrastructural observations on a range of acute, subacute and chronic pneumonias as well as histochemical studies on normal and pneumonic lung tissue. These studies were made in order to accumulate information on the reaction of the ovine pulmonary alveolus to injury and thus gain a better understanding of the pathogenesis of naturally-occurring pneumonia.

MATERIALS AND METHODS

Animals

For the study of acute pneumonia 52 cases from sheep ranging in age from 2 weeks to more than 6 years were examined during the period 1969 to 1974. Also during this period 64 cases of chronic pneumonia were examined together with a further 32 cases of aspiration pneumonia and pulmonary or pleural abscessation. In addition, 280 cases of chronic and subacute pneumonia of varying degrees of severity were collected from 5 to 10-month-old lambs slaughtered at local meatworks. These were obtained at weekly or fortnightly intervals during the December to May period over the years 1969 to 1972.

In only 4 cases were tissues from sheep with acute pneumonia obtained soon enough after death to be suitable for electron microscopy. However, 3 normal sheep lungs and 47 cases of chronic and subacute pneumonia were examined ultrastructurally. The majority of chronic cases and 2 normals were obtained from local meatworks where lung specimens could be collected within 15 to 20 minutes following death from exsanguination. The remaining specimens were collected within 30 minutes after natural death or within 10 minutes after death following intravenous administration of barbiturate (Euthatal⁽¹⁾) and exsanguination via the brachial artery.

Microscopy

Tissues for light microscopy were fixed in 10% formol-saline, processed in the usual manner and embedded in paraffin. They were cut at 4 to 5 μm and routinely stained with haematoxylin and eosin (HE). A selection of normal and pneumonic tissues were also stained with periodic acid-Schiff (PAS), alcian blue, picro-Mallory, Gomori's trichrome, van Gieson, Gordon and Sweet's reticulin, Von Kossa, Geimsa (Culling, 1963) and Humberstone's Gram (Humberstone, 1963). For electron microscopy blocks 1 mm³ were fixed in 3% glutaraldehyde in 0.1M phosphate buffer at pH 7.2 or in modified Karnovsky's fixative (2% formaldehyde and 3% glutaraldehyde

(1) May and Baker Limited.

in 0.1M phosphate buffer at pH 7.2) at 4°C overnight. They were then post-fixed in 1% osmium tetroxide (pH 7.2) at 4°C for 2 to 3 hours. After dehydration through an ethanol series, the tissues were embedded in epoxy resin (Durcupan ACM⁽¹⁾) via a propylene oxide series. Thick sections (1 µm) were cut with glass knives on a LKB ultramicrotome and stained with toluidine blue, phloxine and haematoxylin (Shires et al., 1969) and toluidine blue and basic fuchsin (TbBf) (see appendix 1). From these, appropriate areas were selected for electron microscopy and the blocks trimmed down accordingly. Thin sections were cut and mounted on supported and unsupported copper grids. They were stained with aqueous uranyl acetate or 50% ethanolic uranyl acetate and lead citrate (Reynolds, 1963) and examined in a Phillips EM200 electron microscope.

Histochemistry

Blocks of fresh normal and pneumonic lung 5 mm thick were used for histochemical studies. They were collapsed by evacuation in a 30 ml bottle to 60 mm Hg for a short time after which pressure was allowed to return slowly to normal. Frozen sections were cut at 8 µm on unfixed blocks and blocks fixed in formol-saline at 4°C and then transferred to ice-cold 0.88M sucrose containing gum acacia, as described by Holt and Withers (1958). These were examined for adenosine triphosphatase (ATPase) and adenosine monophosphatase (AMPase) by the methods of Wachstein and Meisel (1956), and for non-specific alkaline phosphatase and acid phosphatase by the naphthol AS methods (Thompson, 1966). In addition, lactic dehydrogenase (LDH), malate dehydrogenase (MDH), succinic dehydrogenase (SDH), isocitric dehydrogenase (ICDH), glutamate dehydrogenase (GDH), glucose-6-phosphate dehydrogenase (G-6-PDH), hydroxybutyric dehydrogenase (OHBHDH) and diphosphopyridine nucleotide diaphorase (DPN) were identified by the methods of Pearse (1960). The sections were examined at x400 and the various cell types were scored from $\pm$ (very weak) to ++++ (very strong) according to the intensity of the histochemical reaction.

(1) Fluka A.G., Buchs, Switzerland.

RESULTS

Normal Lung

The cells lining the alveoli could not be readily identified by routine methods of light microscopy. Alveolar epithelial (type I) cells were often indistinguishable from capillary endothelial cells, and great alveolar (type II) cells were similar in appearance to peripherally located alveolar macrophages. With the better detail afforded by the use of 1 μ m plastic-embedded sections great alveolar cells could be identified with more confidence. They were cuboidal, with a finely vacuolated cytoplasm and projected into the alveolar space although they were firmly attached to the underlying basement membrane. Approximately one of these cells was seen in every three alveoli examined.

Histochemical studies also succeeded in differentiating great alveolar cells from alveolar macrophages. Great alveolar cells could be seen as regularly spaced cells projecting from the alveolar lining and giving strong reactions for alkaline phosphatase, LDH and MDH, and weak reactions to ATPase, alkaline phosphatase, LDH, MDH, SDH, ICDH and G-6-PDH) (Table VI).

Electron microscopy clearly identified the cellular components of the normal ovine alveolus. The alveoli were lined with flattened (type I) epithelial cells similar to those described originally by Low (1952) (Figure 2.1). Great alveolar (type II) cells similar to those described in mice by Karrer (1956) and Policard et al. (1959) were easily recognised (Figure 2.2). They had the following features:-

- (a) Firm attachment to the basement membrane and projection into the alveolar space.
- (b) Numerous short microvilli protruding from the free surface.
- (c) Characteristic lamellated osmiophilic bodies in the cytoplasm.

The majority of these had a vacuolated appearance although some coarsely lamellated bodies, resembling those described in the foetal lamb by Kikkawa et al. (1965) were seen in occasional cells. Sometimes the contents of these bodies were observed being discharged into the alveolar space (Figure 2.3).

- (d) Numerous large, elongated mitochondria, especially at the base of the cell.

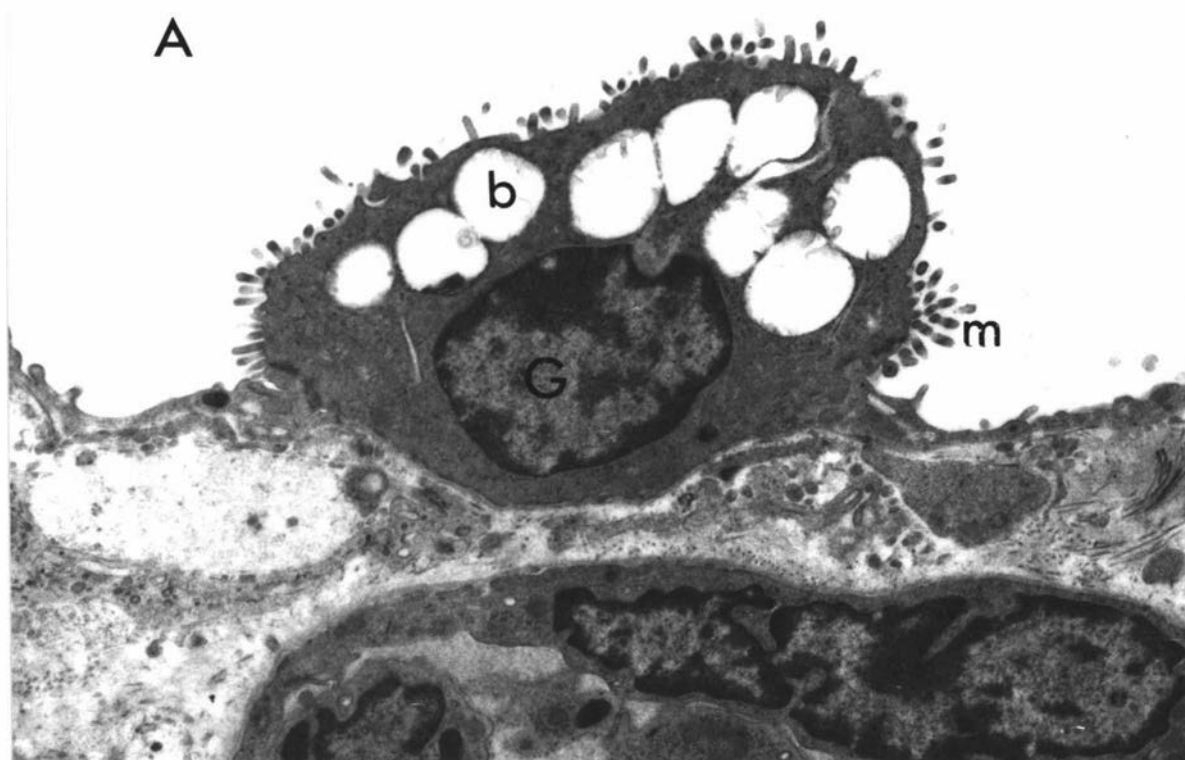
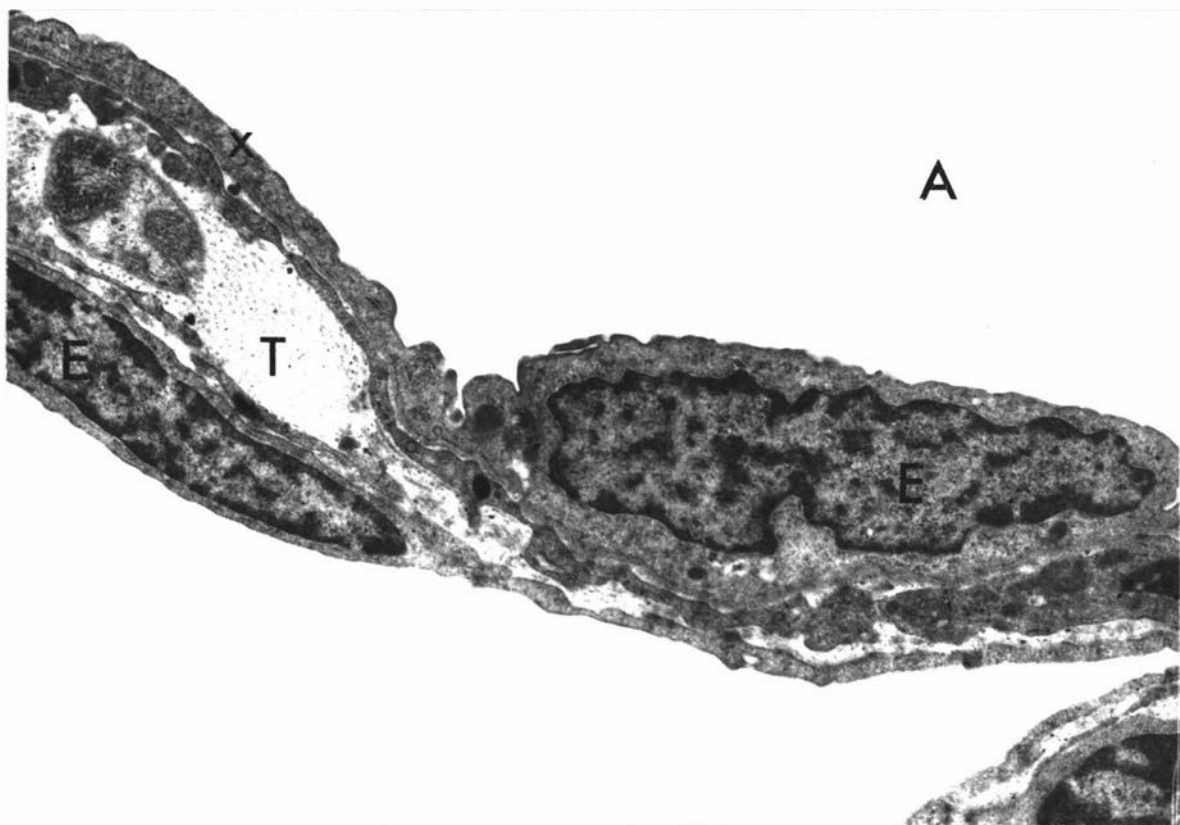
following page 66

Figure 2.1

Normal ovine alveolar type I epithelial cells (E) lining alveolar spaces (A). The cells have a flattened nucleus and thin cytoplasmic extensions (X) which cover the interstitial connective tissue (T). x 12,600.

Figure 2.2

Normal ovine great alveolar type II cell (G) protruding into an alveolar space (A). The cells have numerous short microvilli (m), vacuolated lamellated bodies (b) in the cytoplasm and are firmly attached to the underlying basement membrane. x 12,600.



following page 66

Figure 2.3

Discharge of concentrically lamellated contents from vacuole (V) of an alveolar type II cell into alveolar space (A). The adjacent lamellated body (b) in the cytoplasm contains characteristic coarsely lamellated material seen most often in immature cells. Nucleus (N). x 27,700.

Figure 2.4

Pulmonary alveolar macrophage (M) projecting through a pore of Kohn in the alveolar septum (S) of normal lung. The cell has a moderate amount of cytoplasm and several lysosomes (r) of various types. x 12,600.

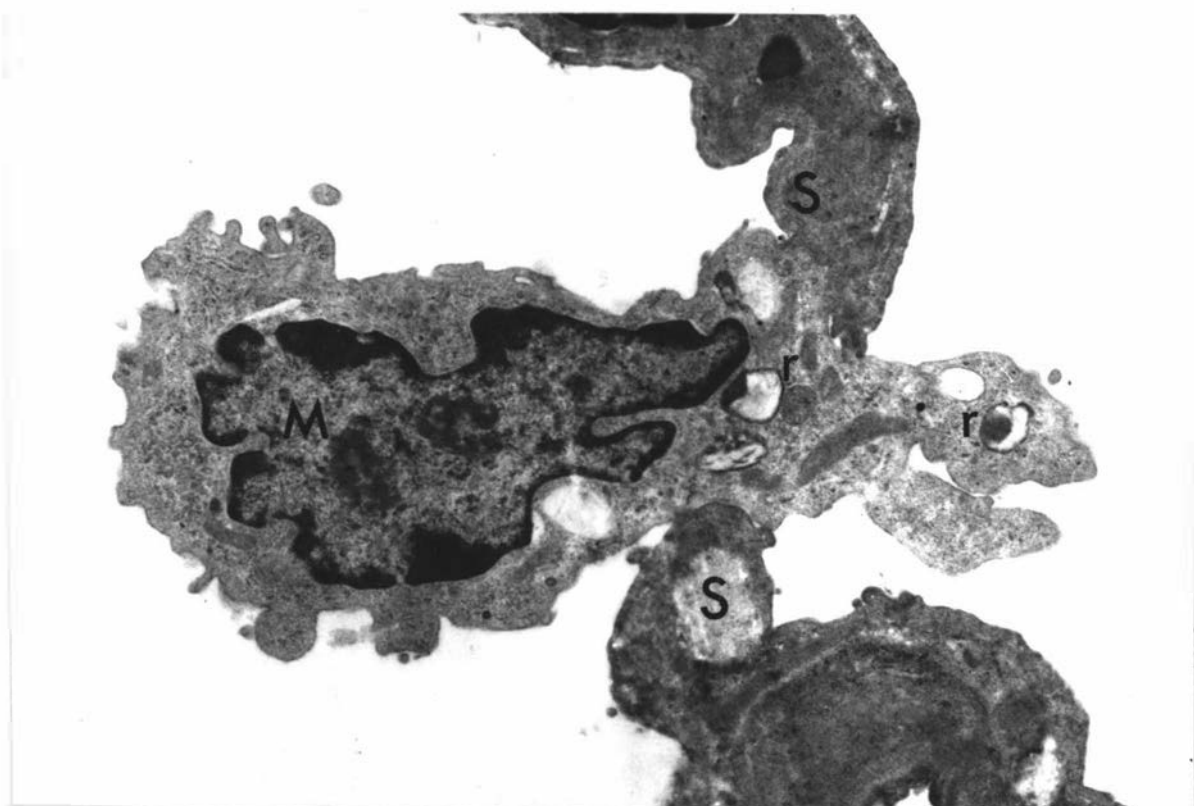
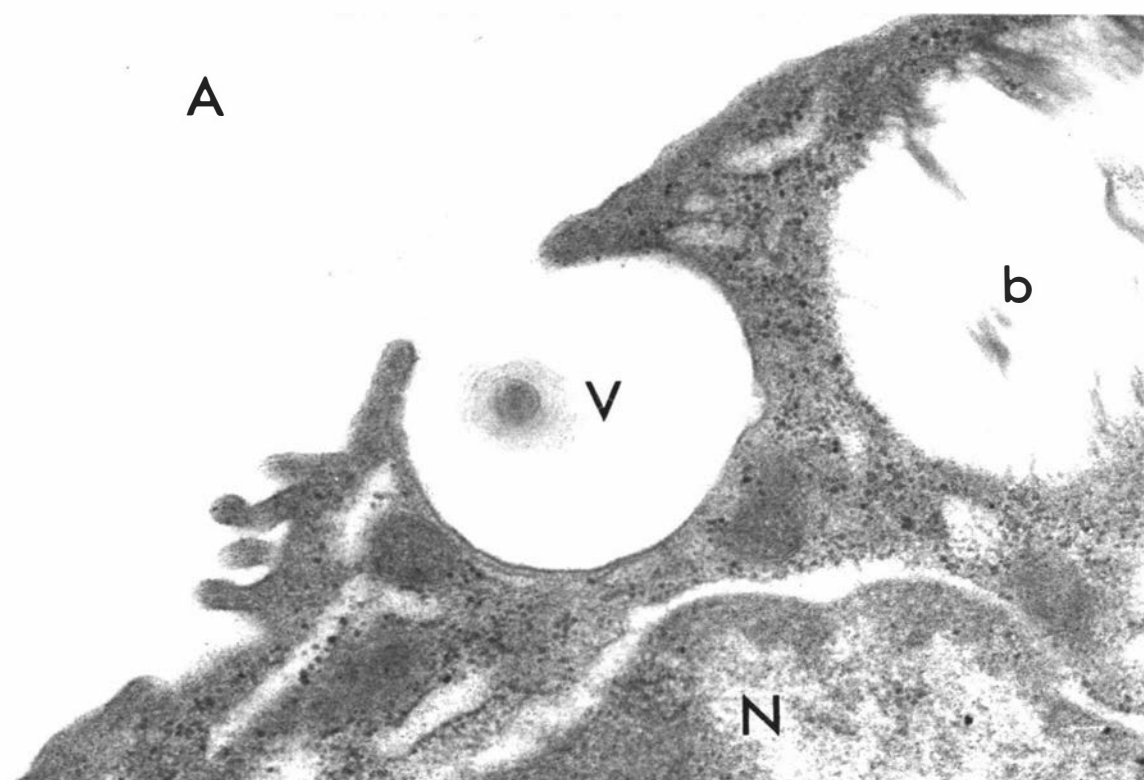


TABLE VI
HISTOCHEMICAL ACTIVITY OF VARIOUS ENZYMES IN THE PULMONARY ALVEOLUS
OF SHEEP WITH CHRONIC PNEUMONIA AND NORMAL SHEEP⁽¹⁾

ENZYMES	TISSUE			
		Great Alveolar (type II cells)	Alveolar Interstitialium	Alveolar Macrophages
<u>PHOSPHATASES</u>				
Adenosine triphosphatase (ATPase)	Normal	+	++	+
	Pneumonic	+	++	++
Adenosine mono-phosphatase (AMPase)	Normal	+	+++	0
	Pneumonic	+	++	±
Non-specific Acid phosphatase	Normal	+	+	+++
	Pneumonic	+	+	++++
Non-specific Alkaline phosphatase	Normal	+++	0	±
	Pneumonic	++++	0	+
<u>OXIDATIVE ENZYMES</u>				
Lactate dehydrogenase (LDH)	Normal	+++	++	+
	Pneumonic	+++	++	+
Malate dehydrogenase (MDH)	Normal	++	+	+
	Pneumonic	++	+	+
Succinic dehydrogenase (SDH)	Normal	0	0	+
	Pneumonic	±	0	+
Glutamate dehydrogenase (GDH)	Normal	+	±	0
	Pneumonic	++	±	0
Isocitric dehydrogenase (ICDH)	Normal	+	0	±
	Pneumonic	+	0	+
Hydroxybutyric dehydrogenase (OHBHDH)	Normal	+	0	0
	Pneumonic	+	0	0
Glucose-6-phosphate dehydrogenase (G-6-PDH)	Normal	+	+	±
	Pneumonic	++	+	±
Diphosphopyridine nucleotide diaphorase (DPN)	Normal	+	+	0
	Pneumonic	++	+	+

(1) The results listed are the average intensity of reaction of 5 lungs with grey-red consolidation and 3 normal lungs.

- (e) Well-developed Golgi apparatus.
- (f) Granular endoplasmic reticulum containing numerous cisternae.

Less frequently seen were alveolar macrophages which were found lying against the alveolar wall or passing through the pores of Kohn. They were readily distinguished from great alveolar cells by their numerous cytoplasmic extensions, lack of attachment to the basement membrane and presence of a variety of residual bodies, lysosomes and phagosomes within the cytoplasm (Figure 2.4).

The structure of the alveolar interstitium was similar to that described in cattle by Epling (1964). Occasional mast cells (Figure 2.5) and fibroblasts were seen but the great bulk of cells present were capillary endothelial cells (Figure 2.6).

Acute Pneumonia

Gross Pathology

The typical gross lesions consisted of red consolidation of the apical, cardiac and ventral diaphragmatic lobes of both lungs (Figure 2.7). Involvement of the diaphragmatic lobes was irregular and often patchy in distribution. The remainder of the lungs were usually congested and oedematous and a blood-stained, frothy exudate was present in the bronchi.

The consolidated areas were swollen and protruded above the surface of the surrounding tissue. They varied from dark red to red-blue in colour and were moderately firm in consistency. Their surface was often covered with thick strands of fibrin which were mildly adherent to the overlying thoracic pleura. In some cases, grey, necrotic areas were present within ventral parts of the consolidated tissue and these areas occasionally involved whole lobes (Figure 2.8).

The thoracic cavity contained a variable amount of clear exudate in which occasional strands of fibrin could be found. Petechial haemorrhages were sometimes seen on the parietal pleura, pericardium and epicardium. The mediastinal and bronchial lymph nodes were usually swollen and red.

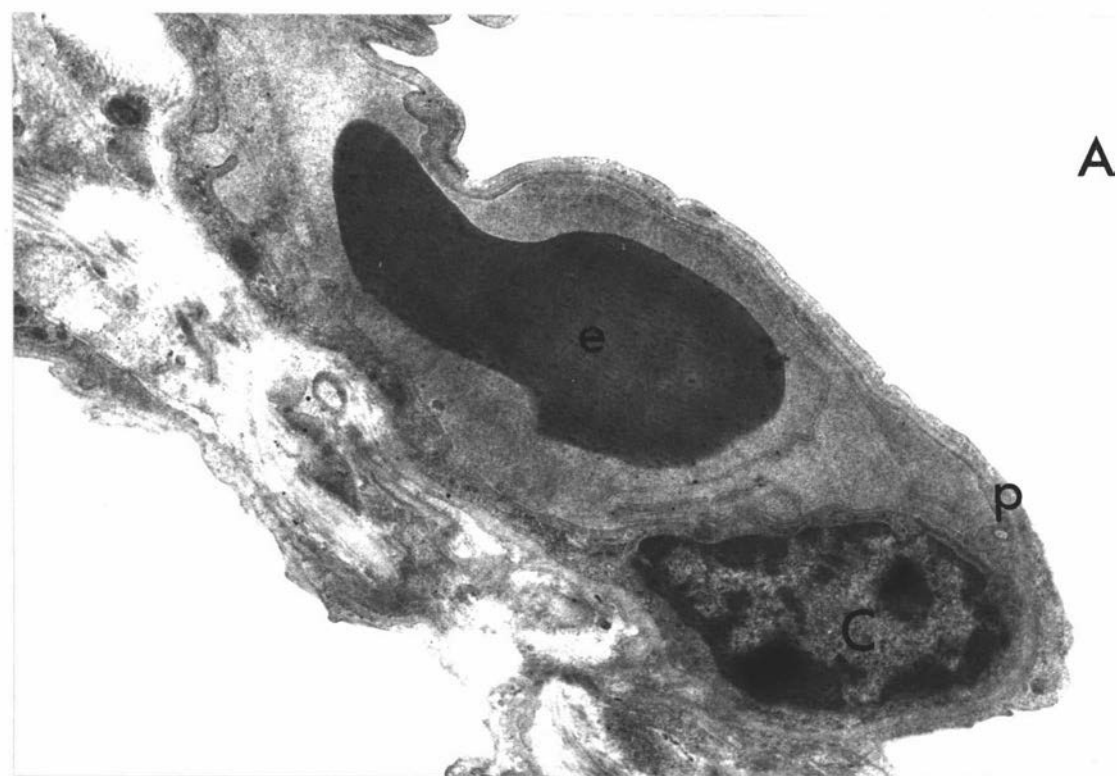
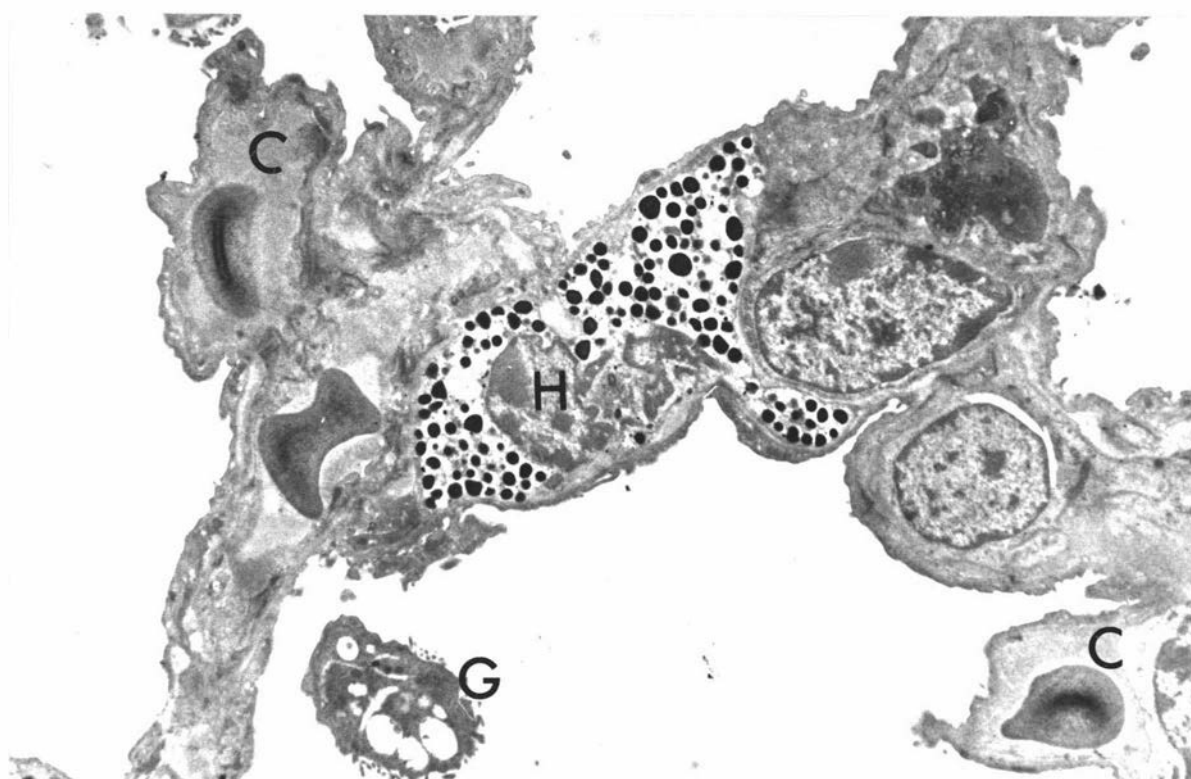
following page 68

Figure 2.5

Mast cell (H) containing dense cytoplasmic granules in the alveolar interstitial space of normal ovine lung. Several capillaries (C) contain erythrocytes and a fragment of an alveolar type II cell (G) can also be identified. x 10,000.

Figure 2.6

Alveolar capillary in a normal lung containing an erythrocyte (e) and lined by capillary endothelial cell (C). The thin blood-air barrier (p) separates the erythrocyte from the alveolar space (A). x 12,600.



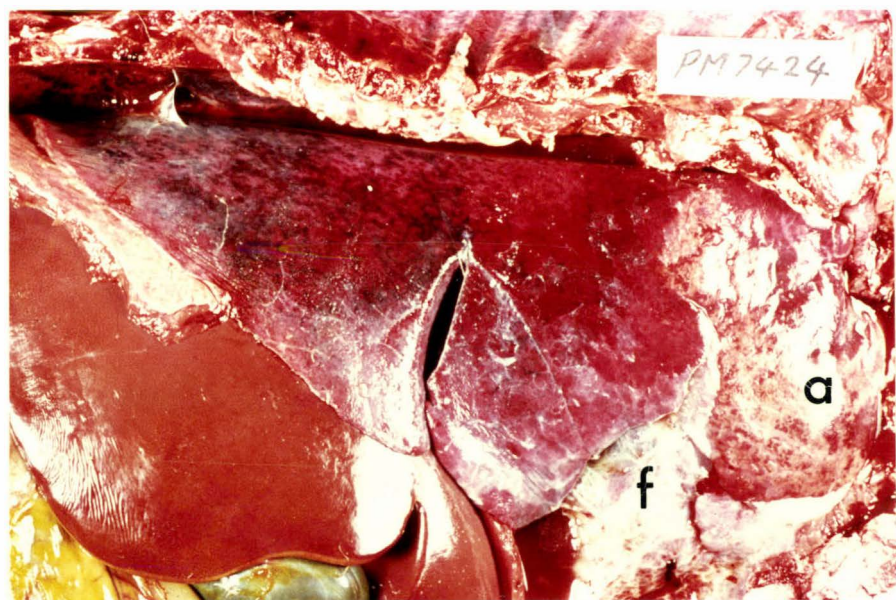
following page 68

Figure 2.7

Acute enzootic pneumonia. The right apical lobes show dark red consolidation and are swollen above the surface of the remaining lung.

Figure 2.8

A more advanced case of acute enzootic pneumonia. The right apical lobe (a) is necrotic and the remaining lung is severely congested. Thick deposits of fibrin (f) are visible between the apical and cardiac lobes and the pericardial sac.



Light Microscopy

The characteristic feature of all the lesions examined was the severe exudation of both cells and fibrin into alveolar spaces. This was accompanied by severe congestion of alveolar capillaries with haemorrhage into alveoli in some areas. The cellular reaction varied considerably in degree but was usually relatively uniform in type.

The most mild changes were seen at the periphery of the consolidated areas and consisted of the accumulation of variable numbers of neutrophils and occasional small macrophages within a proteinaceous exudate in alveoli (Figure 2.9). Sometimes alveolar spaces were distended with randomly distributed necrotic cellular debris (Figure 2.10) but more often they contained densely packed, elongated nuclear material which could frequently be seen "streaming" through pores of Kohn and into alveolar ducts and bronchioles (Figure 2.11 and 2.13). These elongated cells were sometimes embedded in fibrin (Figure 2.12) and occasionally in mucus (Figure 2.13). Large alveolar macrophages with abundant PAS-positive cytoplasmic contents were found in variable numbers in areas away from the main lesions. They were mixed with moderate numbers of neutrophils or embedded in fibrinous exudate (Figure 2.14).

The interstitial tissues were oedematous and the interlobular and pleural spaces were usually distended with a fibrinous exudate sparsely infiltrated by neutrophils and large mononuclear cells in equal numbers (Figure 2.15). In some cases fibrinoid thrombi and moderate numbers of neutrophils were found within alveolar capillaries and interlobular blood vessels.

Areas of necrosis appeared to arise primarily from bronchioles (Figure 2.16) although in some cases they extended widely across whole lobules with only ghosts of alveoli remaining. Such areas were often margined by necrotic cellular exudate or haemorrhage. Bacterial colonies were usually found within these necrotic areas and at the periphery of necrotic lobules (Figure 2.17). Bacteria were also readily identified elsewhere by the use of special stains or in plastic embedded sections. They were present within the necrotic contents of alveolar

following page 69

Figure 2.9

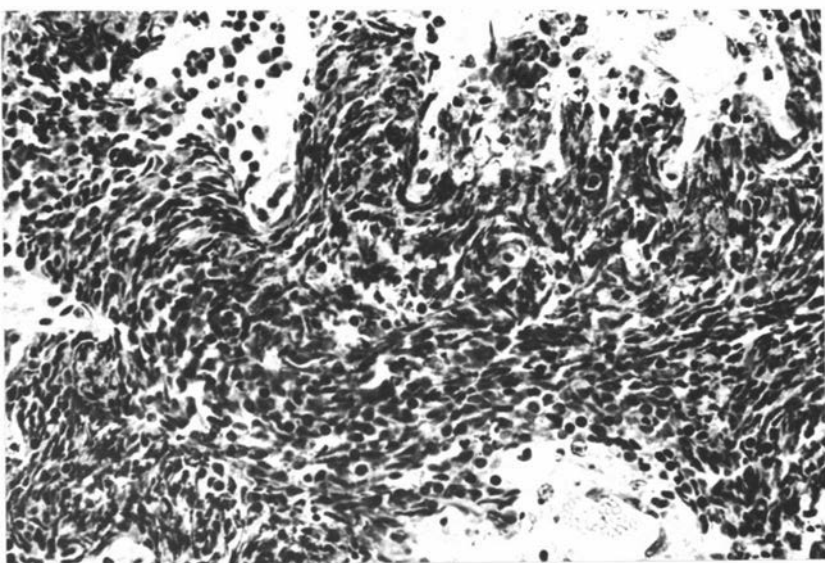
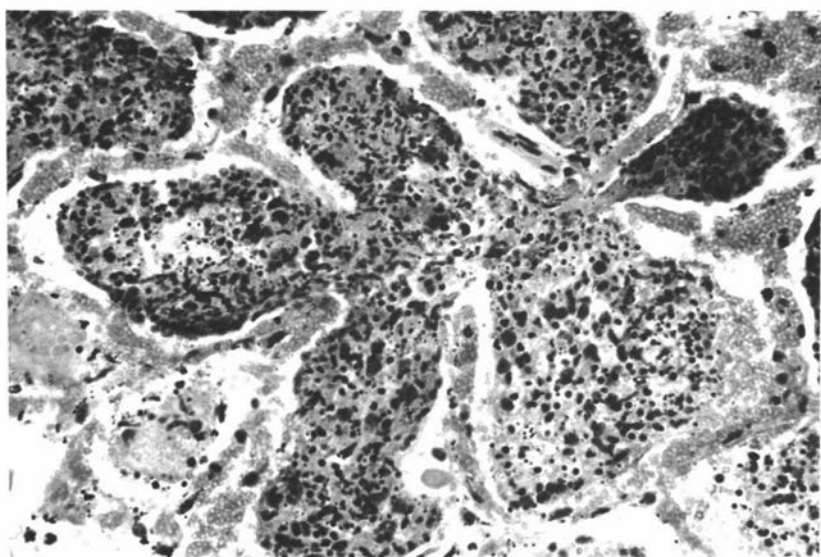
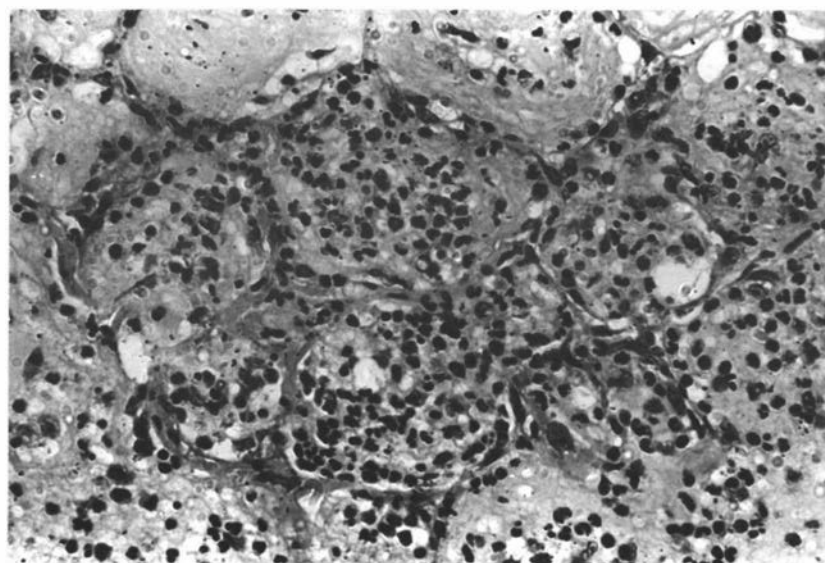
Early accumulation of cellular exudate in acute pneumonia. Neutrophils and small mononuclear cells migrating into alveolar spaces which are filled with protein-rich exudate. HE x 250.

Figure 2.10

Necrosis of cellular exudate. Alveolar spaces are distended with randomly distributed necrotic debris embedded in an amorphous exudate. Alveolar capillaries are severely congested. HE x 250.

Figure 2.11

Parallel elongation of pyknotic nuclei in necrotic cellular exudate. The "streaming" appearance is characteristic of acute enzootic pneumonia in sheep. HE x 250.



following page 69

Figure 2.12

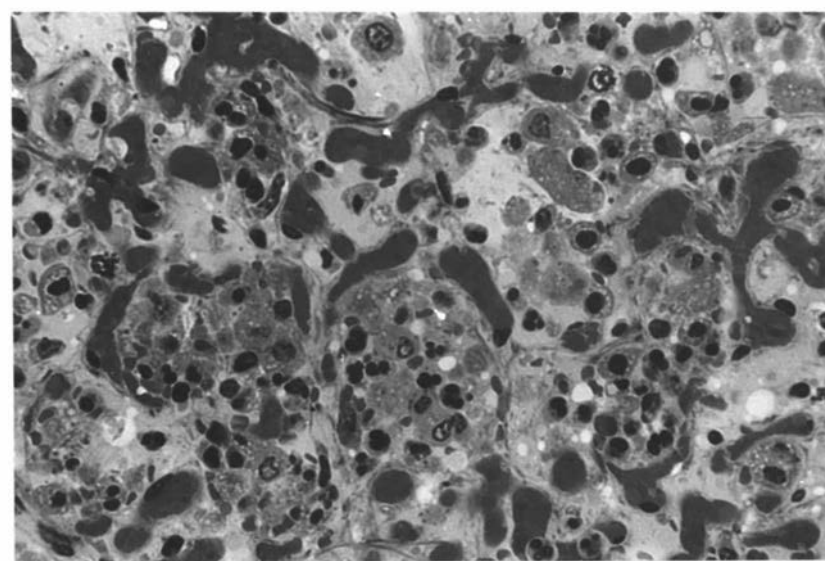
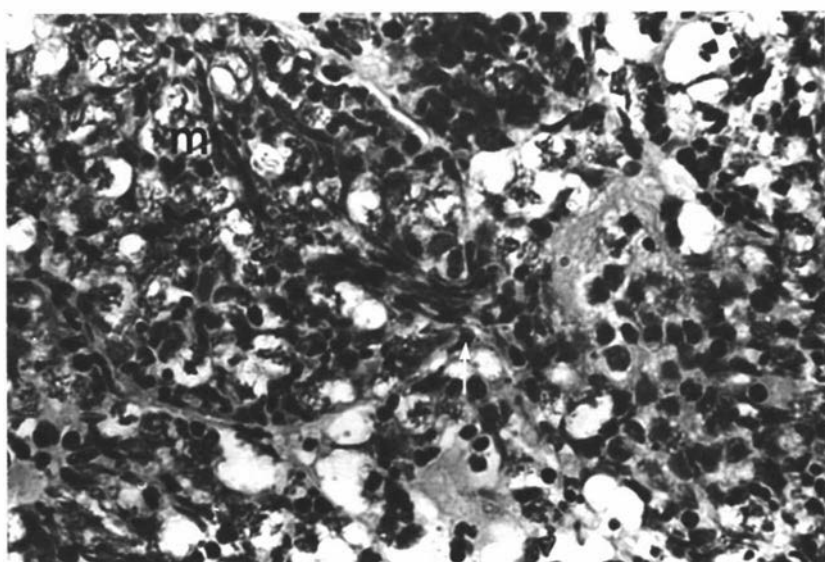
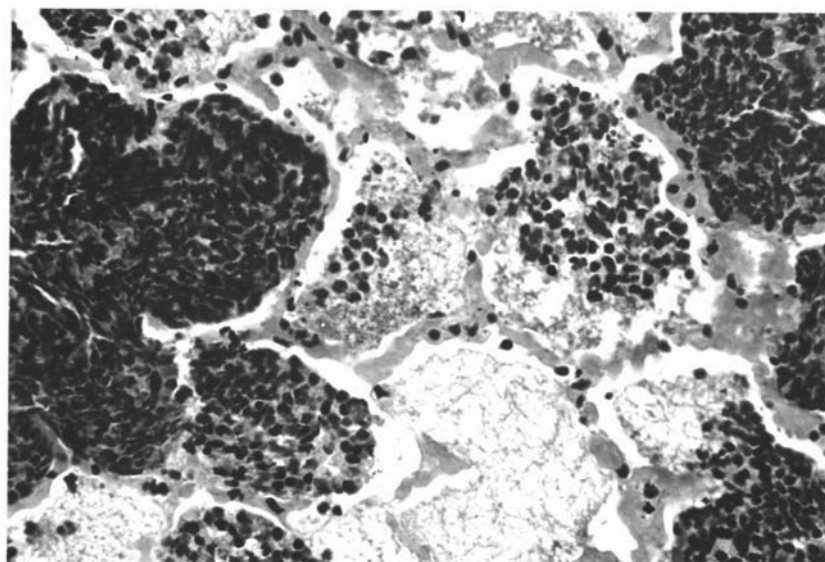
Alveolar spaces adjacent to those containing cellular exudate are distended with variable amounts of fibrin and proteinaceous fluid. Apart from leucocytes within the congested alveolar capillaries, few cells can be recognised in the alveolar septa. HE x 250.

Figure 2.13

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.

Figure 2.14

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



following page 69

Figure 2.15

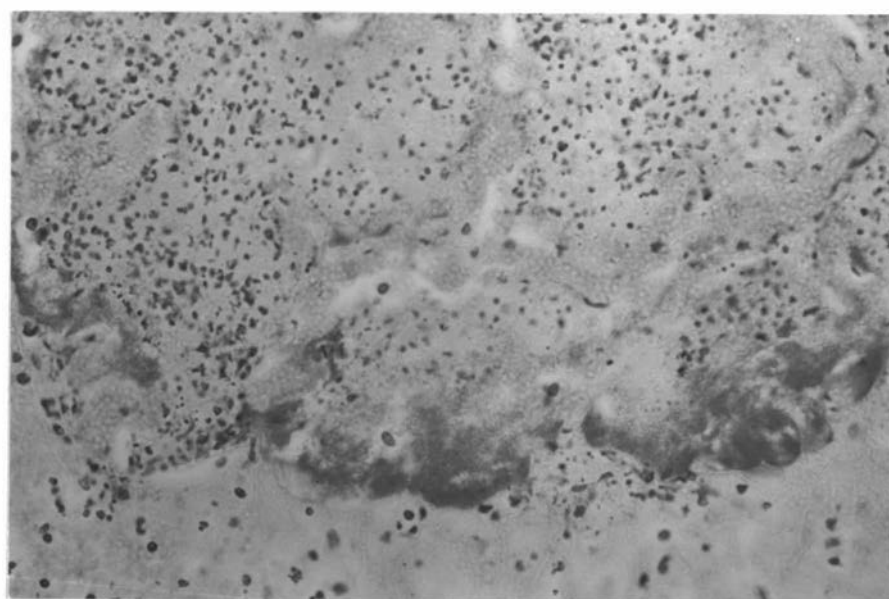
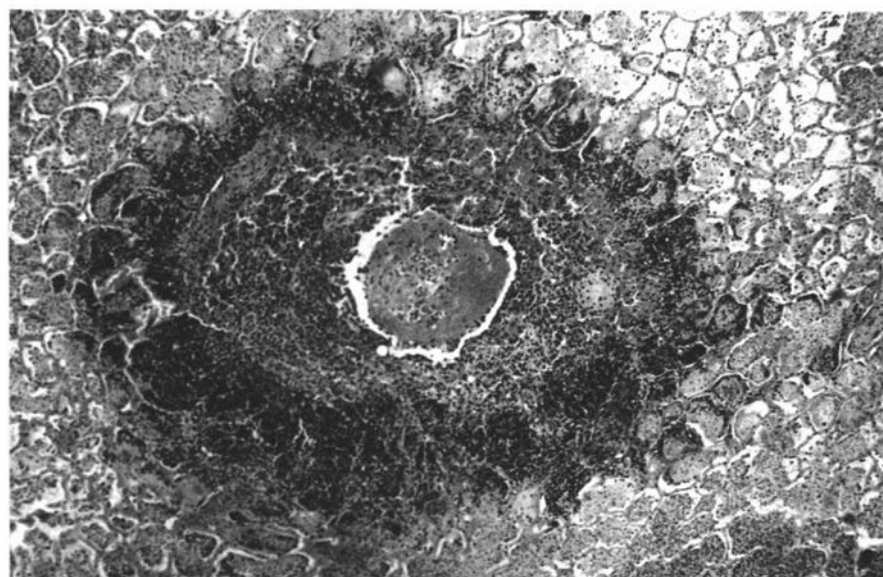
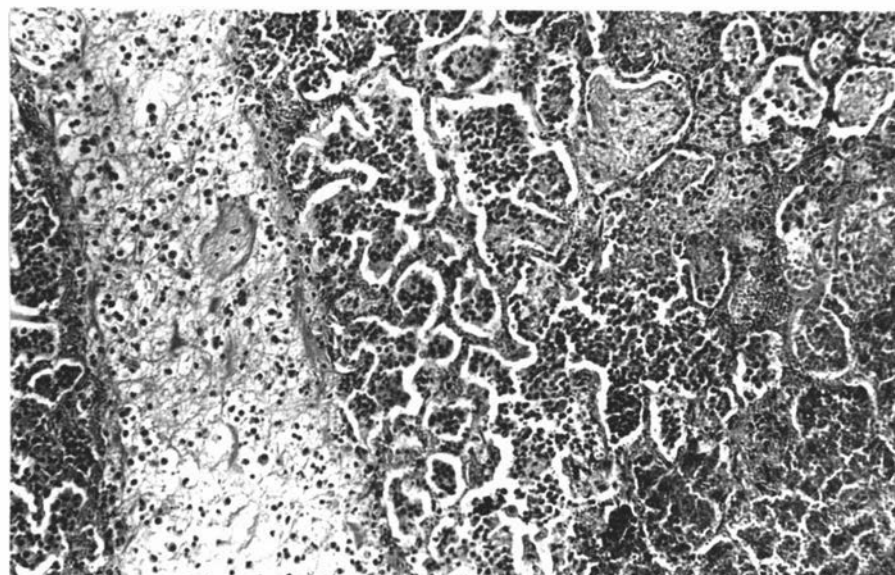
Distended interlobular interstitial space containing strands of fibrin and infiltrations of equal numbers of mononuclear cells and neutrophils. The adjacent alveolar spaces are filled with a mixture of haemorrhagic, fibrinous and cellular exudate.
HE x 100.

Figure 2.16

Area of necrosis surrounding a bronchiole. The necrotic area is demarcated by a margin of severe leucocytic infiltration.
HE x 40.

Figure 2.17

Colonies of Gram-negative bacteria located within alveoli at the periphery of a necrotic lobule. Humberstone's Gram.
x 400.



spaces and bronchioles.

Electron Microscopy

One of the most outstanding features observed in all sections was extensive destruction of the alveolar epithelium. Even in the least severely affected areas alveolar epithelial (type I) cells showed severe damage. This ranged from cytoplasmic vesiculation and blurred cell membranes, to complete sloughing and disintegration (Figure 2.18). A thick proteinaceous exudate often separated the alveolar epithelium from the underlying basement membrane and occasionally macrophages or neutrophils were present within this space (Figure 2.19). In the most severely affected areas where cellular exudate was the main feature, the alveolar epithelium was often completely absent. However, sometimes remnants of epithelium could be detected amongst the necrotic cellular exudate (Figure 2.20).

Great alveolar (type II) cells were sparse and difficult to identify in all the sections examined. The majority of these cells had apparently desquamated in the earlier stages of infection. However, occasional degenerate type II alveolar cells were observed in the least severely affected areas. These had a vesiculated, pale-staining cytoplasm and were often lifting from the basement membrane (Figure 2.21). Their plasma membranes were indistinct and they contained few lamellar bodies. Microvilli were reduced in number but when present they allowed easy recognition of the cells despite the extensive degenerative changes.

The integrity of the basement membrane appeared to be the main factor preventing complete destruction of the alveolar walls in areas devoid of epithelium. Thickening and duplication of the basement membrane were frequently observed in these circumstances (Figure 2.20). When destruction of the basement membrane occurred, fibrin and platelets could be seen passing into the adjacent interstitial space (Figure 2.22). Similarly, with more extensive ruptures, erythrocytes and neutrophils were extravasated into the surrounding tissue (Figure 2.23). In general, the capillary endothelium showed a lesser degree of damage than the alveolar epithelium and was sometimes found intact following rupture of

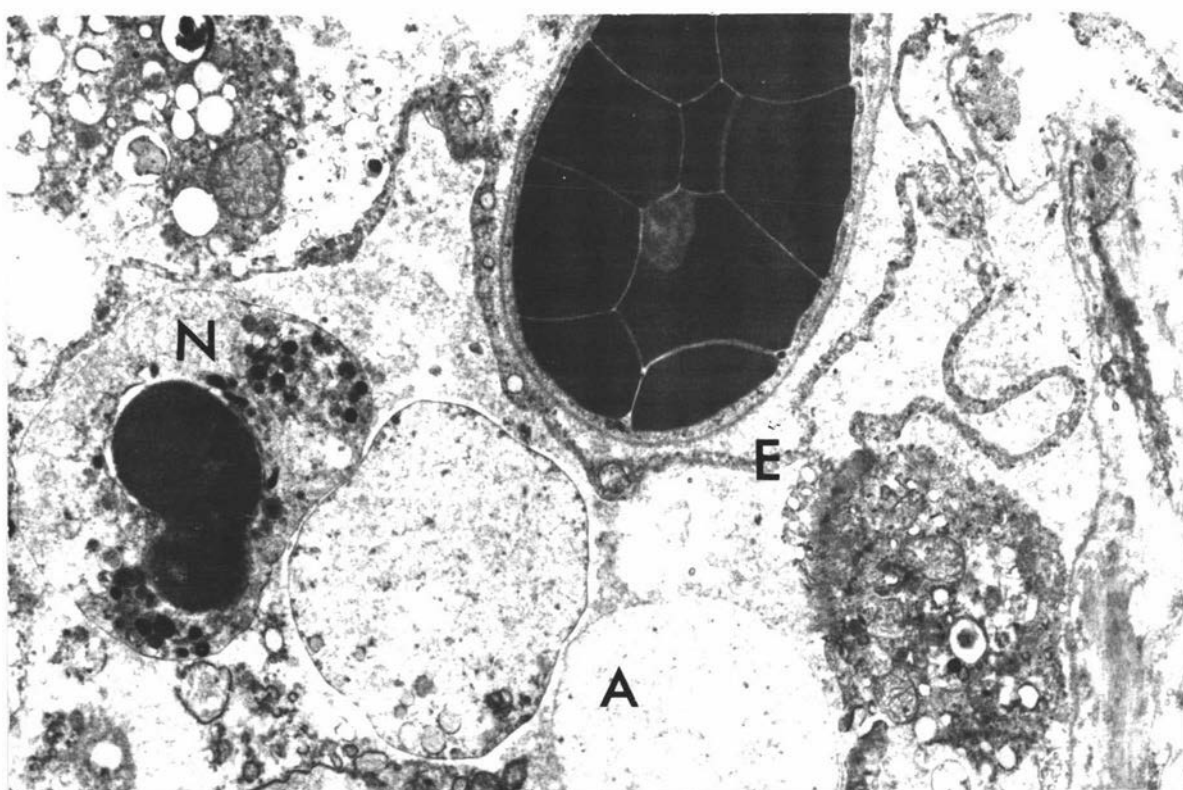
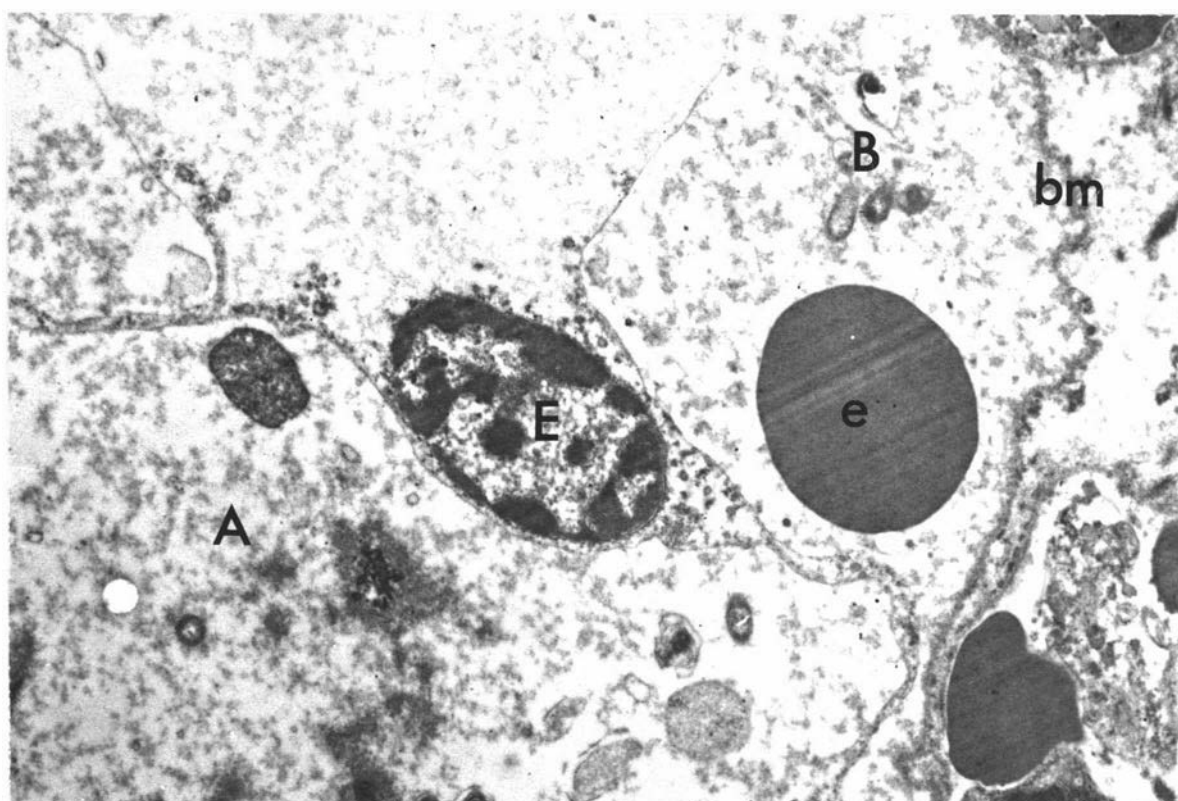
following page 70

Figure 2.18

Disintegration and sloughing of alveolar epithelial type I cell (E) into alveolar space (A). Between the desquamating cell and the underlying basement membrane (bm) is an erythrocyte (e) and bacteria (B). x 9,000.

Figure 2.19

Necrotic alveolar macrophages (M) are present beneath the alveolar epithelial type I cell (E) which is desquamating into the alveolar space (A). Within the alveolus a degenerating neutrophil (N) and other cellular debris are already present. The endothelial lining of the capillary (C) appears relatively undamaged. x 9,000.



following page 70

Figure 2.20

Clumps of bacteria (B), neutrophils (N) and possible remnants of alveolar epithelium (E) are present in the necrotic alveolar exudate. The basement membrane (bm) of the surrounding alveolar wall is exposed and the underlying interstitial tissues are oedematous and contain strands of fibrin.
x 9,000.

Figure 2.21

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.

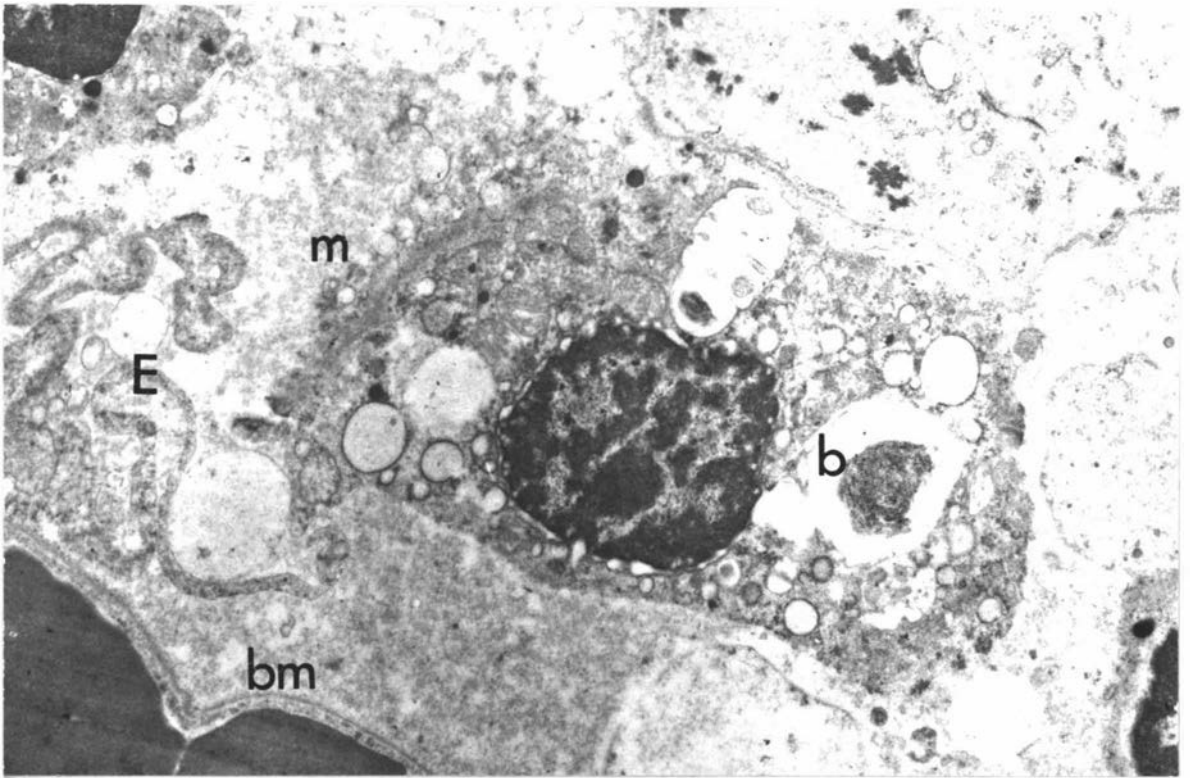
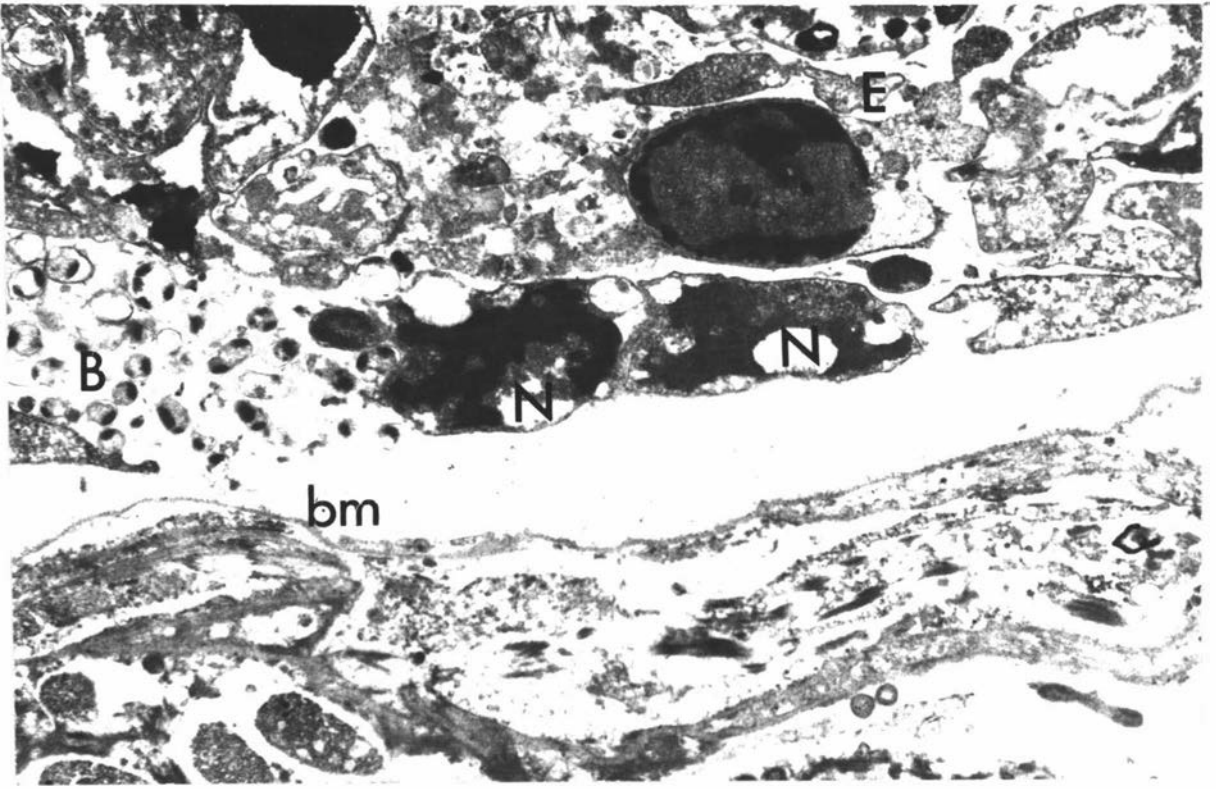
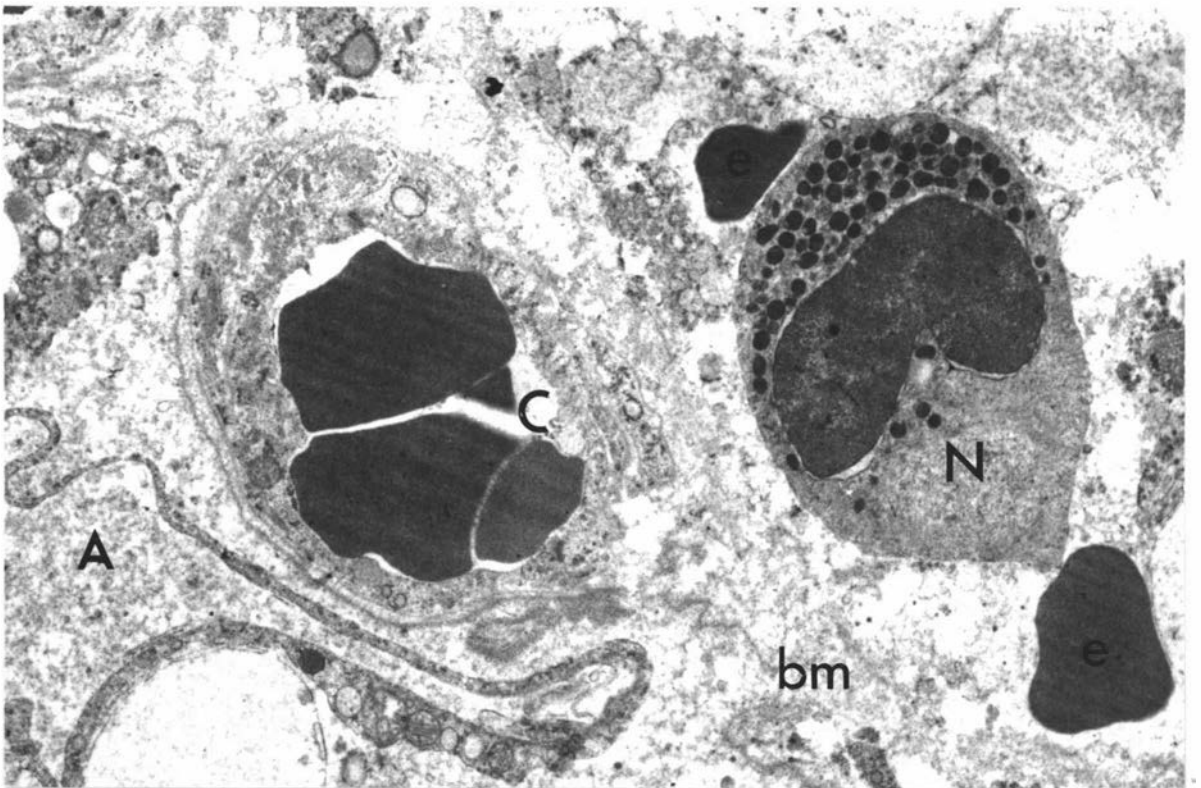
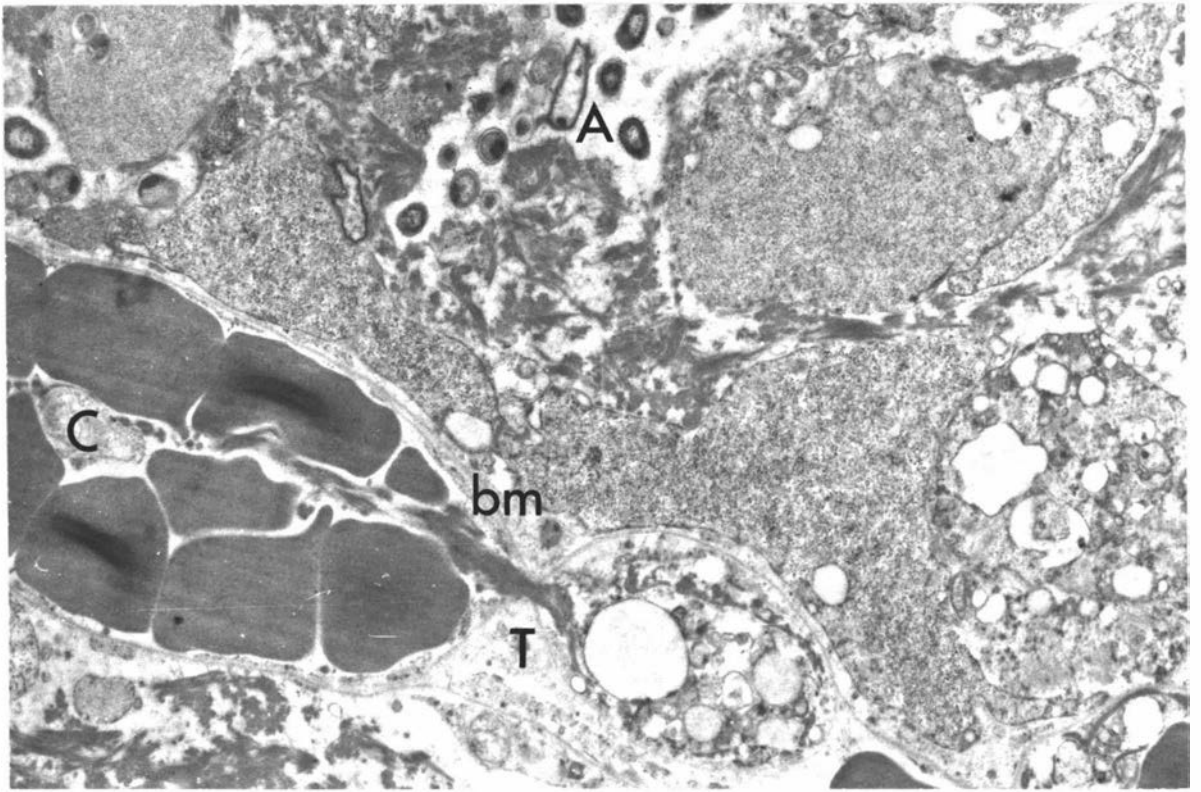


Figure 2.22

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.

Figure 2.23

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.



the basement membrane. Occasionally however, severe vesicular changes and disintegration occurred in parallel with alveolar epithelial damage. This resulted in the endothelial lining being replaced by a thin layer of necrotic debris in which strands of fibrin were often present.

The cellular exudate in the central areas of the alveoli, alveolar ducts and bronchioles consisted largely of compressed necrotic debris which was lacking in fine structural detail (Figure 2.24). Flattened remnants of nuclei with coarsely granular, homogenous contents were embedded in a coagulum of lysed cytoplasmic material. Observations made at the periphery of these areas indicated that several cell types were contributing to the exudate. A large proportion of cells present appeared to be neutrophils (Figure 2.25) but in addition macrophages, small monocytes and alveolar epithelial cells were also identified (Figure 2.20).

Bacteria were always a prominent feature at the ultrastructural level in this series of cases. They were found in the majority, but not all of the alveolar spaces sectioned. In some instances they were present in large numbers at high density (Figure 2.26) but more often they occurred in small aggregations embedded in necrotic cellular exudate or fibrin (Figure 2.20). Their presence was usually associated with severe destructive changes in the alveolar wall and they could sometimes be found embedded in necrotic alveolar epithelium or between the alveolar epithelium and the underlying basement membrane (Figure 2.27).

Subacute and Chronic Pneumonia

Gross Pathology

Fundamentally, the lesions consisted of varying degrees of consolidation of the antero-ventral parts of both lungs. However, a broad spectrum of lesions were encountered varying considerably in colour, consistency and extent. The areas of consolidation were always sharply delineated from the dorsal areas of lung tissue which were usually normal apart from the occasional margin of compensatory emphysema adjacent to the consolidated tissue and the variable presence of Mullerius capillaris and Dictyocaulus filaria lesions.

following page 71

Figure 2.24

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.

Figure 2.25

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

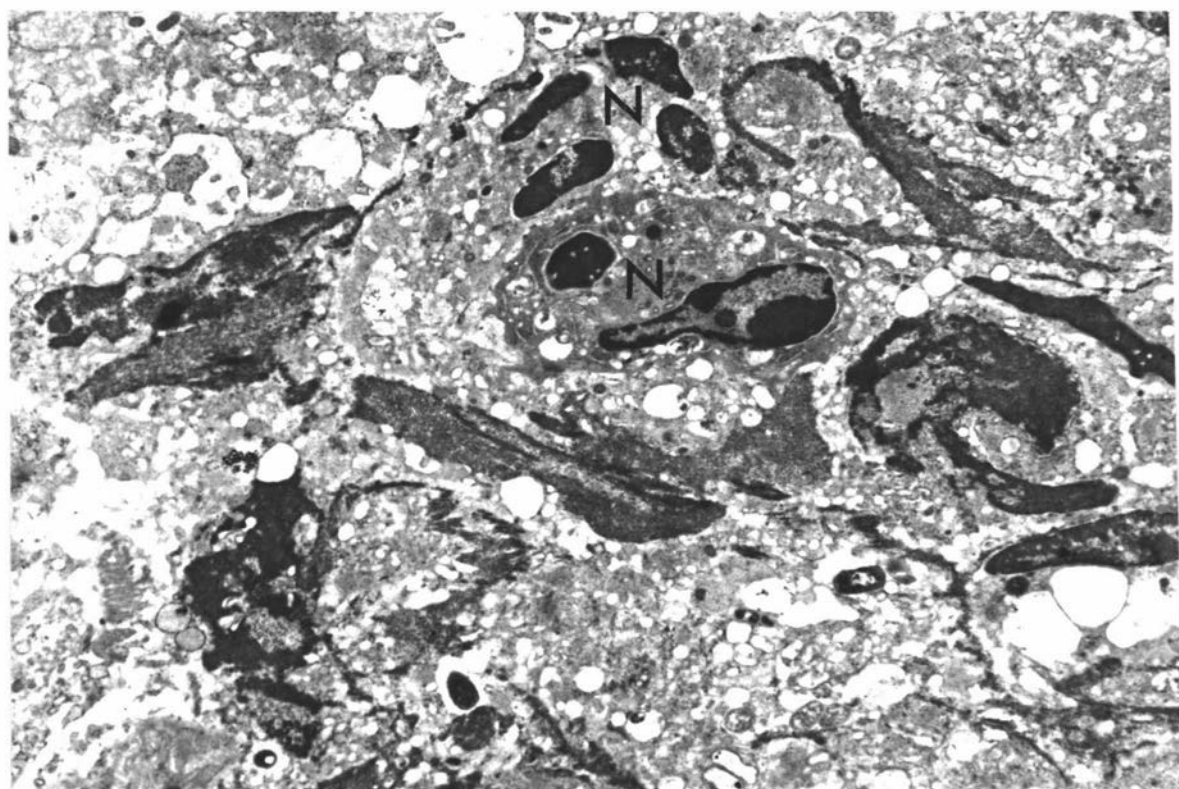
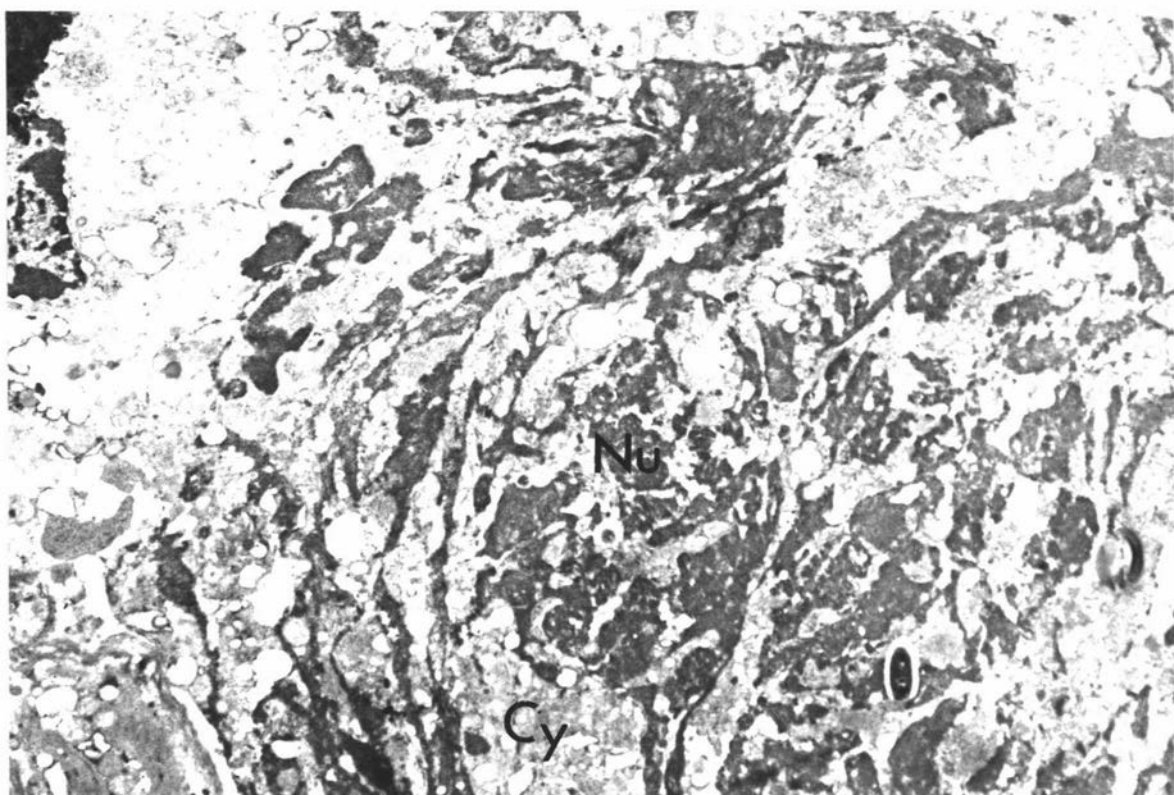
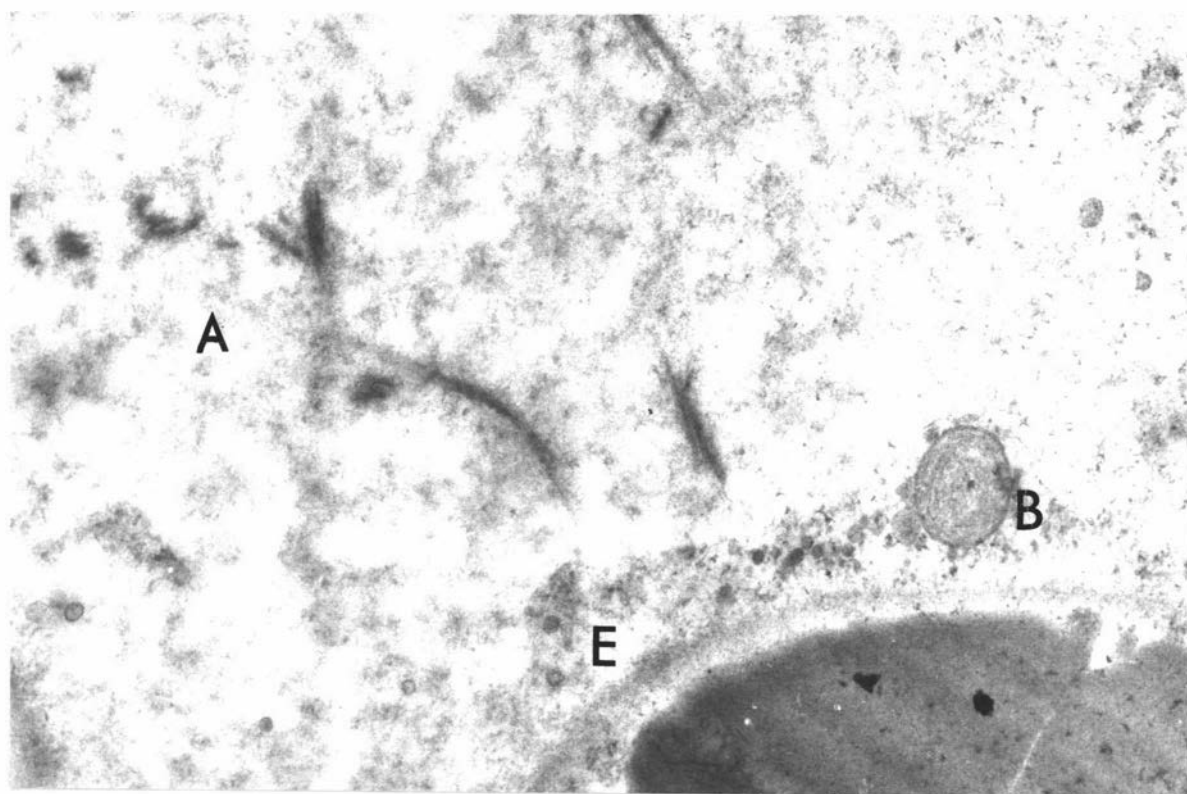
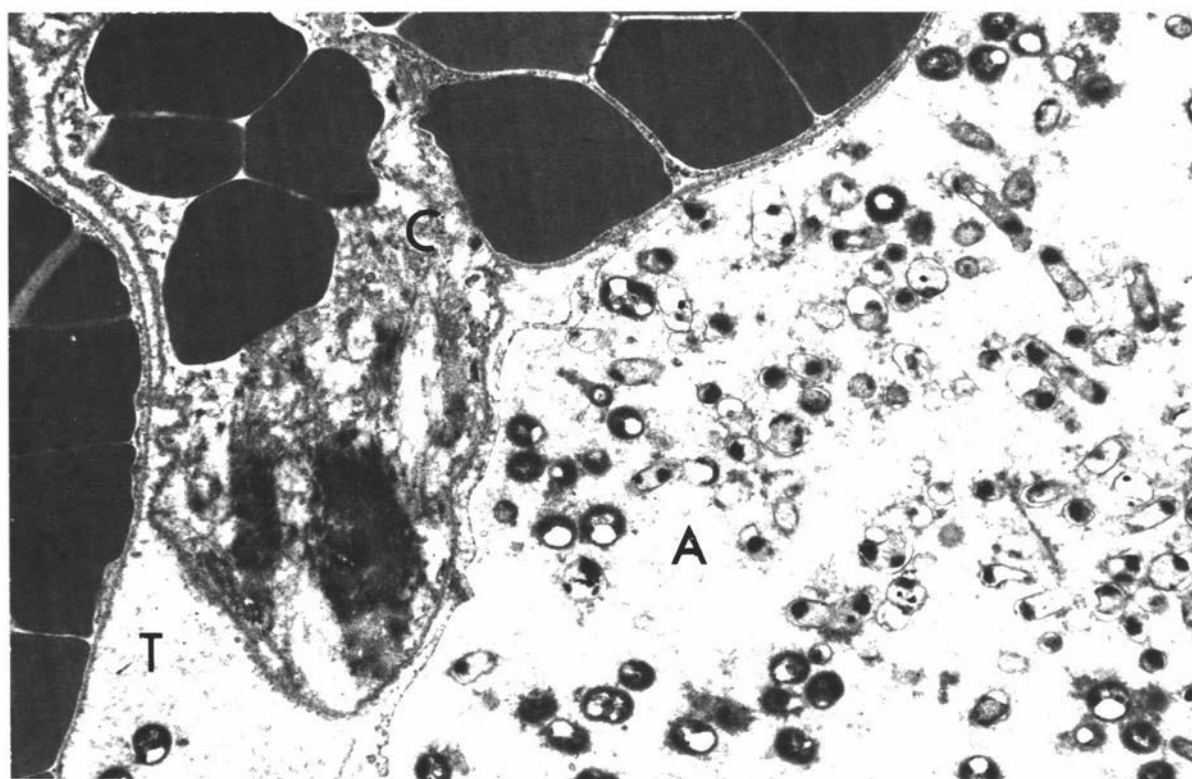


Figure 2.26

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.

Figure 2.27

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.



In order to study the pathology of these lesions in more detail they were divided into 4 categories on the basis of their gross and histological appearance. Because of the overlap of lesions in adjacent categories it was difficult to firmly classify all the affected lungs on gross examination alone. A proportion were therefore classified provisionally at the time of gross examination, and subsequently approximately 10% of all lungs were reclassified on the basis of their histological features.

Type 1 (Dull red consolidation). The consolidated areas were dull red in colour and were found mainly in the ventral apical and cardiac lobes of both lungs (Table VII). They were sunken below the surface of the adjacent normal lung and were of a homogeneous appearance with a smooth pleural surface (Figure 2.28). Dull red, linear areas of collapse, approximately 5mm in width were often found associated with these lesions or were seen in other ventral areas of the lung, however linear lesions of this nature were not included in this category.

Type 2 (Red-grey consolidation). The gross lesions in this category were usually more extensive and consisted of red-grey consolidation of the ventral apical, cardiac and occasionally intermediate lobes. Although they were sharply delineated from the normal lung they did not sink below its surface (Figure 2.29). On close examination numerous grey-white foci, 1-2mm in diameter could often be seen within the consolidated tissue and the pleural surface had an uneven granular appearance in reflected light.

Type 3 (Grey-red consolidation). The lungs in this group showed extensive grey-red consolidation which usually involved whole lobes. In the least severe cases the lesions involved the right apical lobe but more often they extended into apical, cardiac and intermediate lobes and in more than half the cases also involved the ventral diaphragmatic lobes (Table VII). The consolidated tissue had a finely granular, mottled appearance, firm consistency and affected lobes were thicker than normal (Figure 2.30).

Type 4 (Grey consolidation). In this category were placed lungs with

TABLE VII

FREQUENCY OF ANATOMICAL DISTRIBUTION OF LESIONS IN 120 CASES
OF SUBACUTE AND CHRONIC PNEUMONIA

Right Apical Lobe	115	(96%)		
Left Cardiac Lobe	104	(87%)		
Right Cardiac Lobe	103	(86%)		
Left Apical Lobe	83	(69%)		
Intermediate Lobe	67	(56%)		
Right Diaphragmatic Lobe	41	(34%)		
Left Diaphragmatic Lobe	40	(33%)		

	Apical	Cardiac	Right Lung Diaphragmatic	Intermediate
Type 1				
Dull red consolidation	28	23	7	12
Type 2				
Red-grey consolidation	27	27	6	15
Type 3				
Grey-red consolidation	30	28	17	21
Type 4				
Grey consolidation	30	25	11	19
	115	103	41	67

	Apical	Cardiac	Left Lung Diaphragmatic	Total
Type 1				
Dull red consolidation	17	23	4	30
Type 2				
Red-grey consolidation	21	27	8	30
Type 3				
Grey-red consolidation	23	27	16	30
Type 4				
Grey consolidation	22	27	12	30
	83	104	40	120

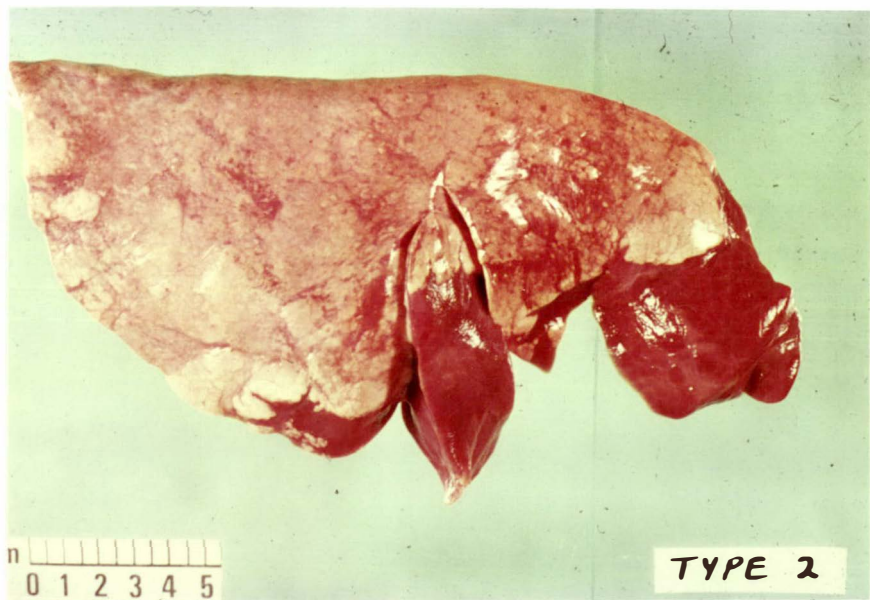
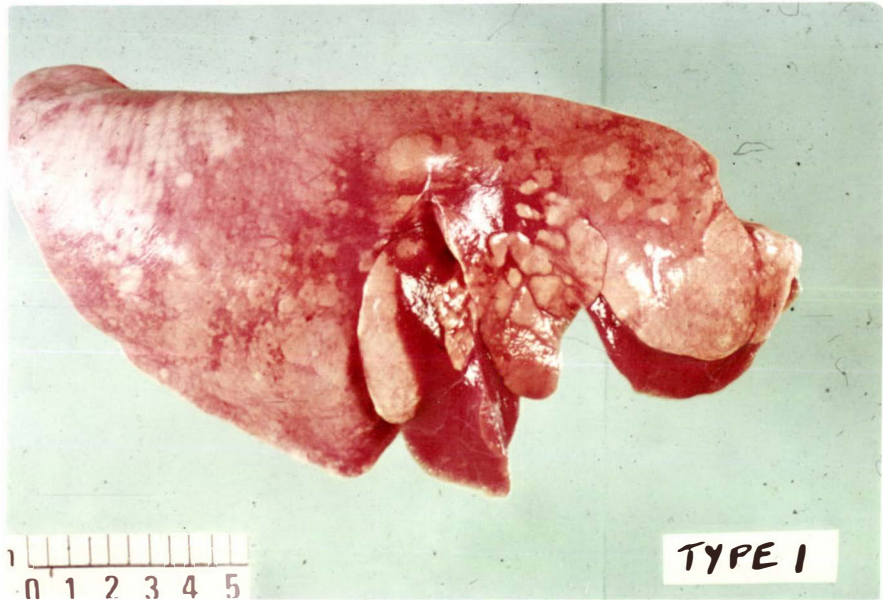
following page 73

Figure 2.28

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.

Figure 2.29

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.



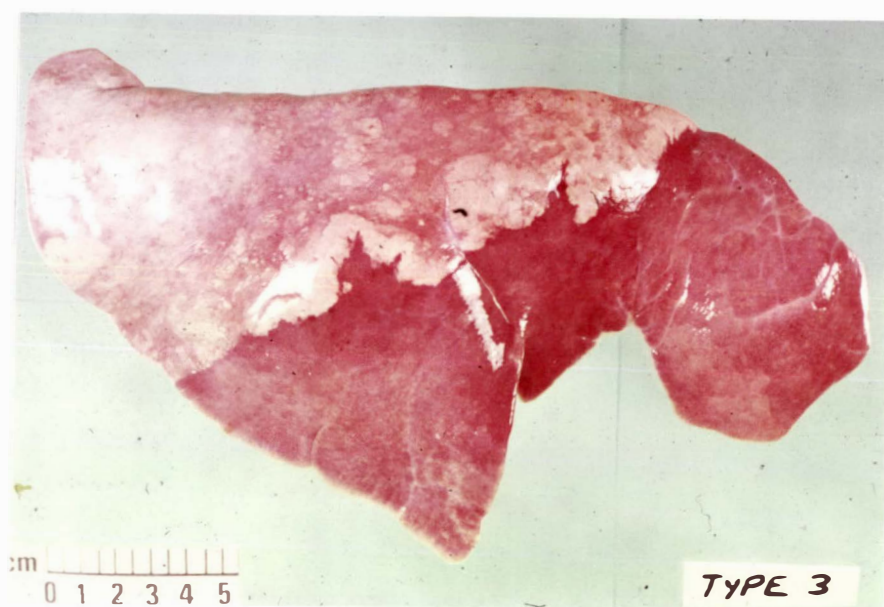
following page 73

Figure 2.30

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.

Figure 2.31

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.



severe grey consolidation which varied in extent but was always confined to the antero-ventral parts of the lungs. The consolidated tissue was very firm and affected lobes were thicker than normal. The cut surface had a finely mottled texture or was homogeneous and pale grey in appearance (Figure 2.31). Occasionally, fibrous adhesions were present between the pleural surface of the affected lobes and the parietal pleura or between the caudal surface of the cardiac lobes and the antero-ventral surface of the diaphragmatic lobes of both lungs (Figure 2.32).

In addition to these 4 types of lesions some lungs were seen in which the ventral margins of apical and cardiac lobes showed dull grey consolidation which merged dorsally with a zone of emphysematous lung in an irregular and patchy manner (Figure 2.33). This sometimes produced islands of aerated tissue, up to 1cm in diameter which were embedded in the margins of the consolidated tissue. Lesions of this nature were interpreted as representing recovery from earlier consolidation. In some cases of near complete resolution, areas of mild emphysema and thin bands of collapse remained in the apical lobes (Figure 2.34). Many of the lungs in which recovery had occurred showed shortening and distortion of the apical lobes or severe adhesions between the ventral lobes which often resulted in the complete fusion of the cardiac with the adjacent diaphragmatic or apical lobes (Figure 2.32, 2.34 and 2.35).

The prevalence of both type 1 lesions and thick linear bands of collapse appeared to be greater during the summer months, at the beginning of each pneumonia season whereas the proportion of type 3 and 4 lesions as well as dull grey areas undergoing recovery appeared to be greater in the late autumn (April-June period). However, a monthly comparison of lesions was not undertaken as the considerable variation in the prevalence of the disease between different lines of sheep would have necessitated the collection of a very large number of samples in order to make such a comparison valid.

Pleural and pulmonary abscesses were occasionally encountered in sheep of all ages. In lambs younger than 3 months they were usually

following page 74

Figure 2.32

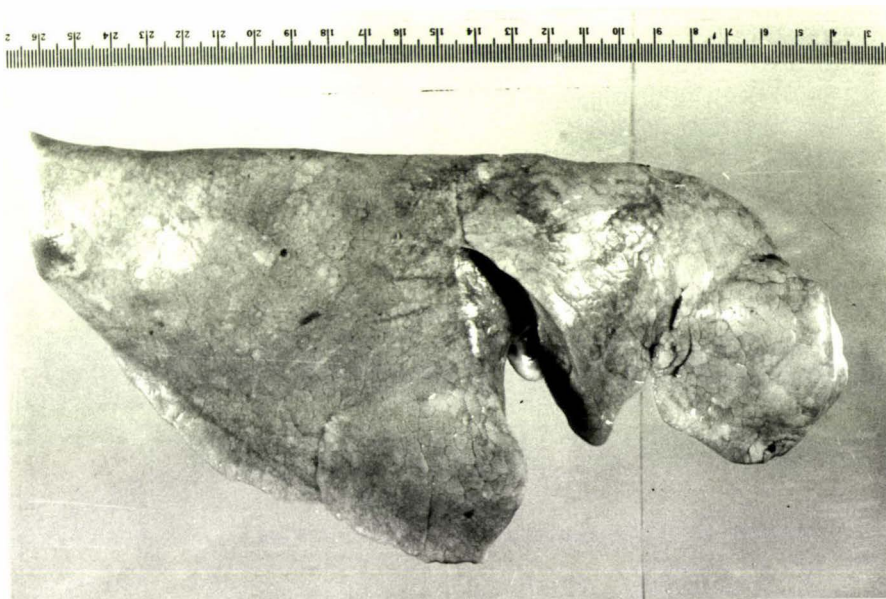
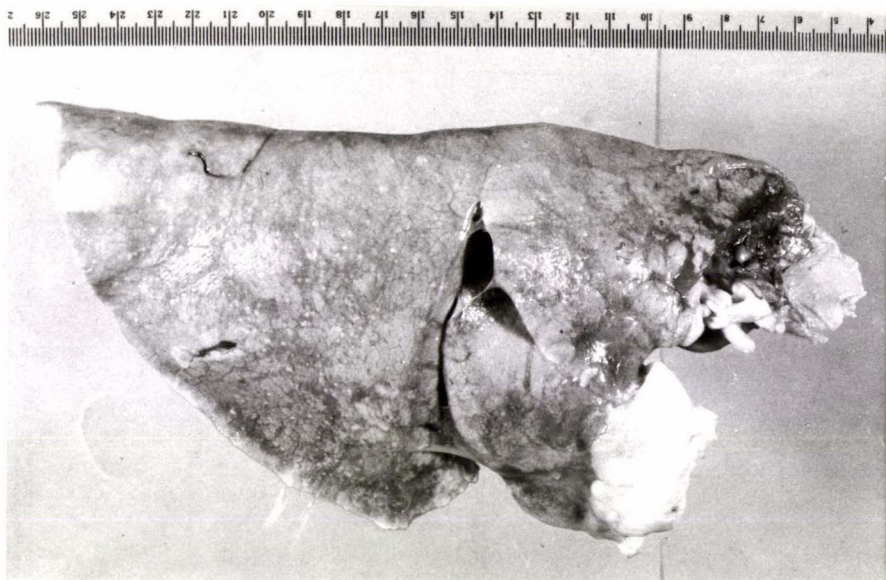
Fibrous adhesions between the pleural surfaces of the apical, cardiac and diaphragmatic lobes of the right lung as well as between the apical and cardiac lobes and the parietal pleura. Lesions of this type are frequently seen in lambs during the April-June period.

Figure 2.33

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.

Figure 2.34

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.



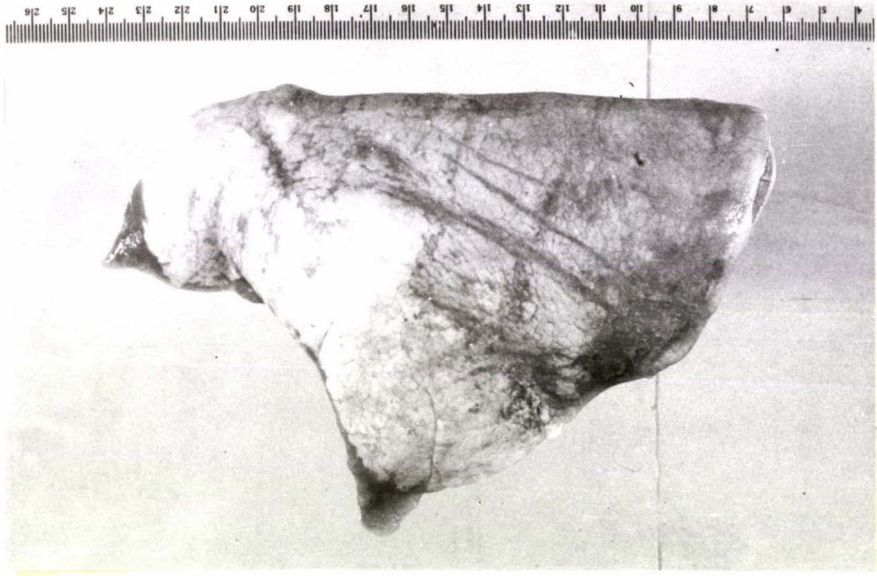
following page 74

Figure 2.35

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.

Figure 2.36

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.



multiple lesions, less than 1 cm in diameter, disseminated throughout the ventral areas of the lung and surrounded by variable areas of red consolidation. In older lambs the abscesses were invariably associated with lesions of a pre-existing chronic pneumonia and therefore occurred most frequently during the late autumn months. Affected animals in this age group were often in poor condition and commonly suffered from concurrent disease problems such as facial eczema (sporodesmin toxicity) or internal parasites. Because of their association with chronic pneumonia the abscesses were usually located towards the periphery of the apical and cardiac lobes of both lungs with resulting adhesions to the parietal pleura which were often firm and extensive. The contents of the abscesses varied from pale cream, semi-caseous pus (Figure 2.36) to yellow, fibrino-purulent gelatinous exudate. In the adult sheep that were examined the abscesses appeared to be more irregularly distributed throughout the lung and in several cases lesions were found in the diaphragmatic lobes. Their appearance and extent in older sheep varied considerably. Sometimes they took the form of well encapsulated, spherical lesions with adhesions to the diaphragm or thoracic wall, but on other occasions they consisted of extensive, foul-smelling, haemorrhagic or necrotic areas. A prior history of drenching, dipping, or surgery under general anaesthesia suggested that many of these cases were the result of the aspiration of irritant liquids or gastric contents.

Light Microscopy

Type 1 (Dull red consolidation). The most prominent histological feature of these lesions was alveolar collapse. This was often severe and extensive so that at low magnifications the tissue had a homogeneous appearance and no alveolar spaces were discernible (Figures 2.37 and 2.38). At higher magnifications small numbers of macrophages were found in alveolar spaces (Figure 2.39). In many cases bronchioles contained moderate numbers of neutrophils which were often embedded in mucus. Occasionally small numbers of neutrophils could be seen infiltrating into alveolar ducts and alveoli surrounding these bronchioles. Reflux of mucus into alveolar spaces which was observed in a small proportion of all types of pneumonic lesions, occurred most often in lesions of this type being present in approximately 30% of the cases examined.

following page 75

The Microscopic Appearance of Type I Lesions in Chronic Pneumonia

Figure 2.37

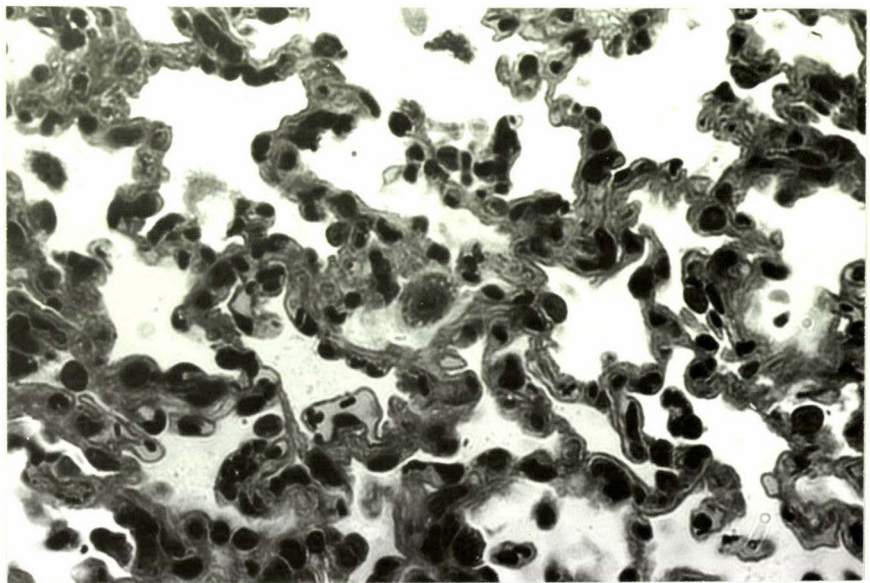
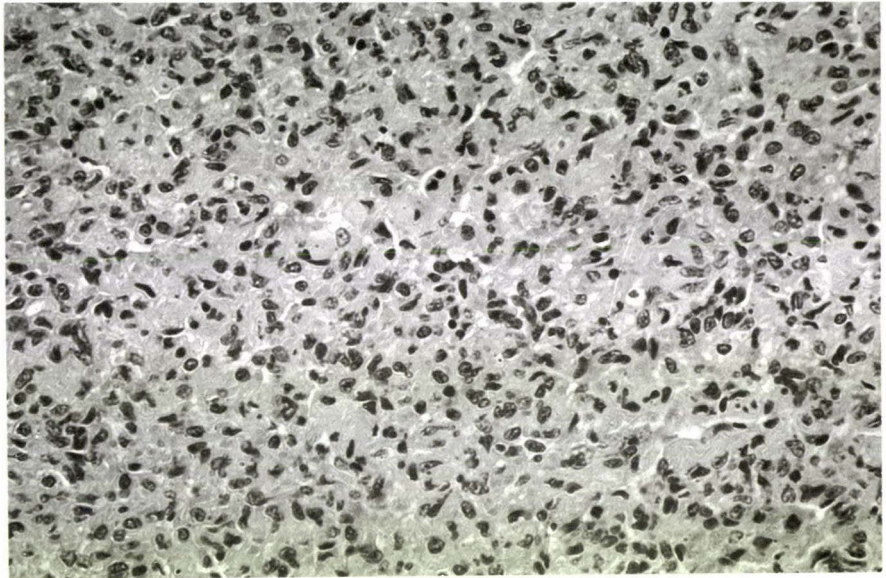
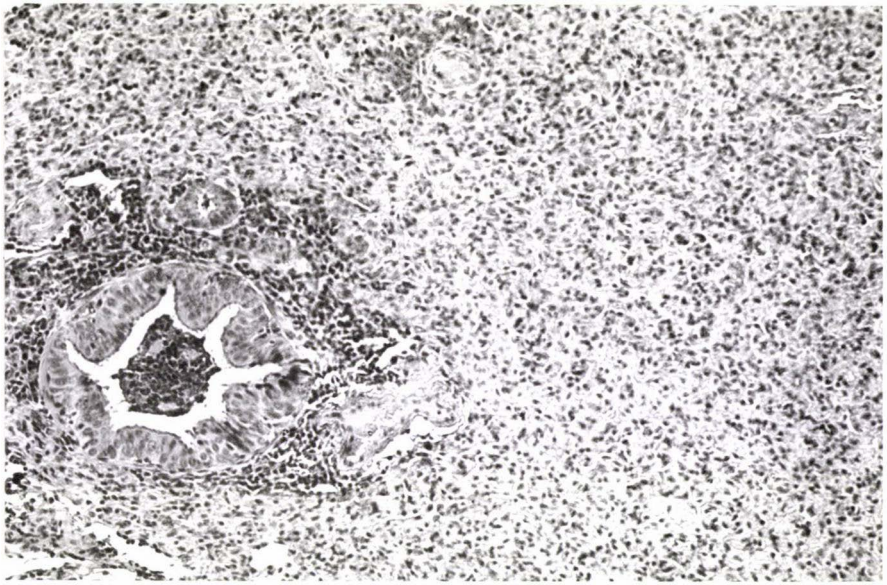
Extensive areas of alveolar collapse with the accumulation of mucus and moderate numbers of neutrophils in terminal bronchioles. HE x 40.

Figure 2.38

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

Figure 2.39

Small amounts of proteinaceous debris and occasional macrophages can be seen in alveolar spaces at high magnifications. Epoxy resin embedded. TbBf x 400.



Type 2 (Red-grey consolidation). Severe infiltrations of neutrophils into groups of alveolar spaces were the main feature observed in lungs of this type. The groups of alveoli involved were disseminated throughout the lesion. Usually adjacent bronchioles also contained an exudate composed largely of neutrophils (Figure 2.40). When severe infiltrations of this type occurred beneath the pleural surface they produced outward bulges in the pleura which were presumably the source of the grey-white foci seen grossly (Figure 2.41). Macrophages were present in moderate numbers (Figure 2.42) and early proliferative changes including bronchiolar epithelial hyperplasia and peribronchial lymphoid hyperplasia were often seen. In some cases prominent cells with large, pale-staining nuclei were observed singly or in pairs closely associated with the alveolar septum. These were later identified by electron microscopy as type II alveolar cells undergoing early proliferation.

Type 3 (Grey-red consolidation). Both exudative and proliferative changes were present in lesions of this type but proliferative changes predominated. Hyperplasia of the bronchiolar and alveolar epithelium were conspicuous features and there was variable peribronchial and perivascular lymphoid hyperplasia (Figure 2.43). The cellular exudate consisted largely of macrophages but these were often mixed with variable numbers of neutrophils (Figure 2.44 and 2.45). The development of fibrous connective tissue in the interstitial spaces around some bronchioles was also noted and could be readily demonstrated with van Gieson and reticulin stains. In occasional lungs there was hypertrophy of the smooth muscle tissue in the walls of small bronchioles and alveolar ducts (Figure 2.49).

Type 4 (Grey consolidation). Chronic proliferative changes were the most pervasive feature in lungs of this type. Severe hyperplasia of both the bronchiolar and alveolar epithelium were constant findings (Figure 2.48). Peribronchiolar fibrosis and alveolar interstitial thickening were also common and in severe cases proliferation of fibrous tissue in the walls of bronchioles resulted in the formation of nodular scars which partially obstructed the bronchiolar lumen (Figure 2.46).

following page 76

The Microscopic Appearance of Type 2 Lesions in Chronic Pneumonia

Figure 2.40

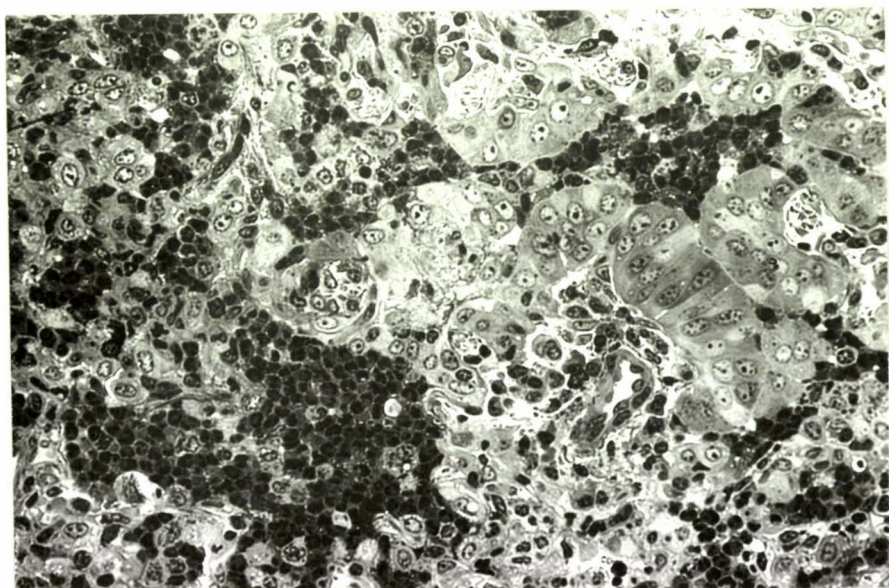
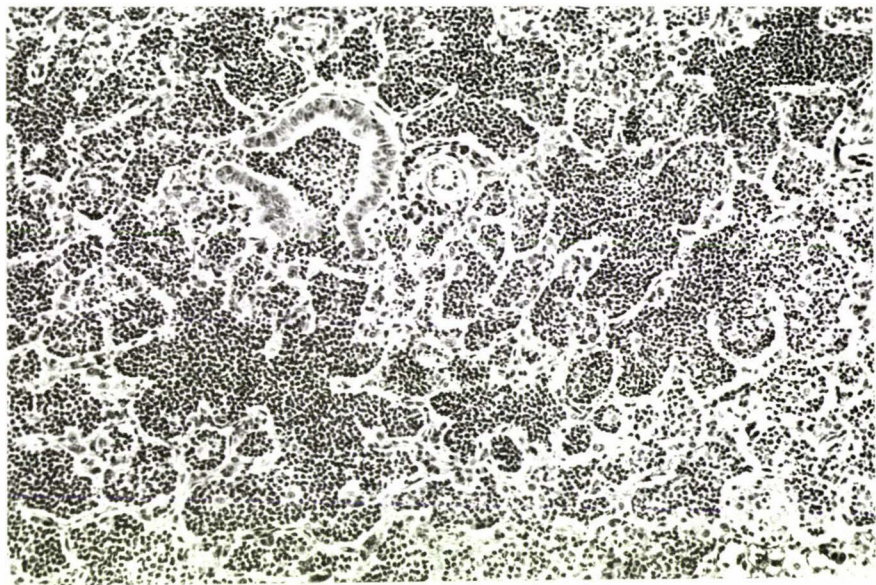
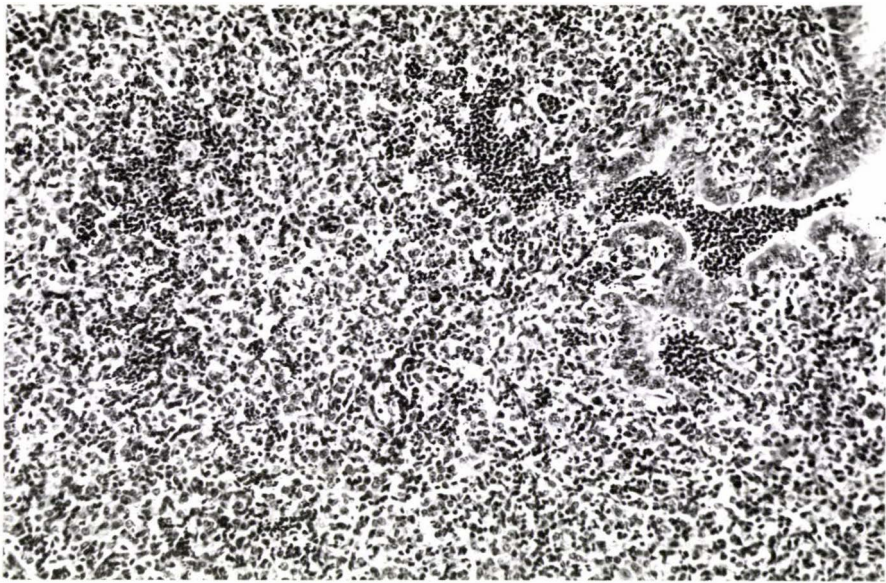
Moderate numbers of neutrophils are present in alveolar ducts and alveolar spaces contain small numbers of neutrophils and macrophages. HE x 100.

Figure 2.41

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

Figure 2.42

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

Figure 2.43

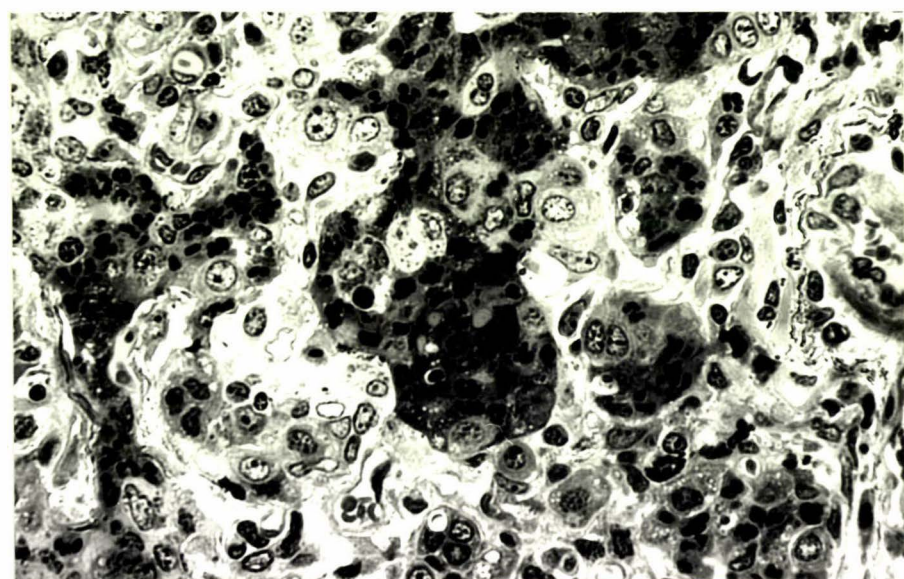
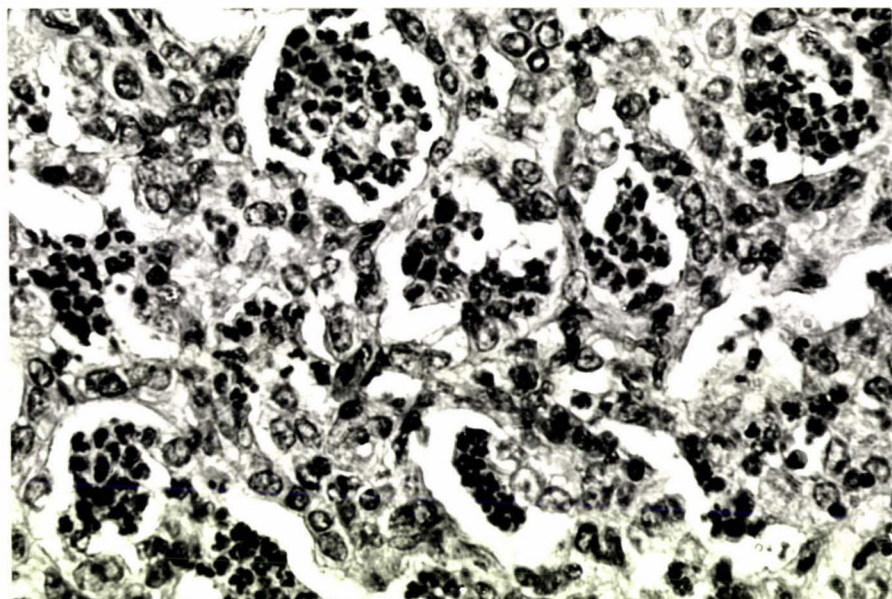
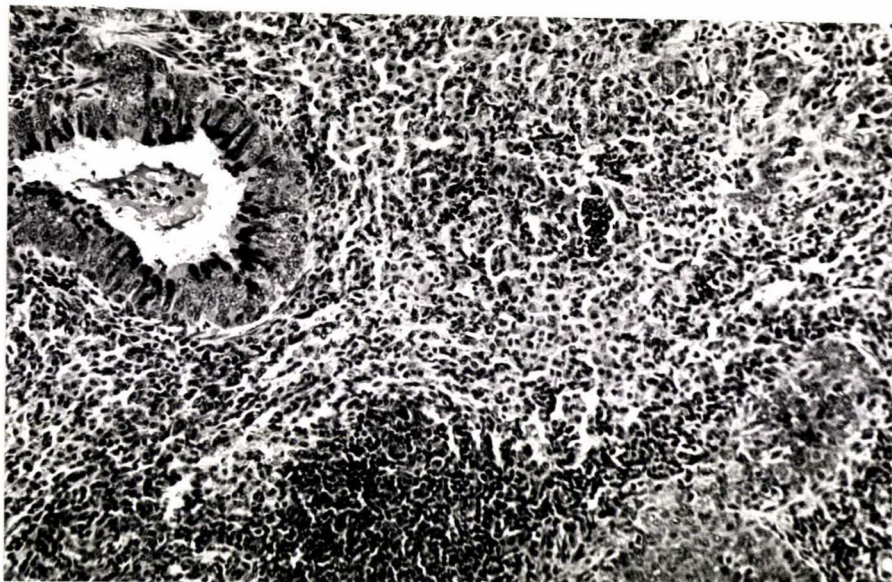
Both exudative and proliferative changes are present. There is a prominent bronchiolar epithelial hyperplasia and an increase in the number of goblet cells. Lymphoid hyperplasia surrounds a bronchiole partially sectioned at bottom right and alveoli are filled with variable numbers of macrophages and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

Figure 2.44

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

Figure 2.45

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



following page 76

The Microscopic Appearance of Type 4 Lesions in Chronic Pneumonia

Figure 2.46

Severe proliferative changes are the main feature including bronchiolar epithelial hyperplasia and peribronchiolar fibrosis with the formation of parabronchial nodular scars. HE x 100.

Figure 2.47

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.

Figure 2.48

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

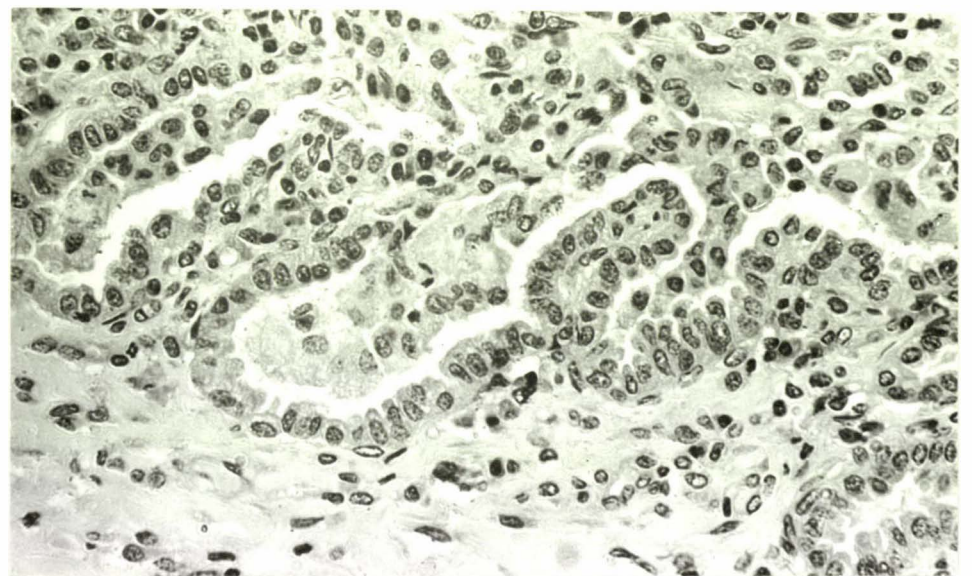
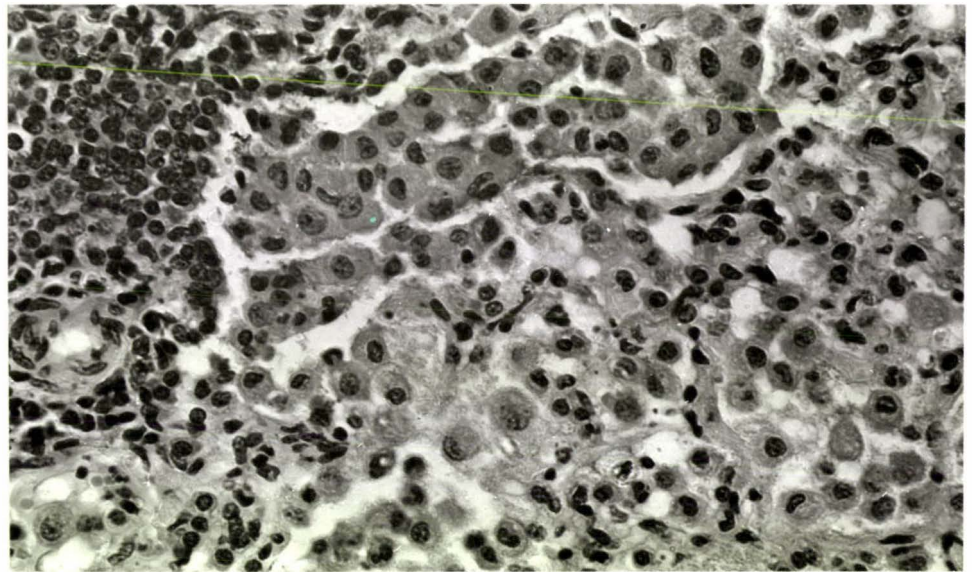
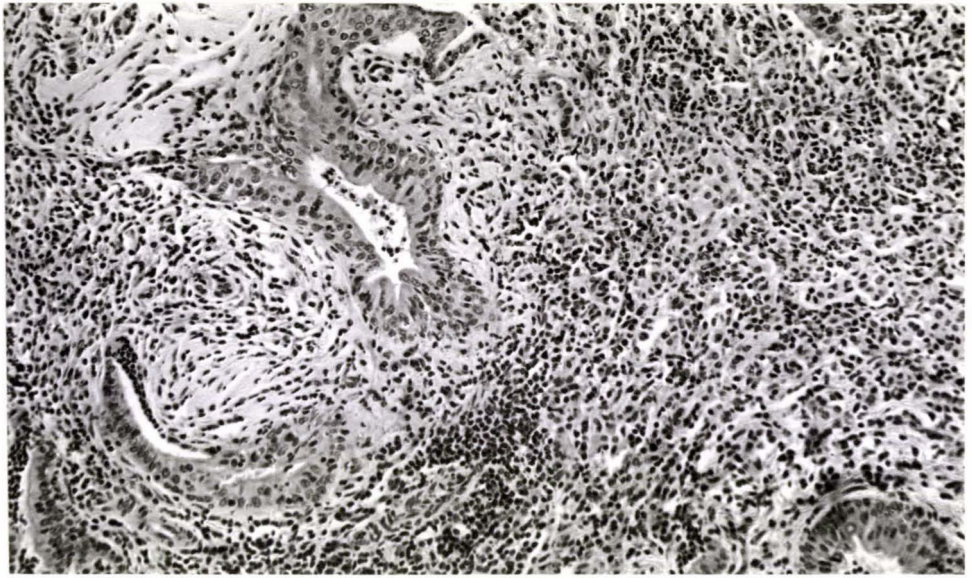


Figure 2.49

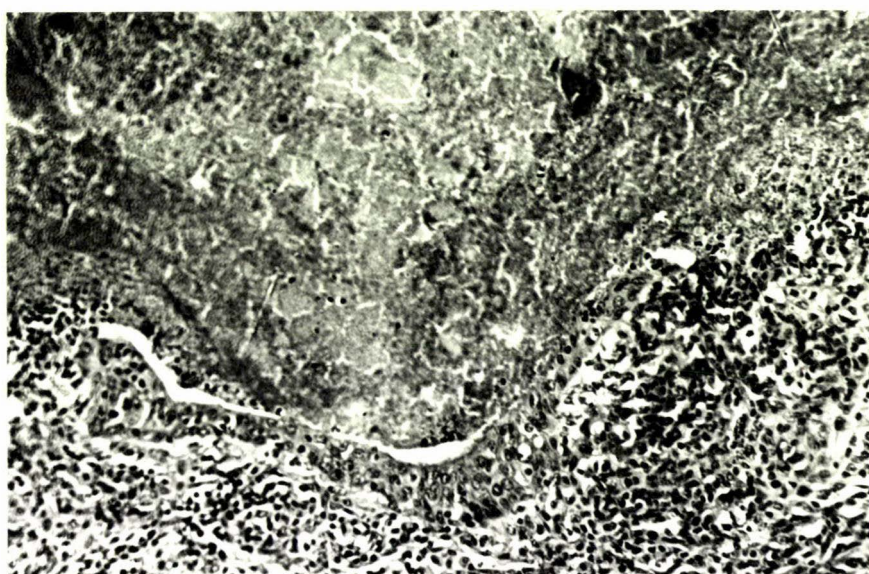
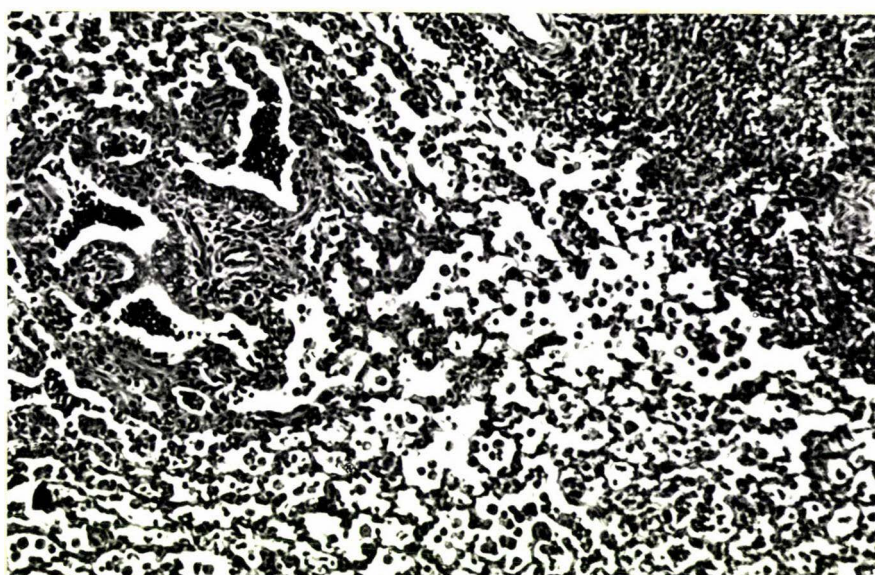
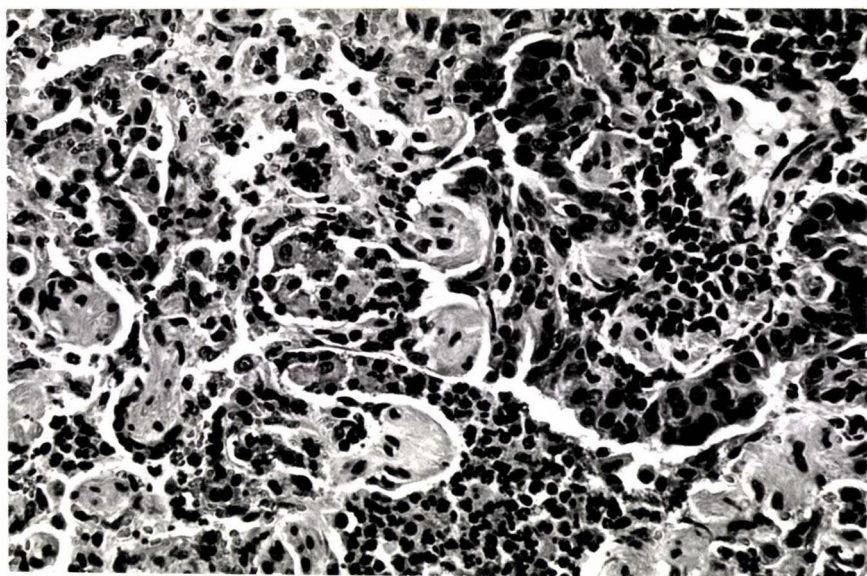
Knob-like swellings of smooth muscle tissue at the ends of interalveolar septa. These were found in some cases of chronic pneumonia but they were not a constant feature. HE x 250.

Figure 2.50

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.

Figure 2.51

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



Peribronchial lymphoid hyperplasia was variable and often severe in extent producing compression of surrounding alveoli and indentations into the lumen of both bronchi and bronchioles. Alveolar macrophages were sometimes present in large numbers (Figure 2.47) but neutrophils were only rarely seen.

In the partially aerated lungs collected during the April-June period, evidence of residual chronic proliferative lesions was commonly present. This usually included severe peribronchial lymphoid hyperplasia together with mild peribronchiolar fibrosis and mild bronchiolar epithelial hyperplasia. The partially aerated alveolar spaces often contained small numbers of macrophages (Figure 2.50). In some cases the close relationship of bronchioles, lymphoid cuffs and interlobular septa indicated that considerable destruction of alveolar tissue had taken place.

The majority of pulmonary abscesses found in young lambs were small, disseminated lesions which appeared to be of haematogenous origin. Those found in older lambs and adult sheep often emanated from bronchi or bronchioles. This was apparent in several cases where sections of bronchiolar epithelium could be found forming part of the wall of the abscess (Figure 2.51). In such cases, the necrotic exudate which usually contained numerous colonies of bacteria, sometimes extended to the pleural surface where it fistulated into the thoracic cavity or became covered by a thickened, granulomatous pleura.

In many of the chronic pneumonias examined occasional stratified, spherical bodies, 30 to 100 μ m in diameter were found in alveoli, alveolar ducts and bronchioles. They were similar in appearance to pulmonary microliths in that they usually had an acidophilic matrix and showed variable numbers of concentric, basophilic outer layers. They gave a strong positive PAS reaction and a weak reaction to alcian blue but usually contained no calcium as indicated by the von Kossa reaction. In this, and other respects they closely resembled the pulmonary corpora amylacea described by Baar and Ferguson (1963) in human pulmonary interstitial fibrosis with organising bronchopneumonia. Various

stages of their formation were often visible in the lungs examined and they appeared to arise from the sequestration of protein-rich exudate into which necrotic cellular elements were sometimes incorporated (Figure 2.52).

Histochemistry

The results listed in Table VI show that increased histochemical activity for a variety of enzymic reactions was observed in pneumonic lungs when compared with artificially collapsed normal lungs. The increased activity was in the majority of cases due to increased numbers of alveolar type II cells, alveolar macrophages and neutrophils rather than increased reactivity within each cell. An exception to this were the enzymic reactions within alveolar macrophages which were invariably increased both in intensity as well as in number (Figures 2.53a and 2.53b). The reactions for alkaline phosphatase, LDH and MDH which served to identify type II cells in normal lungs were less evenly spaced and occurred in scattered irregular aggregations of 2 or more cells in all of the 5 grey-red consolidated lungs that were examined (Figures 2.54a and 2.54b). Thus cells with the histochemical characteristics of type II cells were assumed to be proliferating in pockets within the pneumonic tissue.

Comparison of the intensity of alkaline phosphatase reaction in the grey-red consolidated lungs with that in the dull red and red-grey types of consolidation was also attempted. However, variation in section thickness and the limitations of sensitivity and reliability of the techniques used precluded the accurate assessment of these enzyme levels on a quantitative basis.

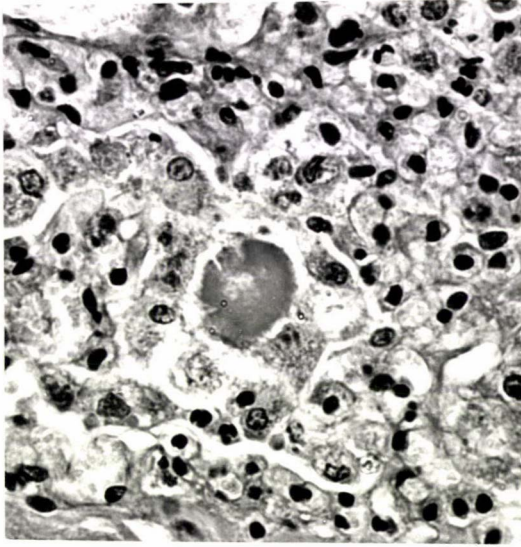
Electron Microscopy

Substantial ultrastructural changes were seen in the lungs with dull red consolidation despite their unremarkable appearance when viewed under the light microscope. One of the commonest changes observed was distension of the interstitial spaces in alveolar septa (Figure 2.55). The distended tissue spaces appeared oedematous and sometimes strands of proteinaceous debris were found within these spaces as well as in the

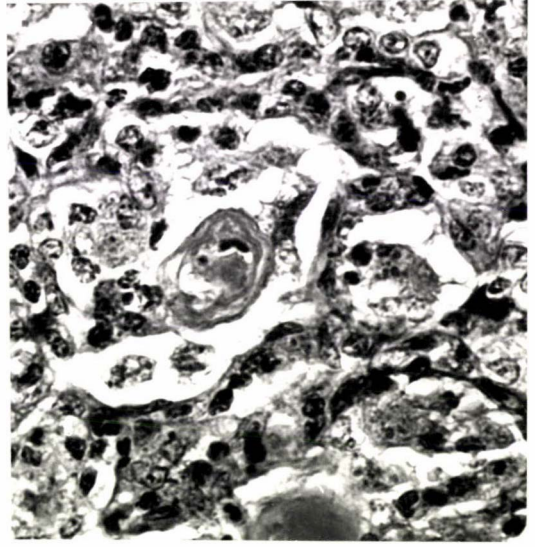
Figure 2.52

Various stages in the development of pulmonary corpora amylacea in chronic pneumonia.

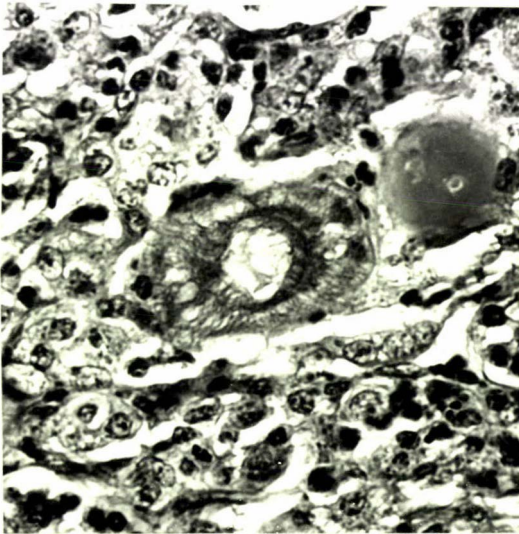
- (a) Sequestered protein-rich exudate surrounded by macrophages in an alveolar duct.
- (b) Necrosis of some of the macrophages in the space surrounding the sequestrum.
- (c) Incorporation of necrotic cellular elements into the outer layers of the sequestrum.
- (d) Well-developed sequestrum showing concentrically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



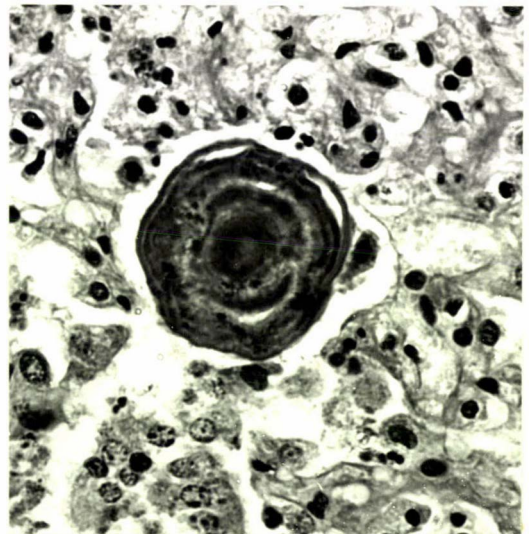
a



b



c



d

Figure 2.53

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.

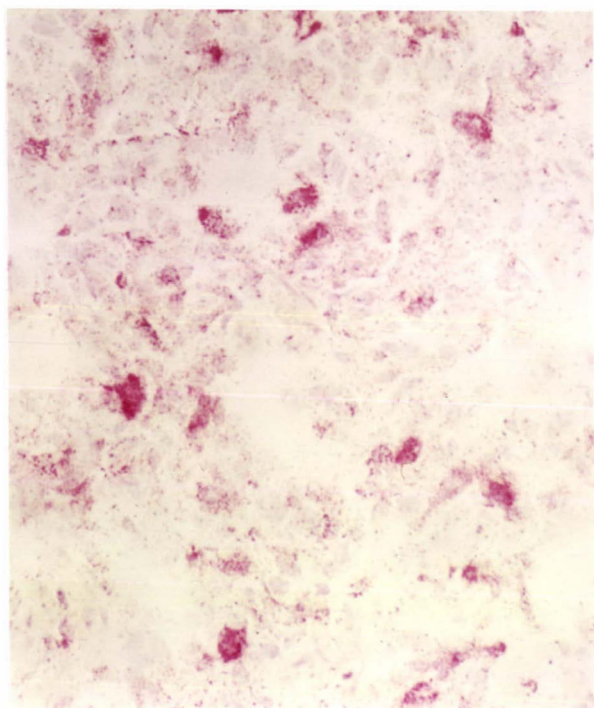
Naphthol AS (NAS) method and haematoxylin. x 250.

Figure 2.54

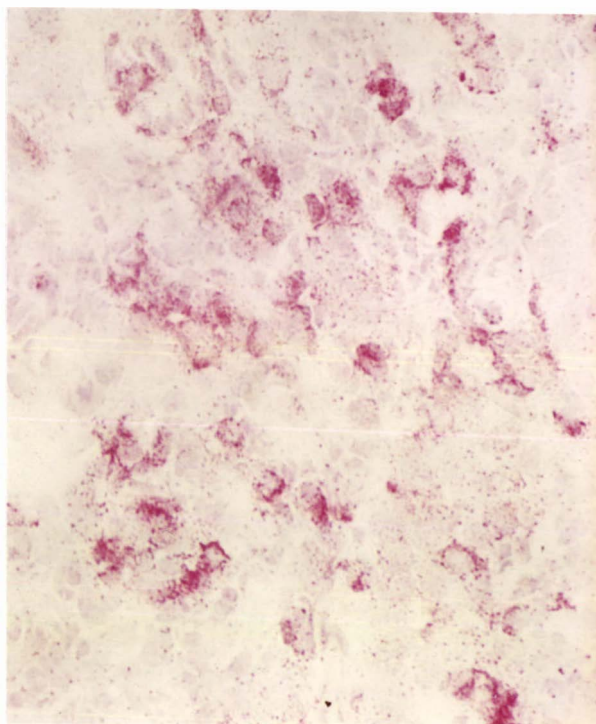
Non-specific acid phosphatase reaction.

- (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.



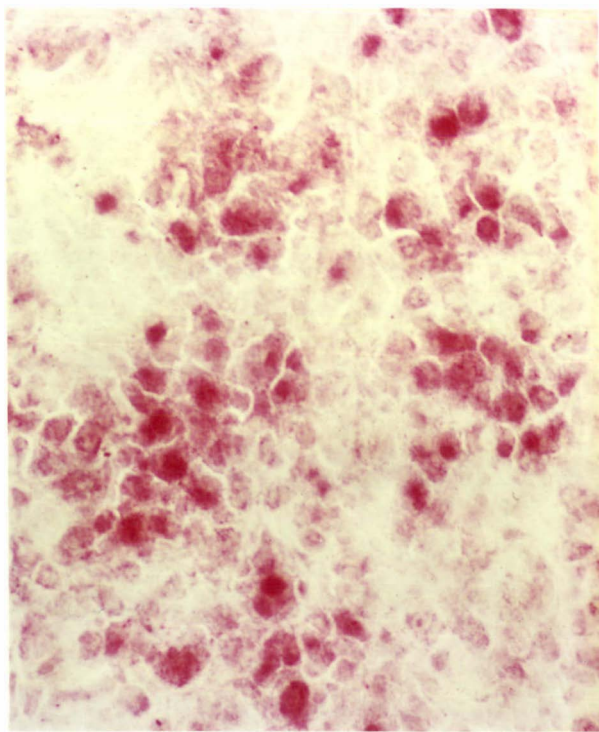
a



b



a



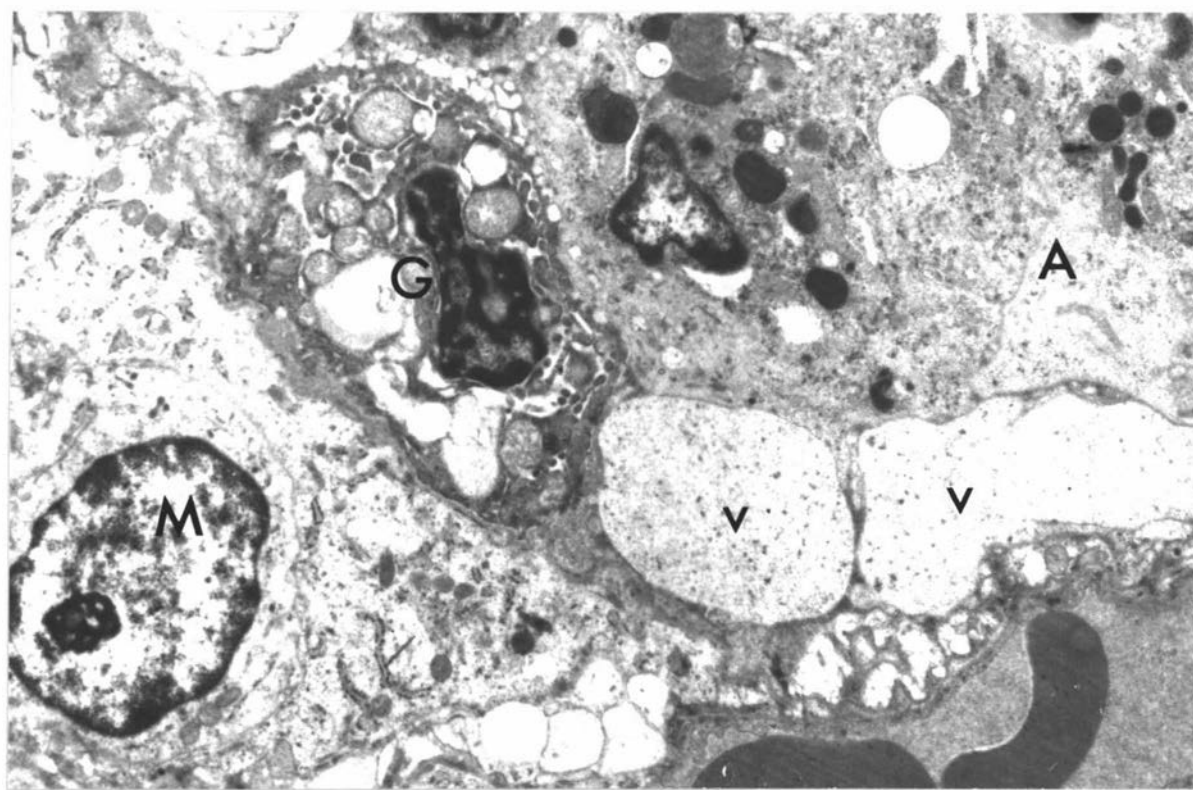
b

Figure 2.55

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.

Figure 2.56

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.



surrounding alveoli. The interstitial swelling was not universal and occurred most often between capillaries leaving large areas of the blood-air barrier apparently unaffected. Occasional large mononuclear cells could be found migrating within these oedematous areas (Figures 2.55 and 2.56).

In some cases active macrophages were found within those alveoli in which proteinaceous exudate was present. They contained numerous large dense bodies, many mitochondria and prominent pseudopodial extensions (Figure 2.57). Increased numbers of macrophages of a similar type were invariably present in lungs with more severe lesions. In these cases the macrophages often had a variety of vacuolated, cytoplasmic inclusions containing cellular debris or whorled myelin-like forms some of which were presumably engulfed secretions of type II cells (Figure 2.58). Many macrophages had been migrating when fixed and showed a broad advancing edge of cytoplasm within which acanthosomes could be seen forming around proteinaceous debris (Figure 2.57). The nucleus was usually displaced towards the rear of these cells and showed an open chromatin pattern typical of metabolically active cells in addition to a prominent nucleolus.

The mildest changes recognised in the alveolar epithelial type I cells were swelling and loss of density in the cytoplasmic extensions together with vesicle formation and loss of continuity of the outer plasma membranes (Figures 2.55 and 2.56). Sometimes large blebs with proteinaceous contents were observed but more often small, clear vesicles were seen which in advanced stages became confluent. Despite these changes the type I cells usually remained securely attached to the basement membrane. In other collapsed alveoli the type I cells often appeared normal (Figure 2.59). However, in some areas the epithelium was thicker than normal and showed small, irregular cytoplasmic projections but these changes were interpreted to be the result of collapse and contraction of type I cell cytoplasm.

Desquamation of alveolar epithelial type I cells was sometimes observed in more advanced cases where neutrophils commonly filled alveolar spaces. This change was usually associated with migrating neutrophils or

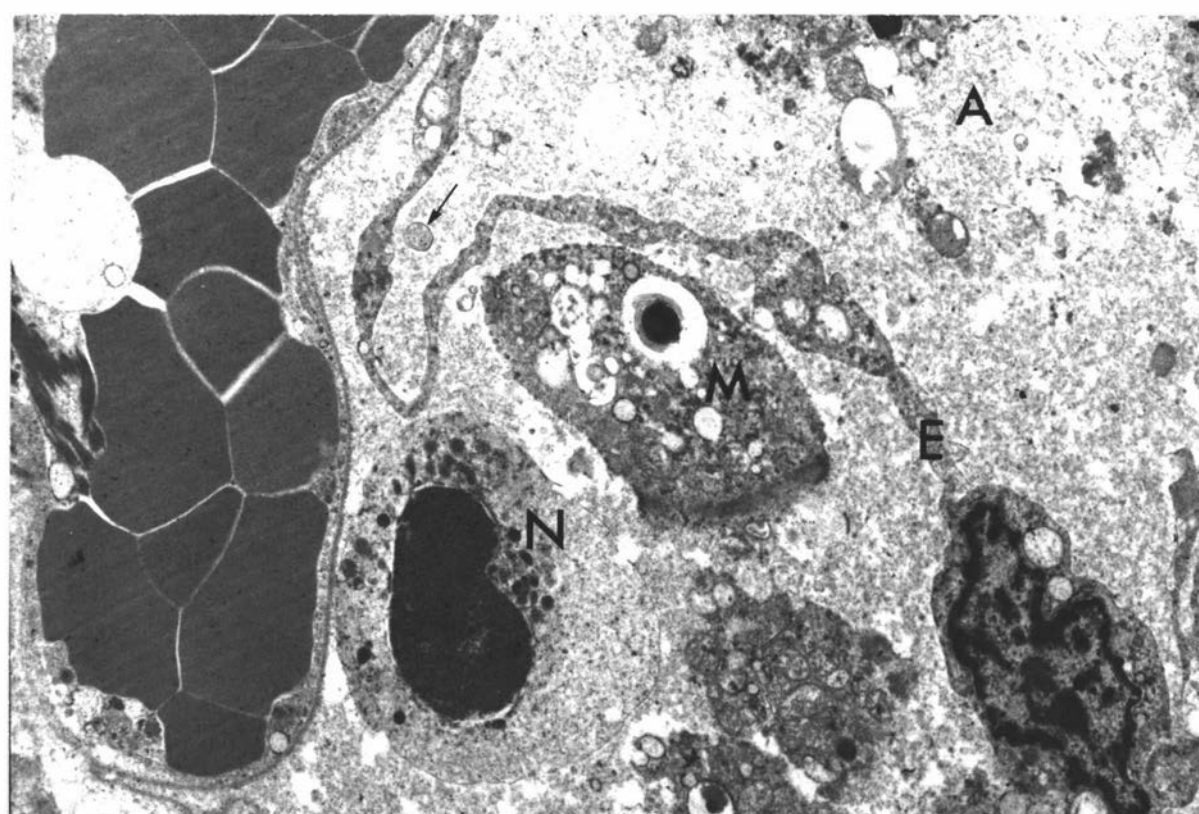
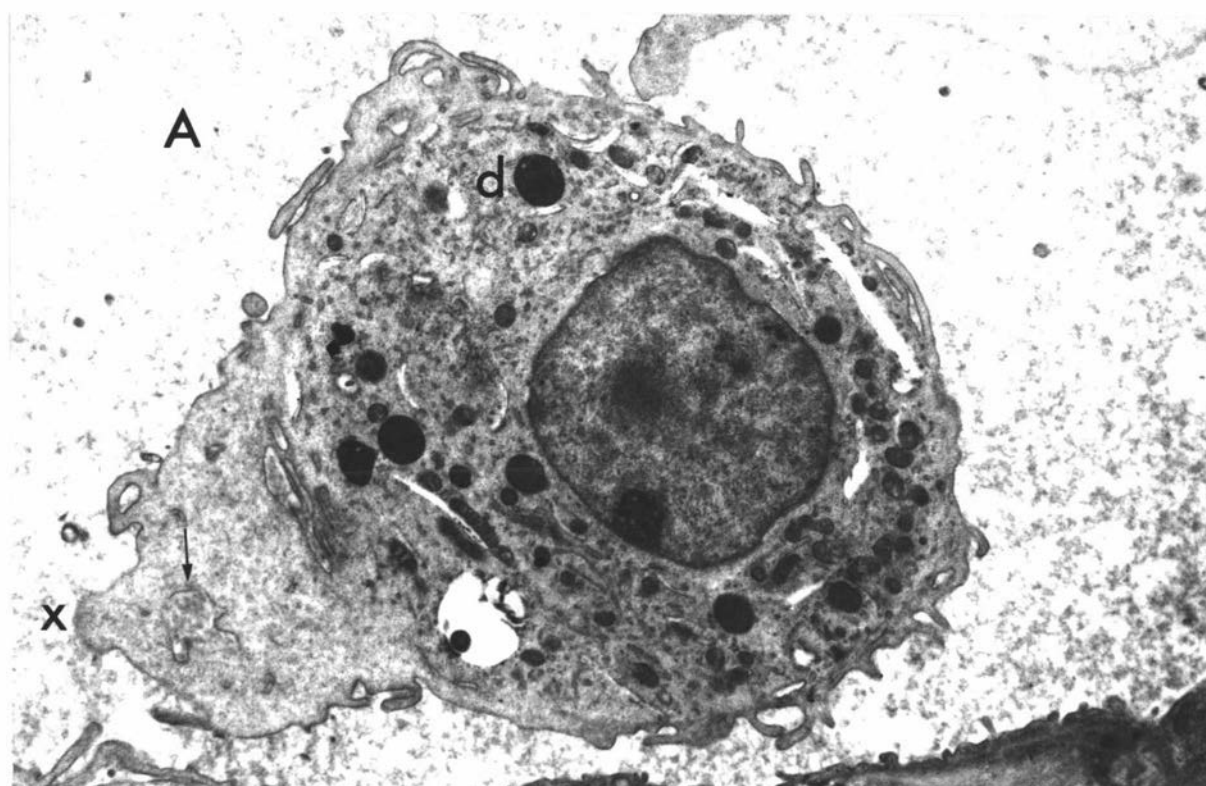
following page 79

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.



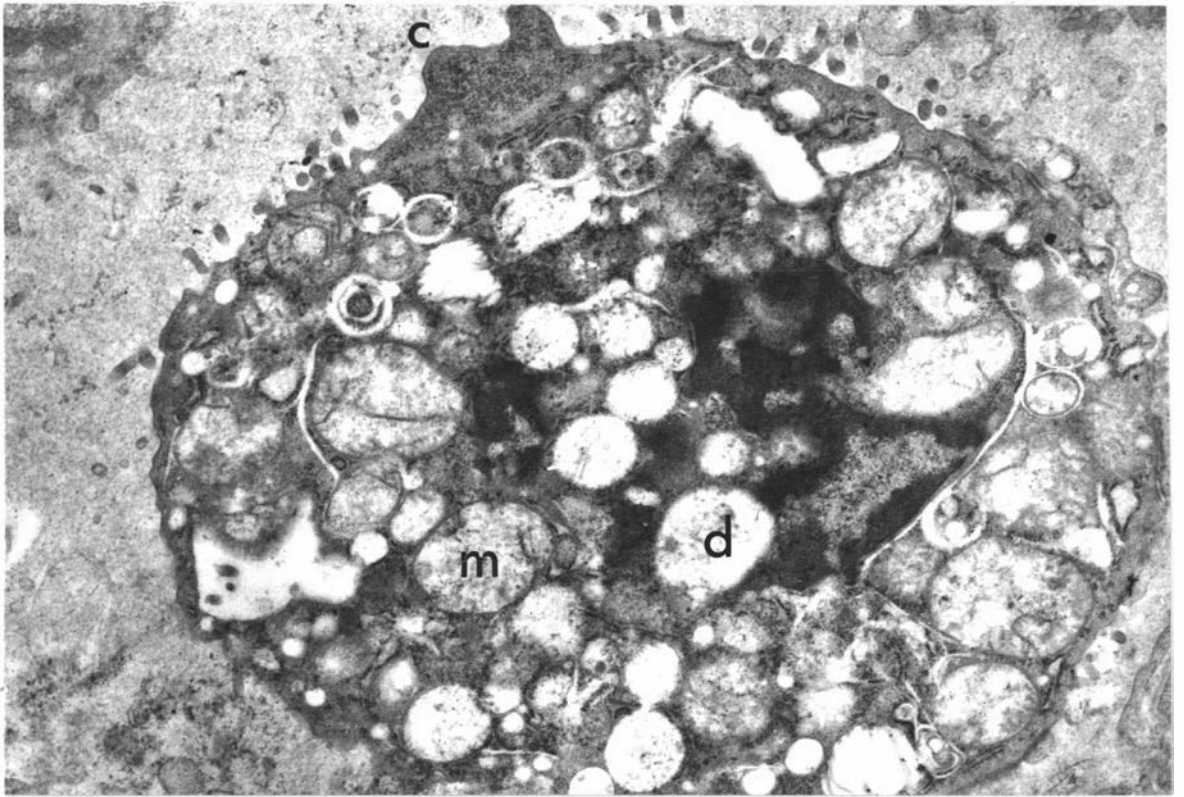
following page 79

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.



macrophages either within the alveolar septum or beneath the detached alveolar epithelium. Despite its predicament the detached epithelium often showed only relatively minor degenerative changes such as mitochondrial swelling and indentation of the nuclear membrane (Figure 2.58). Occasionally small numbers of bacteria were seen associated with these changes but no mycoplasmas were found within alveolar spaces and no viral particles could be seen in alveolar epithelial cells or macrophages.

Degenerative changes in alveolar type II cells were frequently one of the earliest lesions detected in the dull red consolidated lungs that were examined. These changes consisted of swollen mitochondria, mild indentations of the nuclear membrane and moderate dilation of the endoplasmic cisternae (Figure 2.59). As the lesions progressed, cells in which these changes had developed to the point of necrosis were commonly found. They showed severely dilated mitochondria, disruptions of the nuclear membrane, loss of osmiophilic bodies and numerous dilations and vacuolations of the endoplasmic reticulum (Figure 2.60). Sometimes small cytoplasmic extensions of the outer plasma membrane were also seen (Figure 2.60). These were regarded as diminutive forms of more extensive cytoplasmic dilations which were occasionally found in less severely damaged cells in advanced lesions (Figure 2.63). Another common degenerative change found in type II cells was thickening of the superficial plasma membrane with electron-dense material. This was often accompanied by loss of microvilli and early vesicular changes in the cytoplasm (Figure 2.61). Changes of this nature were confined to alveoli in which there were advanced destructive lesions including severe protein exudation and infiltration by macrophages and neutrophils.

Damaged type II cells could still be detected even in the most proliferative types of pneumonia examined. In these cases affected cells showed loss of osmiophilic bodies, severe universal dilatations of the endoplasmic reticulum, swollen mitochondria and severe nuclear indentations. In some of the cells showing these changes and in others in which only mild changes were present, unusual cytoplasmic inclusions were encountered which were not seen in normal cells. They took the form of spherical sacs up to $4\text{ }\mu\text{m}$ in diameter which appeared to arise from dilatations of the endoplasmic reticulum as this often formed circles or semicircles around

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 12,600.

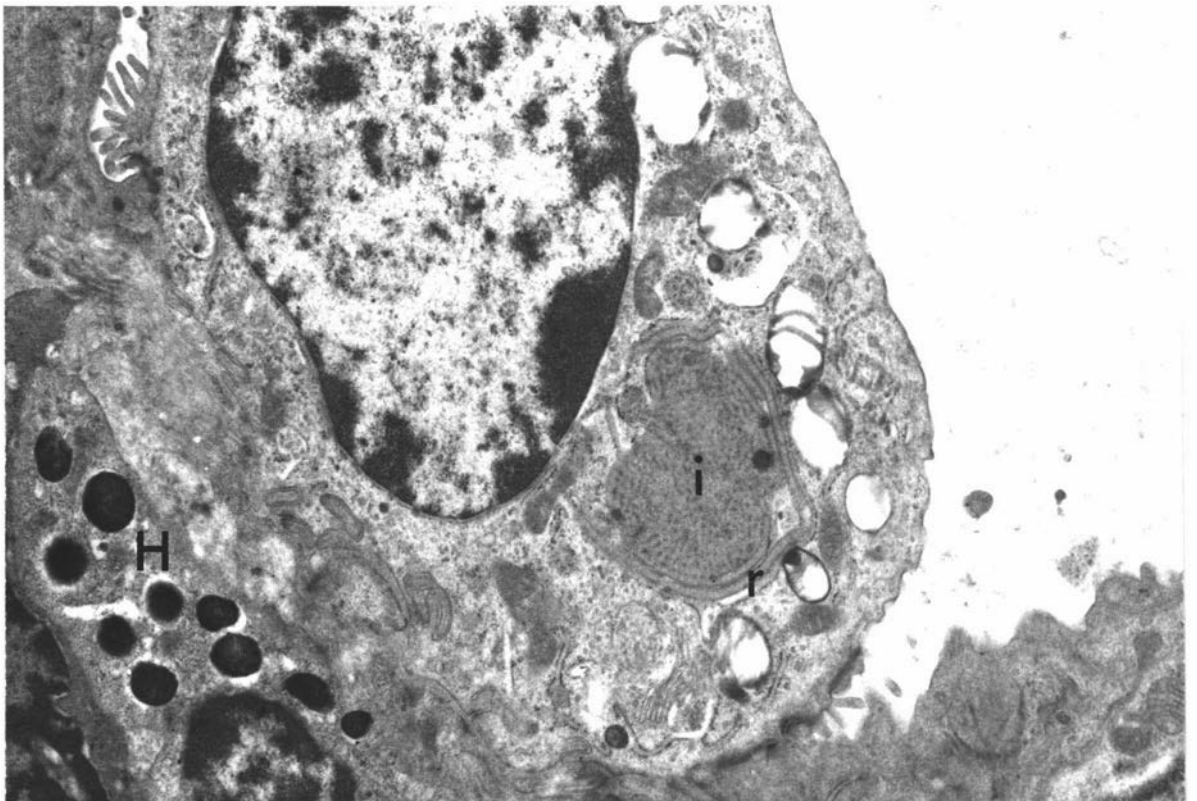
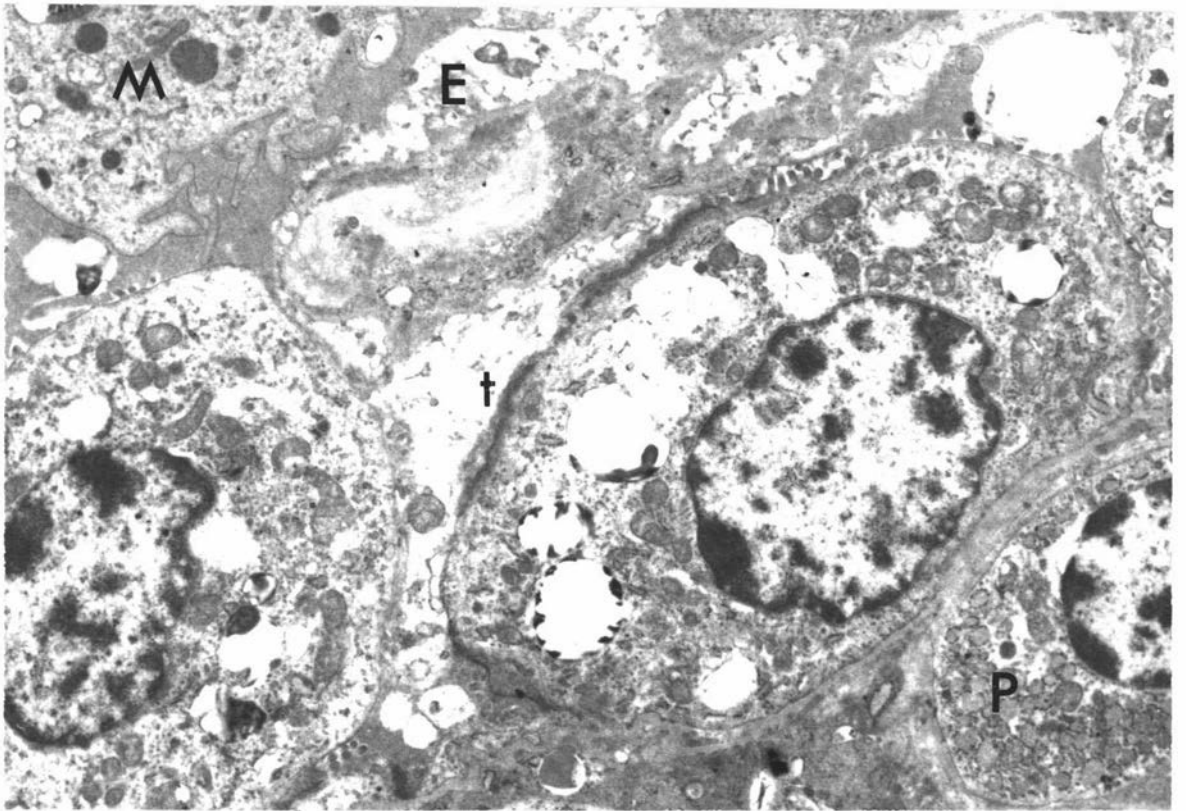
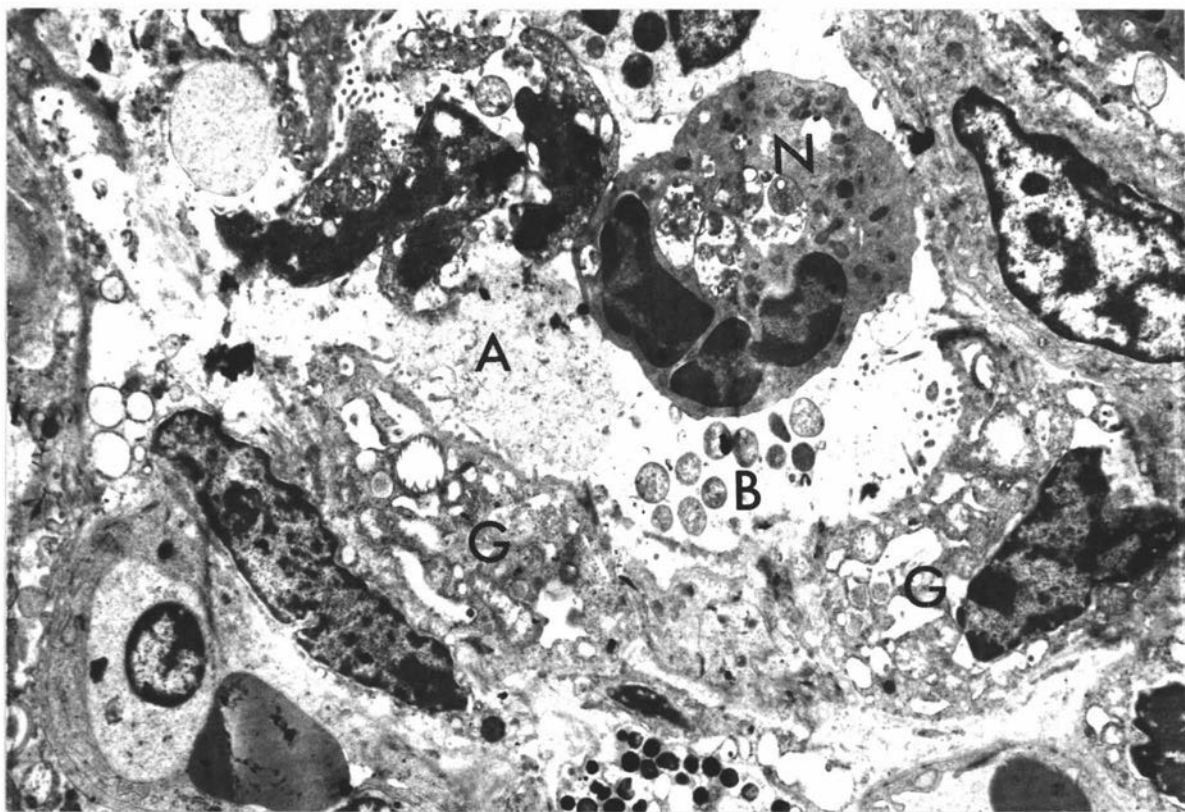
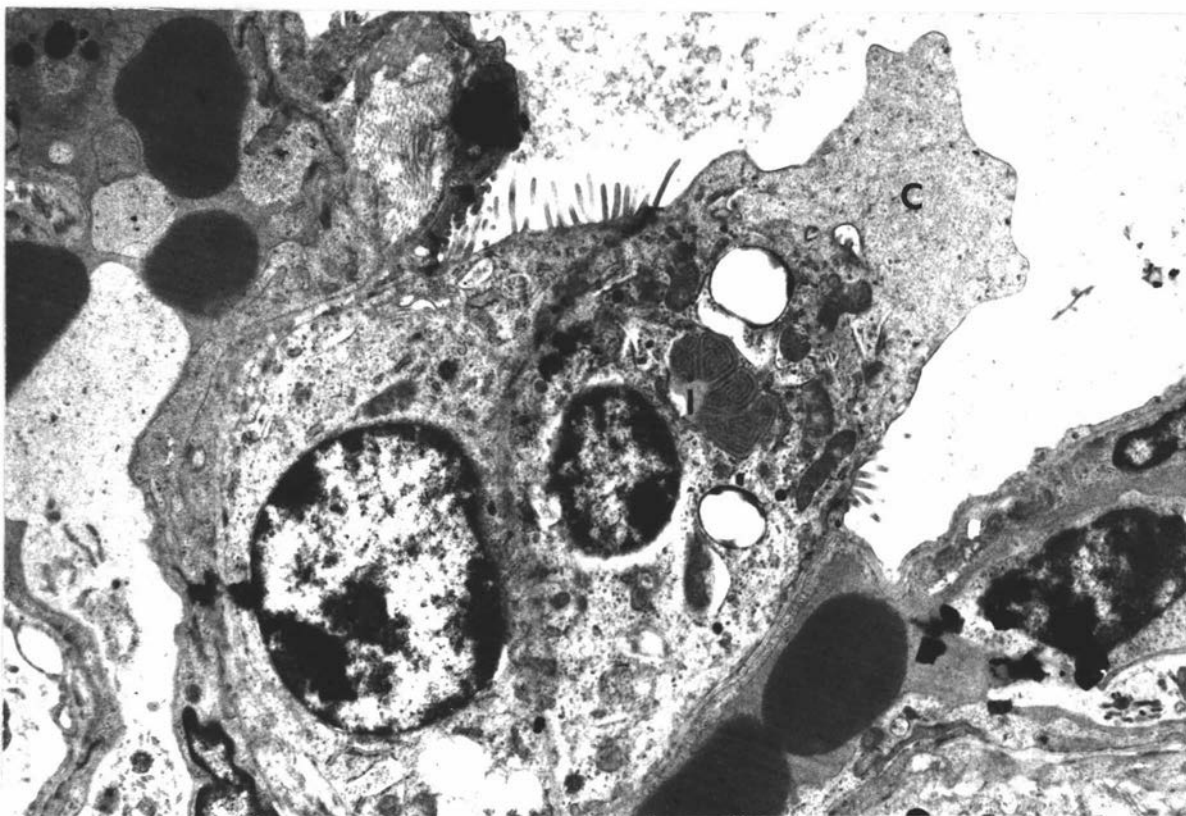


Figure 2.63

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.



them. Their electron density was similar to that of lipid vacuoles but they contained numerous, evenly distributed, dense granules with a periodicity of approximately 80 nm which were sometimes arranged in a linear or circular fashion (Figures 2.62 and 2.63).

Hyperplasia of type II cells was recognised in an early form in lungs showing red-grey consolidation in which there was a large component of cellular exudate. The proliferating cells could be found in groups of 2 or 3 in the corners of damaged alveoli (Figures 2.61 and 2.63). In some lungs of this type, where destruction of the alveolar epithelium had occurred, flattened type II cells were observed lying compressed against the basement membrane and sometimes extending over its surface in a similar manner to the normal posture of type I epithelial cells (Figure 2.64). This phenomenon was also encountered in advanced lesions where proliferative interstitial changes were evident (Figure 2.70).

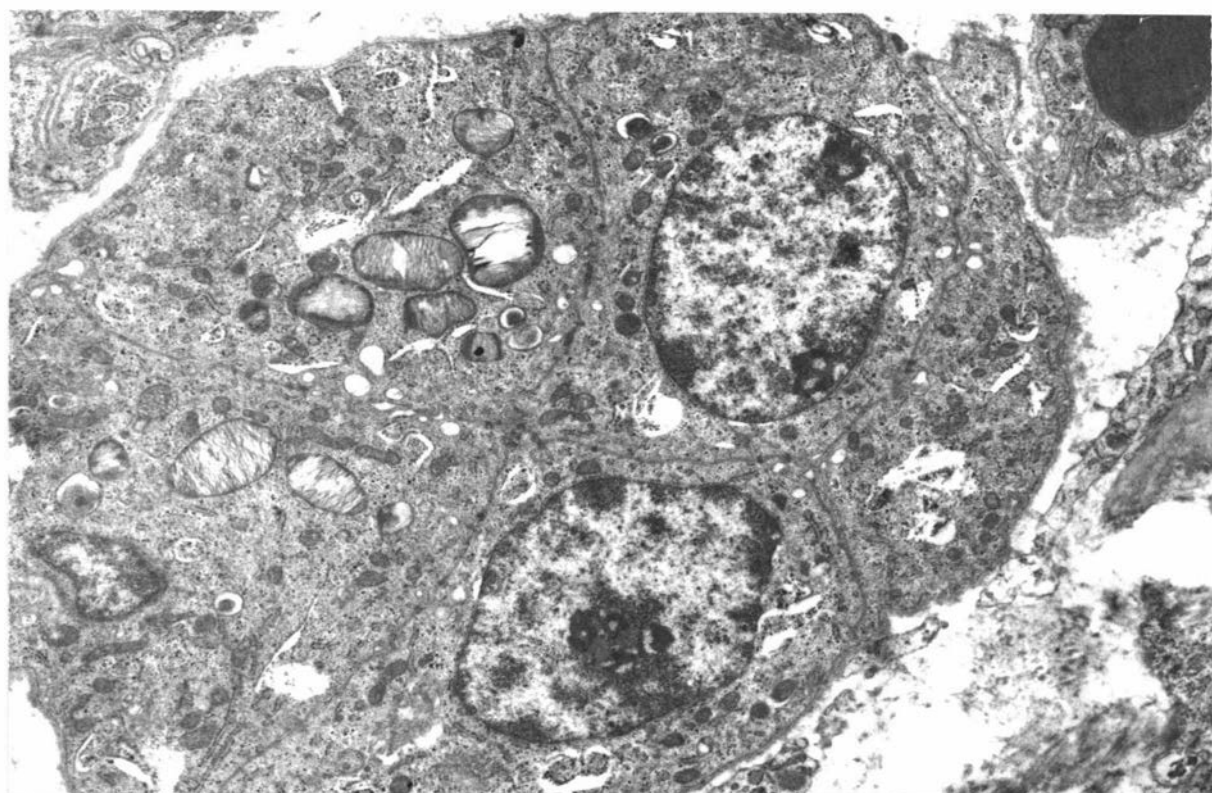
The proliferating type II cells showed considerable variation in ultrastructural morphology. Where proliferation was least severe the cells closely resembled normal type II cells except they usually contained more dilatations of the endoplasmic reticulum into cisternae and smaller numbers of vacuolated osmiophilic inclusions. In those alveoli where there was marked proliferation, the majority of type II cells involved were immature and similar in morphology to those described in the foetal lamb by Kikkawa et al. (1965). They were larger and less electron-dense than mature cells and contained numerous free ribosomes but few cytoplasmic organelles (Figure 2.65). The occasional osmiophilic inclusions that were present were coarsely lamellated similar to those described in foetal type II cells (Kikkawa and Spitzer, 1969). In those lungs where there was extensive interstitial fibrosis with obliteration of alveolar spaces, many of the pockets of cells which lay embedded in fibrous tissue and were of uncertain nature under the light microscope, were identified as alveolar type II cells (Figure 2.66). They often had an abundant cytoplasm with numerous coarsely lamellated inclusions. On some occasions their cytoplasm contained a variety of electron-dense bodies which showed various stages in transformation from lamellated inclusions of normal morphology to small dense bodies containing a spherical, central core.

Figure 2.65

Alveolar epithelialization in chronic pneumonia showing electron-dense mature type II cells (G) and proliferating immature type II cells (F) containing coarsely lamellated oxmiphilic bodies. The alveolar space (A) contains necrotic macrophages. x 9,000.

Figure 2.66

A pocket of proliferating alveolar type II cells in an advanced case of chronic pneumonia. The cells have many coarsely lamellated inclusions and numerous other organelles. x 9,000.



surrounded by an irregular dense ring (Figure 2.67).

Cells intermediate in morphology between type I and type II alveolar cells could occasionally be found attached to the alveolar wall of partially aerated alveoli in some cases of chronic pneumonia. They had plump nuclei, moderate numbers of cell organelles and occasional microvilli but lacked osmiophilic bodies (Figure 2.68). Other cuboidal cells resembling Clara cells but lacking secretory granules, were also seen attached to the alveolar wall on some occasions. In addition, Clara cells attached to the distal end of proliferating bronchiolar epithelia were on rare occasions found to contain lamellated osmiophilic bodies resembling those normally seen in type II cells (Figure 2.69).

In advanced cases of chronic pneumonia, inflammatory cells with the ultrastructural characteristics of lymphocytes, plasma cells, monocytes and fibroblasts were frequently indentified in the alveolar interstitium (Figure 2.71). Mast cells were also seen but did not appear to be significantly increased in number.

Abberant extensions of type I alveolar epithelial cells were occasionally encountered in advanced pneumonic lesions. They took the form of cytoplasmic membranes which covered the free surface of type II cells (Figures 2.72 and 2.73). This covering was sometimes complete resulting in the build up of type II cell lipoprotein secretions beneath the epithelial extensions. The trapped secretions sometimes consisted of vacuoles of variable size containing small, whorled, osmiophilic bodies embedded in a proteinaceous matrix (Figure 2.73). In other cases however, all the secretions were densely osmiophilic and large, concentrically lamellated forms were present within vacuoles (Figure 2.74).

following page 82

Figure 2.67

A group of proliferating alveolar type II cells in the corner of an alveolar space (A). Their cytoplasm contains a variety of osmiophilic bodies ranging from large vacuolated types (m) typical of mature cells, to small irregular bodies (S) with a dense central core. x 9,000.

Figure 2.68

Partially aerated alveoli (A) from an area of resolving chronic pneumonia. A undifferentiated cuboidal epithelial cell (U) is separated by desmosomes (d) from neighbouring alveolar type I cells but lacks the features characteristic of a fully differentiated type II cell. x 9,000.

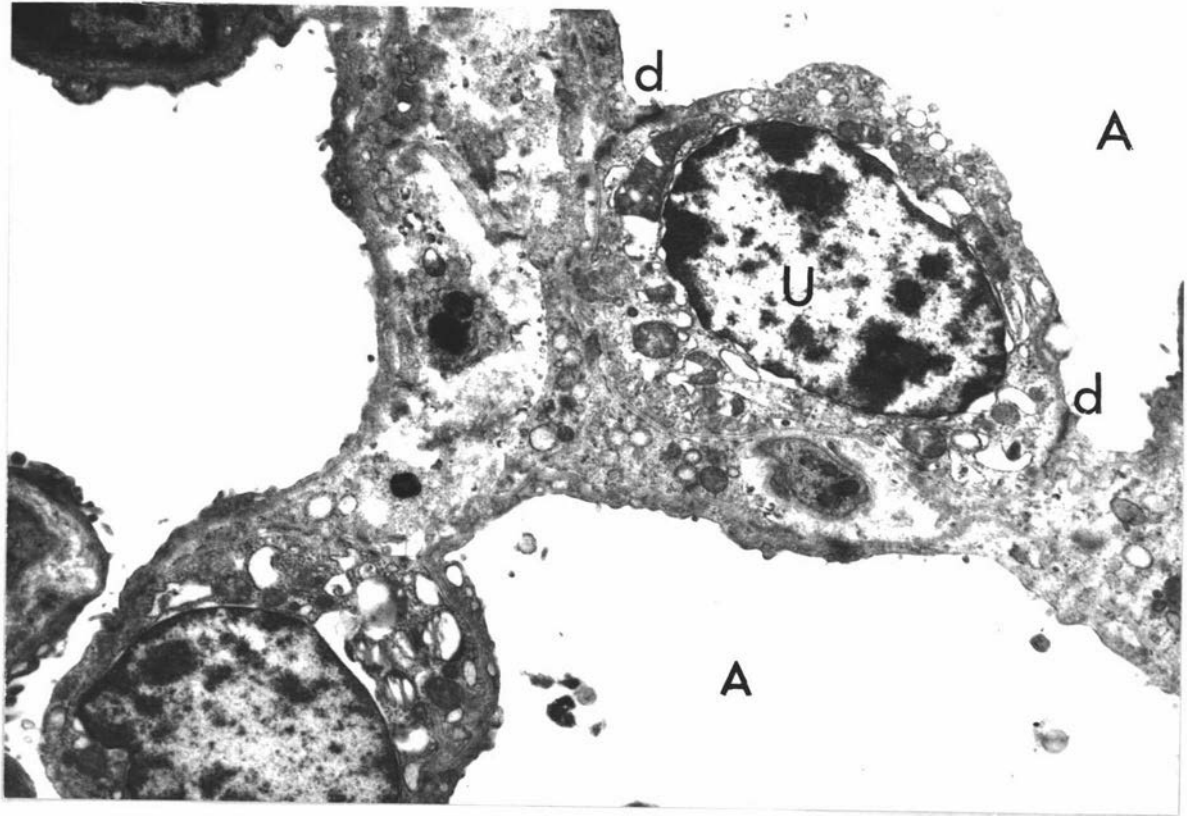
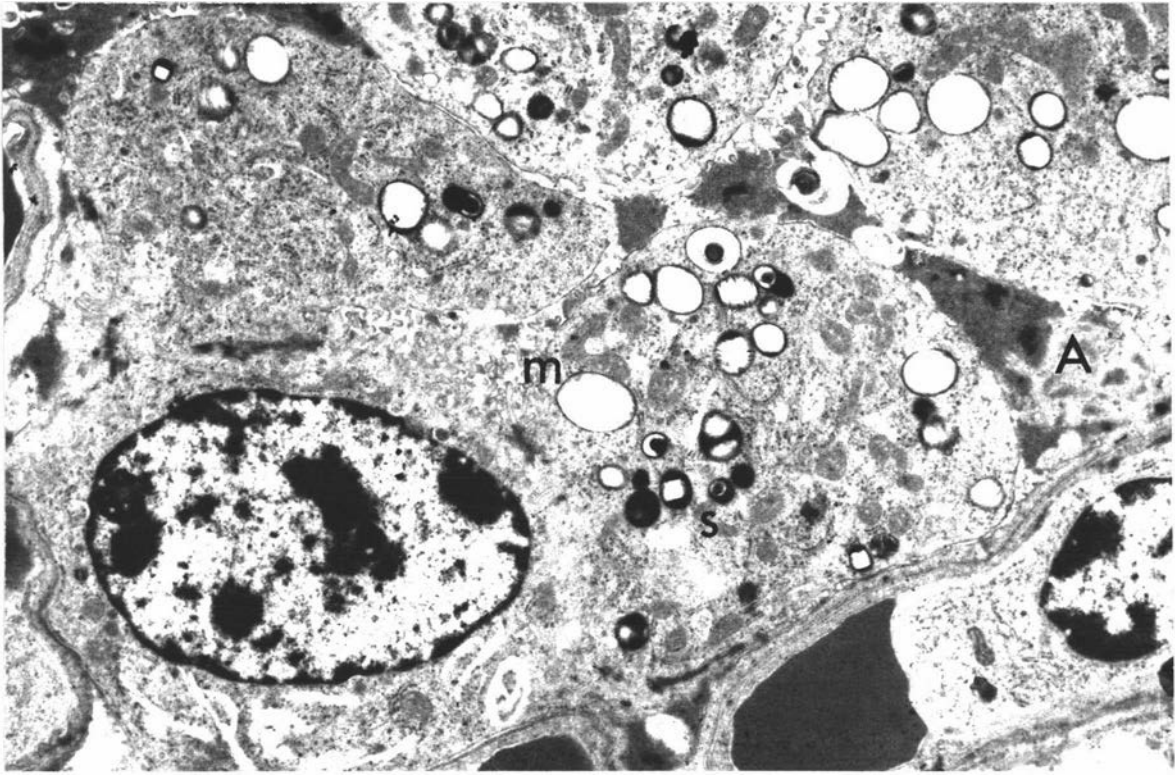
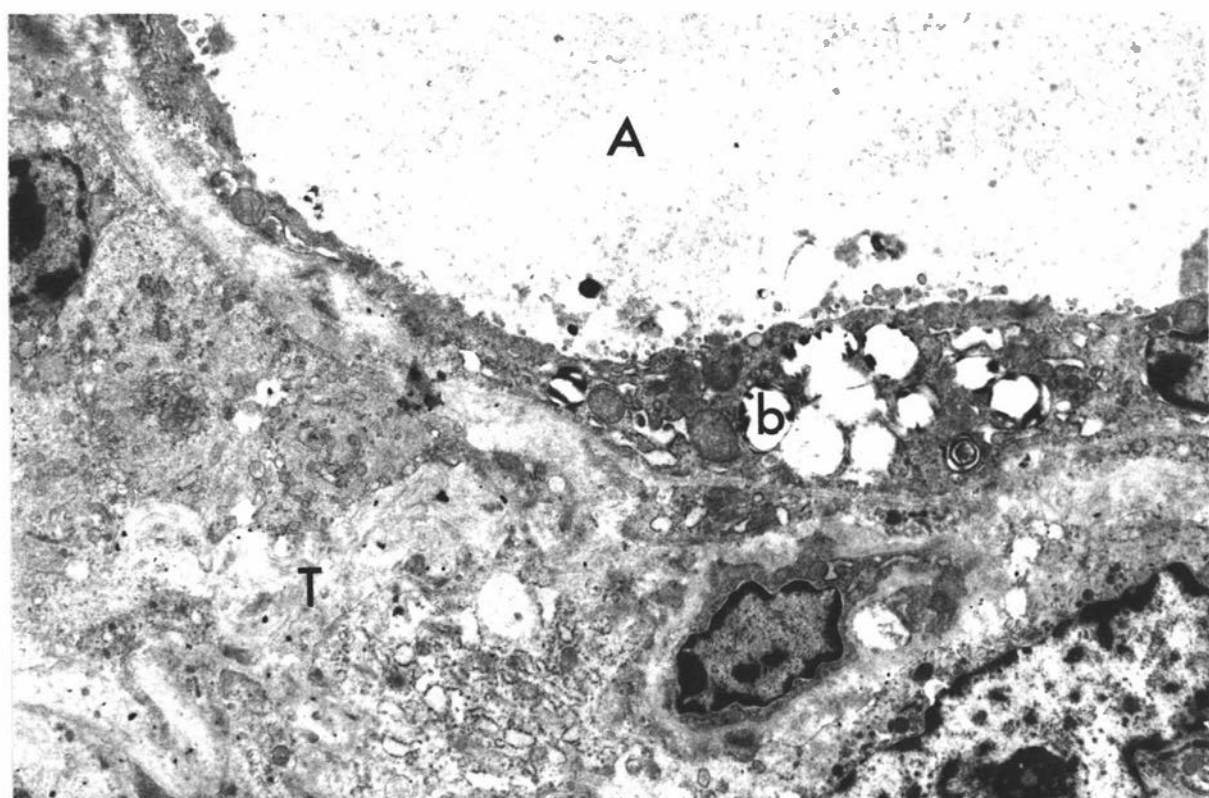
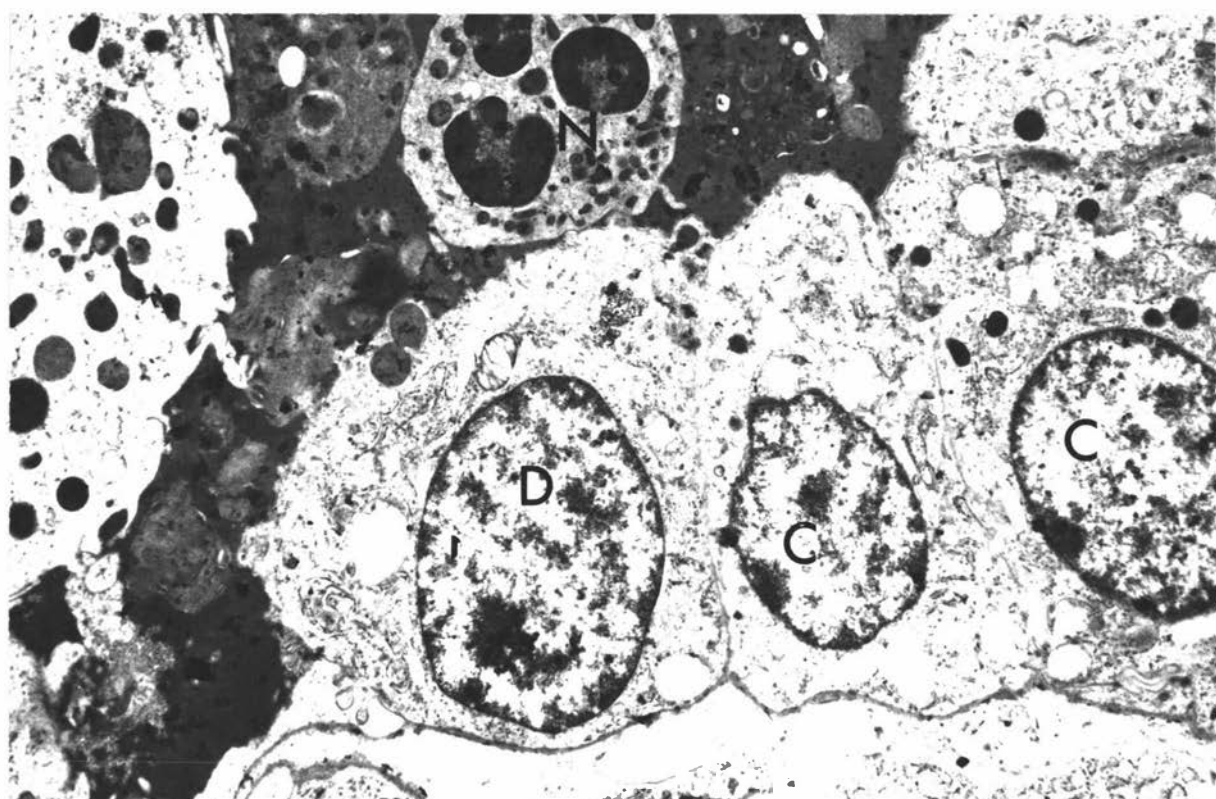


Figure 2.69

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.

Figure 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.



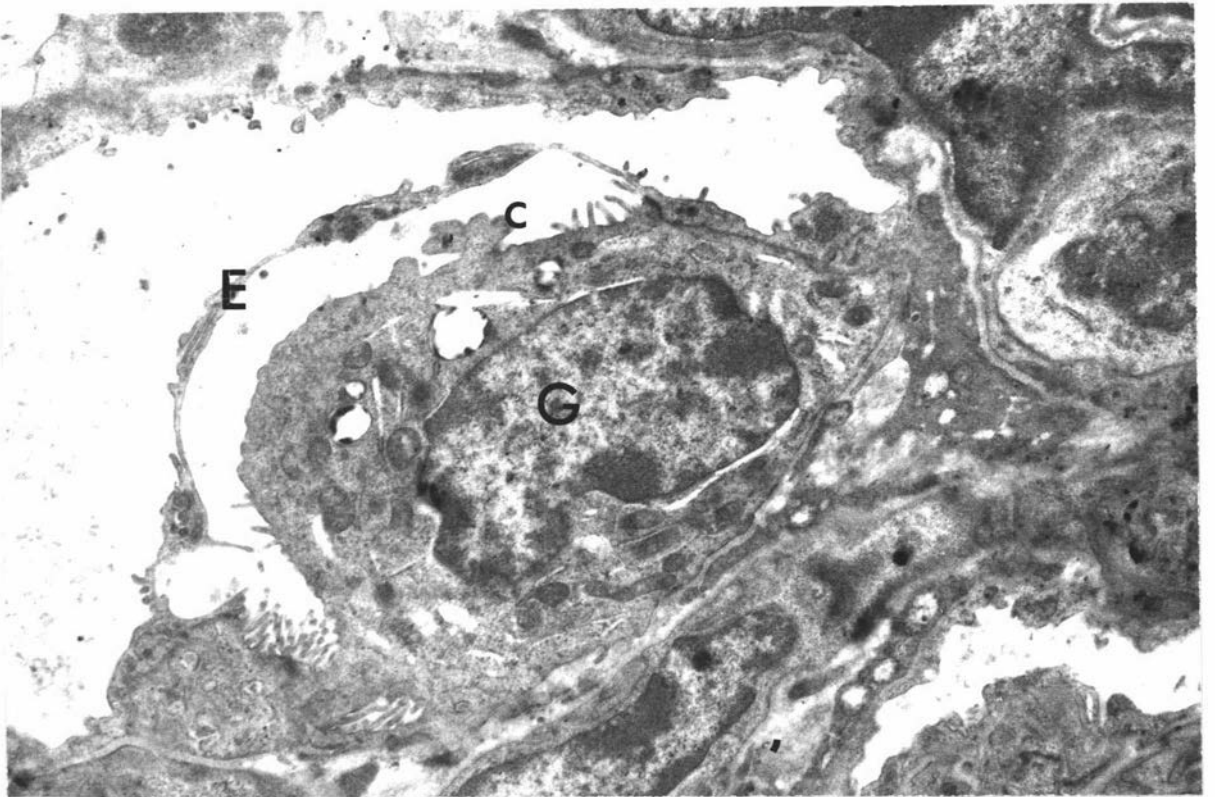
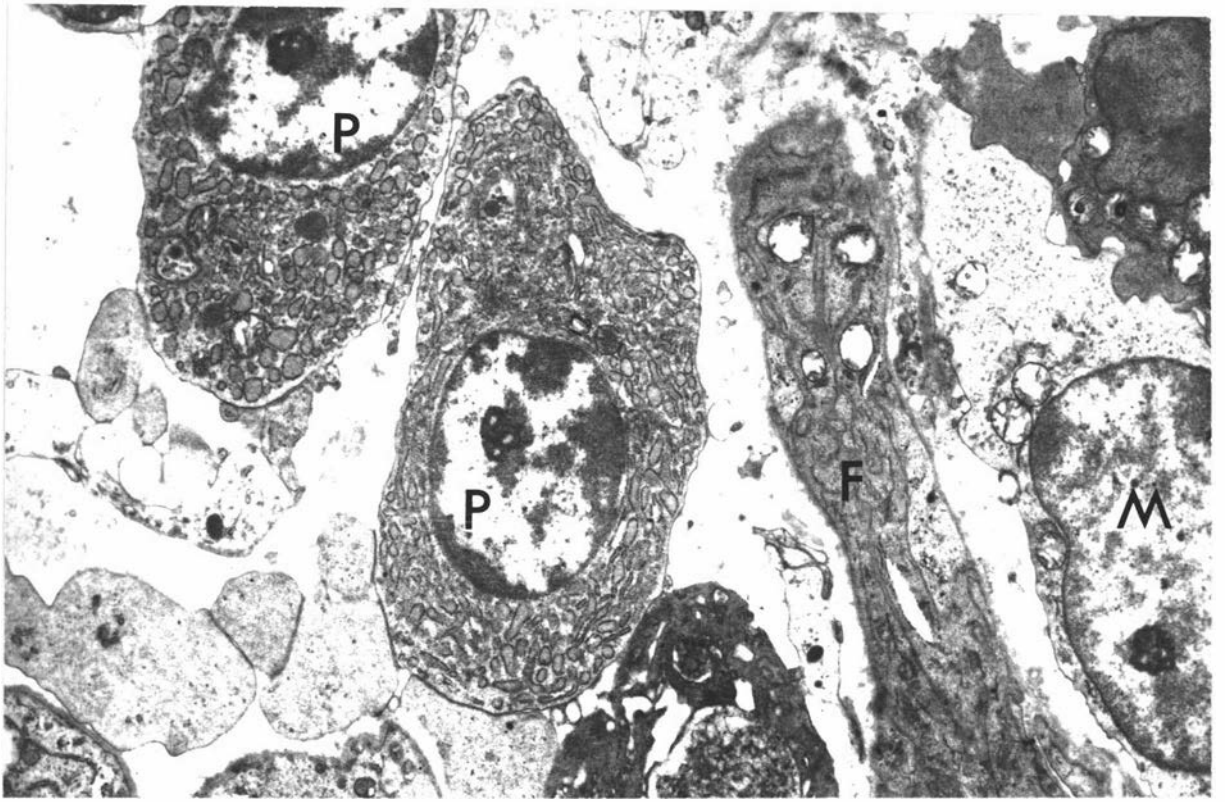
following page 82

Figure 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.

Figure 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.



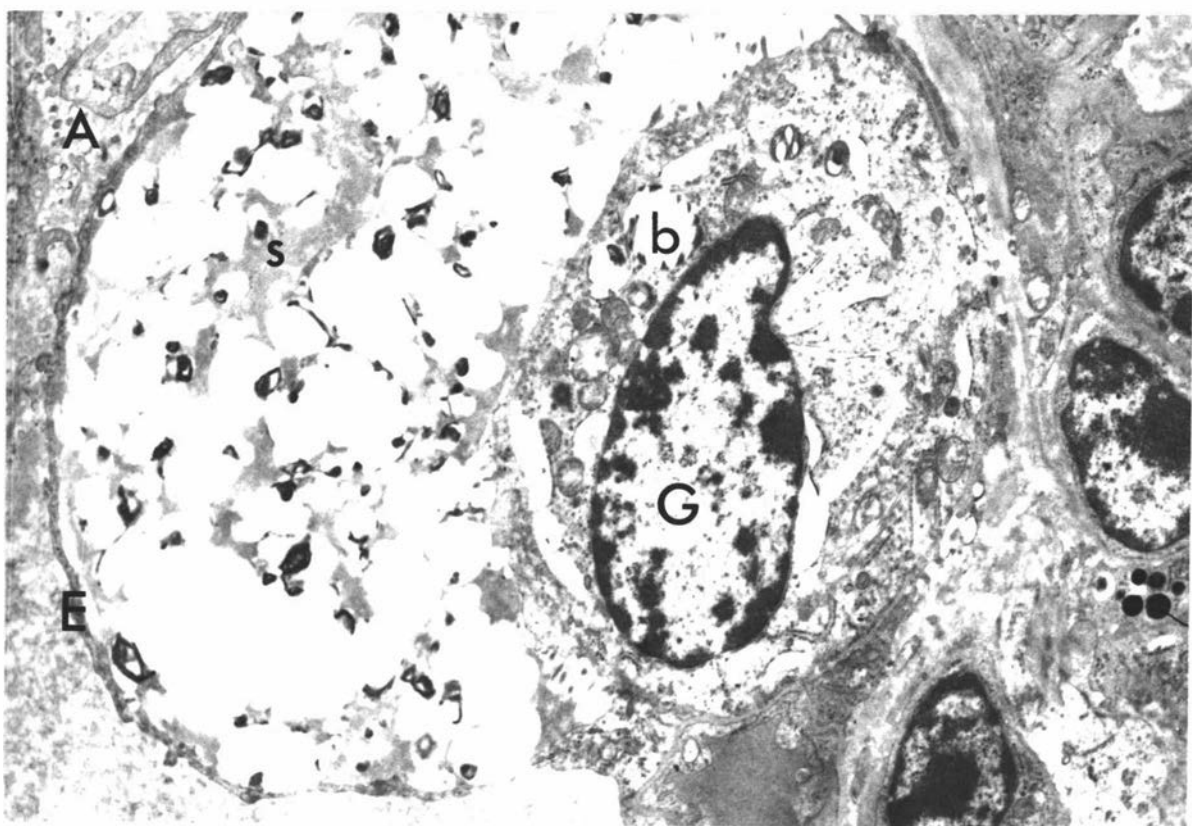
following page 82

Figure 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.

Figure 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.



DISCUSSION

The present study has succeeded in defining pathologically the two main types of pneumonia which are enzootic in sheep in New Zealand. Investigations in the United Kingdom and Australia have also recognised the existence of two similar, separate clinico-pathological entities in the local sheep populations of these countries. Stamp and Nisbet (1963) have used the terms "enzootic" and "atypical" to describe the syndromes which occur in Scotland, whereas St. George and Sullivan (1973) have preferred the terms "acute exudative" and "subacute or chronic (summer pneumonia)" for similar syndromes in Australia. Despite these differences in terminology, the situation in New Zealand with regard to these two pathological entities, appears to be essentially the same as that in both the U.K. and Australia. It is suggested therefore, that the term enzootic pneumonia as used originally in New Zealand by Salisbury (1957) be broadened to its true epidemiological meaning by the inclusion of subacute and chronic pneumonias of the type described in the present investigations.

The distribution of gross lesions in the lungs in acute pneumonia, while invariably more extensive and diffuse than in the subacute and chronic pneumonias, was nevertheless primarily confined to the ventral areas of both lungs. Salisbury (1957) described consolidation and fibrinous pleurisy of a similar distribution; mainly in the apical lobes but extending to the right cardiac and diaphragmatic lobes with acute congestion of the remainder of the lungs. Downey (1957) however, gave a slightly different account of the lesions. While noting that fibrinous pleurisy was a common finding he mentioned that yellow, serous fluid was often present in the thoracic cavity and that congestion and oedema of the lungs were sometimes so intense that it was difficult to demonstrate areas of consolidation.

With the exception of tuberculosis and parasitic diseases, primary involvement of the ventral portions of the lungs is a common event in the majority of pneumonias in most species. It is best explained on the basis of differences in ventilation and perfusion which are related to the gradient in pleural pressure down the lung which in turn is thought to be gravity dependent (Milic-Emili et al., 1968). Under normal

conditions therefore, the alveoli in the dependent portions of lung ventilate more and receive more blood than the upper alveoli thus rendering them more exposed to both endogenous and exogenous insult. The spread of the lesions to the dorsal lobes in many cases of acute pneumonia could therefore be explained either by the possibility that the lesions arise from terminal septicaemic or toxæmic changes or that normal ventilation and perfusion ratios become altered with hyperventilation and physiological stress due to exercise, dipping or shearing with which the disease is often associated.

The histopathology of the acute pneumonias studied in the present investigation does not differ markedly from the description of enzootic pneumonia by Montgomerie et al. (1938) or the contagious pneumonia described in sheep in Iceland by Dungal (1931). The essential features such as the densely packed cellular exudate, fibrin exudation, congestion and intra-alveolar haemorrhage were seen in the majority of the present cases. The occurrence of some other features however, was not as consistent as was indicated by these earlier authors. Haemorrhagic necrosis, although sometimes severe, was not invariably present and fibrinous pleuritis was seen in only approximately half of the cases examined. Nevertheless, the overall pathological picture of the disease in sheep in New Zealand leaves little doubt that it is the same pathological entity as described by these earlier authors and subsequently recognised in many other countries throughout the world (Shirlaw, 1959).

One of the most intriguing questions in the pathogenesis of acute pneumonia concerns the development and composition of the cellular exudate within alveoli that gives rise to the process of "streaming" which appears to be peculiar to sheep pneumonia. Since the initial microscopic observations of Jowett (1930) and Dungal (1931) investigators have consistently regarded this exudate as being largely of mononuclear origin (Montgomerie et al., 1938; Salisbury, 1957). Neutrophils have been observed in capillaries, interstitial spaces and in small numbers in alveolar spaces (Dungal, 1931) but have not been conspicuous within the cellular exudate. The participation of such large numbers of mononuclear cells in what is clinically and pathologically an extremely acute inflammatory reaction is contrary to experimental observations on cellular

migration in acute inflammation (Florey, 1970). Studies on acute inflammation using skin windows in the ears of rabbits (Page, 1972) have shown that polymorphs reach a peak migration rate 6 hours after the initial injury and thereafter migrate at more than double the rate of mononuclear cells at all times up until 72 hours. Mononuclear cells reach their peak migration rate at 32 hours after injury. What is known of the epidemiology of acute pneumonia in sheep from farm histories suggests that it is usually of rapid onset with a short duration and abrupt clinical signs. Salisbury (1957) noted that death usually occurred within 12 hours of the onset of symptoms. It seems unlikely therefore, that a necrotic cellular exudate of the extent observed throughout the lung would have time to accumulate within a 12 to 48 hour period if it was composed only of mononuclear cells. This information therefore supports the present ultrastructural observations which indicate that neutrophils and detached alveolar epithelial cells may contribute to at least part of the cellular exudate. More detailed studies of cell migration in acute inflammation in the ovine lung will be necessary however before firm conclusions can be drawn.

The chronic and subacute pneumonias examined in the present investigation showed histopathological features which most closely resembled the lesions of atypical pneumonia as described by Stamp and Nisbet (1963). Many of the features mentioned by these authors, including interstitial mononuclear cell infiltration, lymphoid hyperplasia, alveolar epithelialization, bronchial epithelial hyperplasia and peribronchiolar fibrosis with the formation of nodular scars were regular features of the present cases. None of these proliferative changes appear to be associated with specific aetiological agents in pulmonary pathology but rather they represent the response of pulmonary tissue to any long term irritation. In the sheep, interstitial thickening, lymphoid hyperplasia and bronchial epithelial hyperplasia are all features of maedi (Georgsson and Pálsson, 1971). However, smooth muscle hyperplasia in the walls of alveolar ducts was only seen occasionally in lungs in the present series and was not as common a finding as it is in maedi. Similarly, the degree of interstitial mononuclear cell infiltration was only mild when compared to maedi. Peribronchial and perivascular lymphoid hyperplasia was variable and was sometimes absent from lungs in which many of the other proliferative

changes were prominent features. Alternatively, it could sometimes be found well developed in lungs where there was only minimal proliferative change and could even be found in occasional lungs from normal lambs collected during the latter half of the pneumonia season. The histological division of pneumonic lungs into lymphoid and interstitial types as was attempted by Stamp and Nisbet (1963) did not seem warranted in the present study as there was considerable overlap of lesions of this type in many of the pneumonias. Instead an attempt was made to divide the lungs into 4 groups according to their gross lesions and the degree of exudative and proliferative changes which were present microscopically. Although no definitive boundaries could be drawn between the lesions in the 4 different groups the exercise was considered useful as it highlighted what is probably the natural progression of the disease from early (type 1) through to advanced (type 4) lesions. It should be noted however, that this suggestion does not encompass the notion that a progression of this kind is inevitable once type 1 lesions occur. On the contrary, the recovery of lungs following type 1 lesions without undergoing severe destructive and proliferative changes remains a real possibility which requires further investigation.

Comparison of the histopathology of the subacute and chronic pneumonias studied here with those reported in Queensland by Sullivan et al. (1973a) and thought to be associated with Mycoplasma ovipneumoniae infection is of considerable interest. Unfortunately detailed microscopic observations on the full spectrum of lesions encountered in the Australian situation have not yet been published. However, the disease has been described in general terms as a proliferative interstitial pneumonia with septal thickening and perivascular and peribronchial accumulations of lympho-reticular cells. Infiltration by neutrophils was a constant feature and alveolar epithelialization and bronchiolar epithelial proliferation were also observed. Although no mention was made of macrophage infiltration this description is similar in many respects to a large proportion of the present cases. Sullivan et al. (1973a) commented on the histological similarity of this condition to atypical pneumonia in British sheep (Stamp and Nisbet, 1963) although St. George (1972) had earlier found no resemblance between pneumonia in sheep in South Australia and Victoria and that described by Stamp and Nisbet (1963).

The clear pathological differences between the two types of enzootic pneumonia seen in New Zealand are best explained on the basis of differences in degree and rapidity of destruction of pulmonary alveolar tissue. These differences were most readily appreciated at the ultrastructural level. Although degeneration and desquamation of alveolar epithelial type I cells was seen in both acute and chronic forms of the disease there were vast differences between the two forms in the severity and extent of this change. In acute pneumonia there was universal sloughing and disintegration of type I cells while type II cells were difficult to locate as they too had sloughed into alveolar spaces and become indistinguishable from necrotic alveolar macrophages. Changes of this severity were not seen in subacute and chronic forms of the disease. In those lungs with chronic pneumonia where there were marked destructive changes, epithelial desquamation was sometimes seen as a focal event but more common findings were degenerative epithelial changes such as vesiculation and bleb formation. Severe destructive changes of a similar nature have recently been reported in experimental bacterial pneumonia in rats (Pine et al., 1973) and were already well advanced 24 hours after the intra-tracheal instillation of Proteus sp. However, reports of ultrastructural changes of this severity in naturally occurring diseases appear to be lacking at the present time.

Severe interstitial changes which were not easily recognised under the light microscope were a conspicuous feature of the ultrastructure of acute pneumonia. Severe oedema of the interstitium, destruction of the capillary endothelium, fibrin and platelet accumulation and eventual rupture of the basement membrane were commonly encountered. Although changes of this nature were not seen in chronic pneumonia, isolated instances of fibrin and platelet accumulation and interstitial oedema were seen in those lungs with a large component of neutrophils.

A further striking difference between the two forms of pneumonia was the presence of large numbers of bacteria within alveolar spaces in the acute disease. Bacteria have often been reported within the alveolar spaces of sheep with acute pneumonia (Dungal, 1931; Salisbury, 1957). Although they could be readily demonstrated in the present cases by the use of Gram or Giemsa stains the electron microscope enabled better

visualization of their location. It indicated that they were closely associated with destructive changes within alveoli as they could be found within necrotic alveolar macrophages and beneath desquamating type I epithelial cells. Surprisingly few bacteria were found in the subacute and chronic forms of pneumonia, but when present these too were associated with destructive events such as loss of alveolar epithelium and necrosis of infiltrating leucocytes.

No single, universal feature could be considered characteristic of the ultrastructural appearance of the early (type 1) lesions in which alveolar collapse and slight macrophage infiltration were the typical light microscope findings. However, several significant changes were found irregularly distributed in both interstitial and alveolar epithelial tissues. One of these changes was mild interstitial oedema in perivascular spaces which was similar but less severe than that described in the unventilated, collapsed lungs of dogs in the early stages of oxygen poisoning (Coalson et al., 1971). In the experiments with dogs, the oedema was invariably accompanied by capillary endothelial damage but this was not a feature of the present cases. It is possible that the lesions found in the present study resulted from patho-physiological factors related to alveolar collapse such as increased pulmonary vascular resistance, congestion and increased capillary permeability. A further change which was commonly encountered was vesiculation and bleb formation in the alveolar epithelium. Blebs or "blisters" have been seen in a variety of circumstances in type I alveolar cells (Manktelow, 1967b; Reidbord, 1967; Coalson et al., 1971; Finlay-Jones et al., 1971), type II alveolar cells (Harrison, 1970), Clara cells (Wang et al., 1971) and ciliated bronchiolar cells (Baskerville, 1972). They are thought to be caused by degenerative changes in cell membranes due to non-specific toxic factors (Zollinger, 1948).

Possibly one of the more important early changes that were detected were the degenerative lesions exhibited by type II alveolar cells. They ranged from mild changes to severe lesions typical of the subcellular reaction to lethal injury described by Trump and Ginn (1969). They included mitochondrial swelling, dilatation and vesiculation of the endoplasmic reticulum, loss of continuity of plasma membranes, indentation

of nuclear membranes and clumping of nuclear chromatin. Changes in type II cells of this nature have been previously reported in such diverse conditions as oxygen poisoning (Adamson et al., 1970b), carbon dioxide exposure (Schaefer et al., 1964), ozone administration (Bils, 1970), phosgene inhalation (Diller et al., 1969), dilute nitric acid instillation (Greenberg et al., 1971), kerosene, sodium arsenite, and distilled water instillation (Manktelow, 1967b), Paraquat administration (Manktelow, 1967b; Vijeyaratnam and Corrin, 1971; Smith and Heath, 1974) and adrenalin administration (Wang et al., 1971). Other features such as the loss of microvilli and disruption of osmiophilic bodies which are more specific to type II cells are also mentioned by many of these authors and were frequently encountered in the present study. One change often observed in both acute and chronic pneumonia which does not appear to have been previously reported was the formation of thickened, electron-dense plasma membranes. Because this change always occurred in the most superficial areas of the cell membrane it was assumed to result from the direct effects of toxic substances in the alveolar space.

The common occurrence of moderately electron-dense inclusions in damaged and proliferating type II cells is an unusual finding. The material involved is presumably a lipoprotein and appears to have accumulated in cells in which few osmiophilic bodies are present. It is possible therefore, that the material has become sequestered in autophagocytic vacuoles because of a metabolic defect of some sort in the normal secretory mechanisms of pulmonary surfactant. Recently, Stephens et al. (1973) have reported the presence of a similar substance in the type II cells of dogs following long term exposure to ozone. Although the material observed by these authors was of similar electron density and distribution it contained bar-like structures which had a periodicity of 75.4 nm. It seems likely that the two substances are of a similar nature and that the differences in periodicity and morphology are due to species differences or differences in fixation and embedding techniques.

One of the major features of the repair phase of chronic pneumonia was hyperplasia of the alveolar epithelium which was seen in the majority of lungs examined. The finding that alveolar type II cells are the main

cell contributing to this repair has been reported by Alley and Manktelow (1971) and has since been confirmed by Baskerville and Wright (1973) in porcine enzootic pneumonia. In human lungs this phenomenon was first noted by Okada and Genka (1966) and has subsequently been observed in desquamative interstitial pneumonia by various authors (Brewer et al., 1969; Leroy, 1969; Shortland et al., 1969). Cells similar to type II cells have also been identified as the main contributor to the neoplastic epithelial proliferations of pulmonary adenomatosis in sheep (Nisbet et al., 1971; Wandera and Krauss, 1971) although many of the cells involved in the intra-bronchiolar proliferations in this disease closely resembled Clara cells of the terminal bronchioles.

Although the involvement of type II cells in alveolar epithelial repair in many mammalian pneumonias is now well established the question of origin of the proliferating cells remains a source of controversy. The finding of cells intermediate between type I and type II in the present study lends support to the suggestion by Evans et al. (1973) that type II cells may transform into type I cells during the later stages of epithelial repair. Similar observations have been made by Faulkner and Esterly (1971) in pneumonia in rabbits induced by the administration of Freund's adjuvant and by Greenberg et al. (1971) in rats following the intra-bronchial instillation of dilute nitric acid. In the latter case however, the authors advanced the reverse hypothesis; namely that type II cells may be formed by transition from type I cells. Convincing evidence favouring the former hypothesis has come from recent autoradiographic studies by Adamson and Bowden (1974) on alveolar epithelial regeneration in mice following oxygen exposure. These studies have shown increased tritiated thymidine labelling and mitosis in type II cells soon after injury without a concomitant response in type I cells. However, on the 3rd or 4th day after injury there was an increase in labelling of type I cells which was coincident with a halving in grain counts in type II cells. While this explanation of the phenomena observed during alveolar repair seems plausible the possibility that replacement of type I cells in the normal lung is accomplished by the multiplication and transformation of type II cells remains a matter for speculation at the present time.

A further possible source of proliferating type II cells worthy of consideration is that those in the peribronchiolar regions may arise from de-differentiation and migration of Clara cells from the terminal portion of respiratory bronchioles. This view is supported by the finding of occasional undifferentiated cuboidal cells having the morphological appearance of Clara cells but lacking secretory granules, lining alveolar spaces in some cases of chronic pneumonia. In addition, a few lamellated structures resembling osmiophilic bodies of type II cells were occasionally encountered within Clara cells at the distal end of respiratory bronchioles. Such a view would be in keeping with the acknowledged close embryological relationship between the alveolar and bronchiolar epithelia (Emery, 1969) and the biochemical similarities which have been established between type II cells and Clara cells (Azzopardi and Thurlbeck, 1969). In this context also, the recent findings of Baskerville et al. (1974) following experimental influenza infection in hamsters and mice are of great interest. It was found that alveolar epithelialization in this situation was due to the downward extension of both Clara cells and ciliated bronchiolar cells. Admittedly however, the lesions induced by Baskerville et al. were of a more bizarre proliferative nature than those seen in the present study as they included complete loss of type II cells from some areas and squamous metaplasia of non-epithelialized areas.

What ever viewpoint one takes regarding the origin and turnover of alveolar epithelial cells during chronic pneumonia it seems clear from the present work that the subdivision of cell types into rigid categories, incapable of de-differentiation and transformation during repair processes is no longer justifiable.

SUMMARY

There are two main types of enzootic pneumonia occurring in sheep in New Zealand. These are respectively acute and chronic in nature and they have been pathologically defined by a study of more than 400 naturally-occurring cases.

Acute pneumonia in sheep of all ages is characterised by congestion, 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. Bacteria are closely associated with destructive changes such as alveolar epithelial desquamation, necrosis of leucocytes and macrophages and interstitial oedema.

The subacute and chronic pneumonias in lambs show varying degrees of dull red to grey consolidation of the anteroventral lobes. They can be divided into 4 groups according to their gross appearance and the degree of exudative and proliferative change observed histologically. It is suggested that these groups represent stages in the natural progression of the disease although resolution of early lesions could presumably occur if destruction was minimal.

The major factor underlying the pathological differences between the two forms of pneumonia is considered to be the severity and extent of damage to the alveolar epithelium. In the acute form, detachment and necrosis is universal whereas in the subacute and chronic forms, slower more localised changes allow time for repair to occur.

No specific ultrastructural lesion are considered characteristic of the early subacute lesions but a variety of degenerative changes in the interstitium and in alveolar type I and II cells are described. The nature and sequence of subcellular type II cell degeneration has been studied and several features not previously reported in mammalian pneumonias have been observed.

Proliferation of alveolar type II cells is the main method of repair of the alveolar epithelium. Electron microscopy reveals that undifferentiated cells resembling those found in the foetal lamb can be seen in cases

where proliferation is marked. Several histochemical reactions have been shown to serve as a further useful means of studying these changes by differentiating alveolar macrophages from type II cells in both normal and pneumonic lungs.

The origin and dynamics of type II cell hyperplasia are discussed. Because cells intermediate between type I and II were sometimes observed it is suggested that a transition from type II to type I cells may occur as part of the normal mechanisms for alveolar repair. It is concluded that the alveolar epithelium has a high degree of adaptive and proliferative capacity which has not been previously appreciated.

CHAPTER 3

EXPERIMENTAL INJURY TO THE OVINE LUNG

INTRODUCTION

During the preceding study of the pathology of sheep pneumonia a great variety of changes were observed in the pulmonary alveolus. Various cell types were found undergoing degeneration and necrosis while others were seen in various stages of infiltration or proliferation. Although these studies were valuable they were not sufficiently illuminating to define the time sequence in which events occurred or to indicate whether the injuries arose from endogenous or exogenous insults.

The following experimental studies were instigated to explore more fully the nature and development of alveolar injury in sheep. They sought to study the pulmonary damage induced in sheep by specific chemical and bacterial agents and compare this with the lesions of naturally-occurring ovine pneumonia. Apart from the inoculations of the various micro-organisms listed in Chapter 1, there have been no detailed experimental investigations of alveolar injury reported in sheep up until the present time. The majority of studies of this type have been carried out in laboratory animals so these must serve as the basis for comparison with the pulmonary lesions induced in the current investigation.

EXOGENOUS AGENTS: DILUTE NITRIC ACID PLUS CARBON BLACK

In order to study the effects of exogenous agents on the pulmonary alveolus and minimise the effects of systemic factors, it is necessary to use a system in which lung injury is localised and not sufficiently severe to cause respiratory embarrassment. At the same time, the agent used must be capable of inducing sufficient damage to allow the normal mechanisms of inflammatory response and repair to proceed in an overt manner.

The use of 1% nitric acid by intrabronchial instillations as demonstrated by Greenberg et al. (1971) in rats, fulfills these

requirements. In addition, it has been postulated that weak solutions of nitric acid are responsible for disrupting portions of the alveolar septum in man and therefore have a role in the pathogenesis of emphysema (Boren, 1964). The weak solutions of nitric acid in this situation are thought to result from the combination of cellular water in the alveolar space and inhaled nitrogen dioxide which is a prime constituent of air pollution in industrialised countries (Hyadon *et al.*, 1965). Thus any information gained from the present investigation may be relevant to the pathogenesis of chronic respiratory disease in man.

Besides its obvious value as a marker, carbon black was included in the present study because it has been shown by Boren (1964) that carbon may act as a carrier mechanism for irritant substances such as absorbed nitrogen dioxide and this in turn may produce focal lung damage following phagocytosis.

Material and Methods

The animals used in this experiment were 2 to 3-month-old worm-free, colostrum-fed lambs which had been housed in isolation from other sheep since 1 to 2 days of age and artificially reared on milk substitutes, hay and a proprietary pelleted sheep diet.

Table VIII summarises the experimental design and shows the time intervals at which the animals were sacrificed. The animals received a suspension of 1.8 ml of 1% nitric acid and 0.2 ml India ink⁽¹⁾. Two animals served as controls and received 0.2 ml of India ink suspended in 1.8 ml of 0.85% (normal) saline.

Endobronchial instillation was accomplished by inserting a 12 gauge hypodermic needle into the midline of the trachea 3 cms distal to the larynx under local anaesthesia. The fluid suspension was instilled by means of a 4 FG gauge plastic canula on which the point had been sealed and 5 to 6 pinpoint holes made around the circumference

(1) Pelikan, Gunther Wagner.

TABLE VIII

SUMMARY OF HISTOLOGICAL FEATURES FOLLOWING INSTILLATION OF
DILUTE NITRIC ACID AND CARBON

1% NITRIC ACID + CARBON

Lamb No.	Duration of lesion	Alveolar collapse	Fibrin	Neutrophils	Alveolar macro- phages	Type II cells	Fibrosis
E211A	2 hours	+	+	+	0	0	0
211	3 hours	±	+	++	+	0	0
212	6 hours	+	+++	++++	++	0	0
213	12 hours	++	++	++	++	±	0
214	1 day	+++	+	++	+++	+	0
215	2 days	++	++	+	+++	+	0
216	3 days	++	++	+	+++	++	0
217	5 days	+++	0	+	++	++	0
218	7 days	+++	0	0	++	++	+
219	10 days	+	0	0	+	+	+

0.85% (NORMAL) SALINE + CARBON

210A	1 day	0	0	0	+	0	0
210	5 days	+	0	+	++	+	0

immediately behind this so that the fluid emerged in a fine spray. In this way as even as possible distribution of liquid was obtained within a localised area. With the animal held in dorsal recumbancy the catheter was inserted through the needle until peripheral resistance was met. The animal was then moved to a vertical upright position before the suspension was delivered slowly through a 2 ml syringe.

The lungs of all animals were examined grossly following euthanasia by intravenous barbiturate and a range of 5 to 6, 1 mm³ blocks were collected from the ink-marked areas for electron microscopy. The remainder of the ink-marked tissue was preserved in 10% formol-saline for histology. Light microscopy was performed both on formol-saline fixed, paraffin embedded sections stained with haematoxylin and eosin (HE) and on 1 µm Karnovsky fixed, epoxy embedded sections stained with toluidine blue and basic fuchsin (TbBf). Thin sections for electron microscopy were cut and stained as previously detailed in Chapter 2.

Pulmonary surfactant activity was studied on all lungs by the use of the frozen section method of Manktelow (1967a). Small 6 to 8 mm³ blocks of fresh tissue from unaffected (normal) areas and affected (pigmented) areas of the lung were collapsed and mounted together on the same chuck. Frozen sections were cut at 20 µm, air-dried and covered with air-saturated water. The distribution and stability of air bubbles in the affected and unaffected areas was then observed under the light microscope.

Results

Gross Pathology

No consolidated areas were seen in the lungs from the control animals or in the lungs from animals in the study group killed at 2, 3 and 6 hours following instillation of dilute nitric acid. The distribution of the fluid suspension as indicated by the presence of carbon marker, was usually in one lung and within that lung it was mainly confined to 1 or 2 lobes. In some cases however, black particles were found in the ventral areas of the other lung where presumably they became lodged following coughing and inhalation. The cut surface of

these lungs showed severe deposition of black particles in some lobes and this appeared to be centred mainly around bronchioles.

The first macroscopic evidence of lung injury was seen in the animal killed at 12 hours following nitric acid administration. This took the form of several 1 to 2 cm diameter areas of red consolidation on the dorsal surfaces of the right apical and left diaphragmatic lobes of the lungs which were surrounded by irregular, patchy areas of consolidation (Figure 3.1). In the animal sacrificed at 24 hours several similar areas were present in the left diaphragmatic lobe and patchy areas of congestion associated with carbon deposition were found in the right apical lobe. Small wedge shaped areas of dark red consolidation up to 3 cms in width were found in the ventral areas of the right apical, right cardiac and left cardiac lobes in the animals killed on days 2 and 3. These were invariably associated with carbon deposition in the most dorsal parts of the lesion and to a varying degree elsewhere in the surrounding lung.

The most severe consolidation was found in the lamb killed on day 5 (Figure 3.2). Here the ventral areas of the right apical and left cardiac lobes showed dull red consolidation and there was patchy congestion of the dorsal and ventral areas of the left diaphragmatic lobe. In the lamb killed at 7 days, patchy red depressed areas, up to 2 cms in diameter which were associated with carbon deposition were found over an 8 cm² area in the left dorsal diaphragmatic lobe. Similar areas were found in the left apical and cardiac lobe of the animal killed at 10 days.

Light Microscopy

For descriptive purposes the histological findings may be divided into 3 overlapping stages. The first stage from 2 to 12 hours was mainly exudative in nature. The second stage from 1 to 3 days was both exudative and proliferative while the third from 5 to 10 days was mainly concerned with repair. The degree of inflammatory response varied considerably between individual animals but despite this it was possible to obtain a reasonably clear view of the basic sequence of events. Table VIII summarises the main histopathological features

following page 98

Figure 3.1

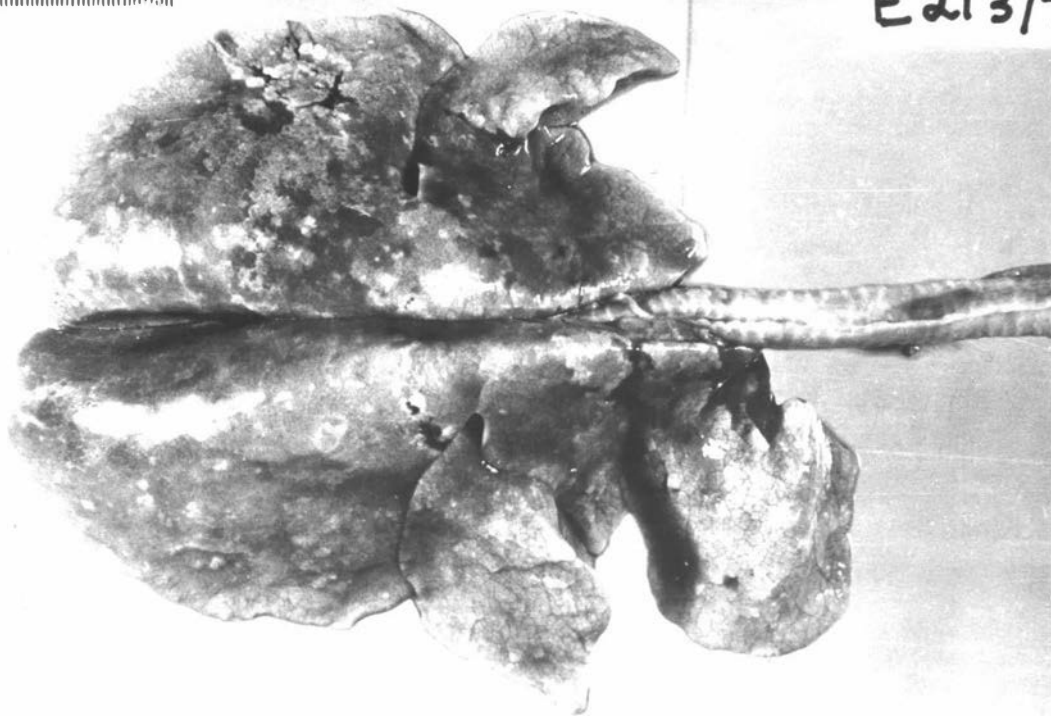
Twelve hours after instillation of dilute nitric acid and ink. Several irregular foci of dark red consolidation and patchy areas of congestion are present on the dorsal surfaces of the right apical and left diaphragmatic lobes.

Figure 3.2

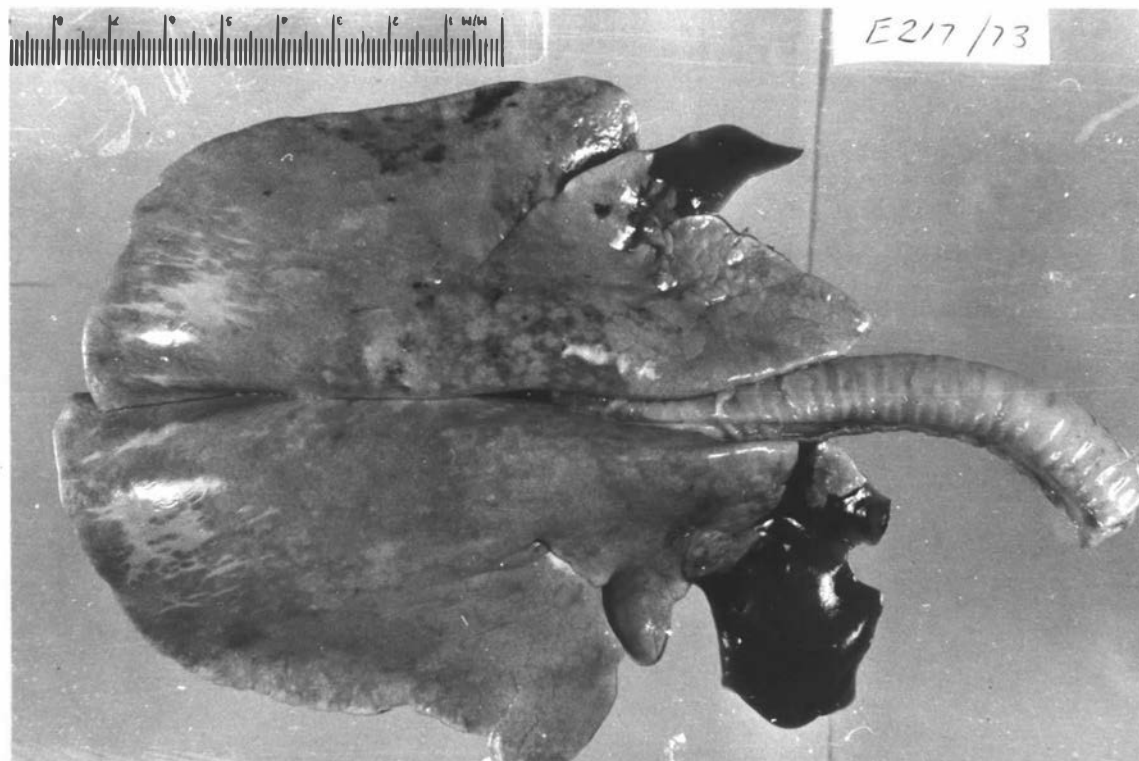
Five days after instillation of dilute nitric acid and ink. The ventral areas of the right apical and left cardiac lobes show dull red consolidation and there is patchy congestion of the dorsal and ventral areas of the left diaphragmatic lobe.



E213/-



E217 /73



seen in both the control and treated groups.

0 to 12 hours. The most striking change observed in the first animal sacrificed at 2 hours after administration was collapse of the alveolar spaces surrounding terminal bronchioles (Figure 3.4). Some of these alveoli contained small amounts of proteinaceous exudate within which carbon particles were situated. There was moderate congestion of alveolar capillaries and some contained small numbers of neutrophils which were adherent to the vessel walls. At 3 hours greater numbers of neutrophils were seen in alveolar capillaries and these first appeared in alveolar spaces at 6 hours (Figure 3.5). Alveolar macrophages were first observed actively phagocytosing carbon particles at 3 hours when large carbon-laden macrophages were found surrounding clumps of ink in both bronchioles and alveoli. However, a large proportion of the carbon remained unphagocytosed at 6 hours and lay mixed with mucus and fibrin in alveolar spaces and bronchioles. Fibrin exudation was very severe in some areas at 6 hours and occasionally haemorrhage into alveolar spaces also occurred. Cellular exudation was also severe at this time and consisted of approximately 70% neutrophils, 20% small macrophages and 10% large macrophages.

In the animal killed at 12 hours some alveolar spaces still contained large numbers of neutrophils (Figure 3.6). Other alveoli were distended with large carbon-laden macrophages and it was estimated that at this stage approximately 50% of the ink present had been phagocytosed. Taken overall, neutrophils and macrophages were present in approximately equal numbers at this time although monocytes predominated in the interstitial tissues.

1 to 3 days. Exudative changes were still a prominent feature of the inflammatory response at 1 day following nitric acid administration but macrophages now outnumbered neutrophils in the cellular exudate. At this stage carbon-laden macrophages were observed within interstitial tissue for the first time. They were found in small numbers in interstitial tissue depots around respiratory bronchioles (Figure 3.7). Occasionally carbon-laden macrophages were also found traversing the epithelium of respiratory bronchioles adjacent to the peribronchiolar

following page 99

Figure 3.3

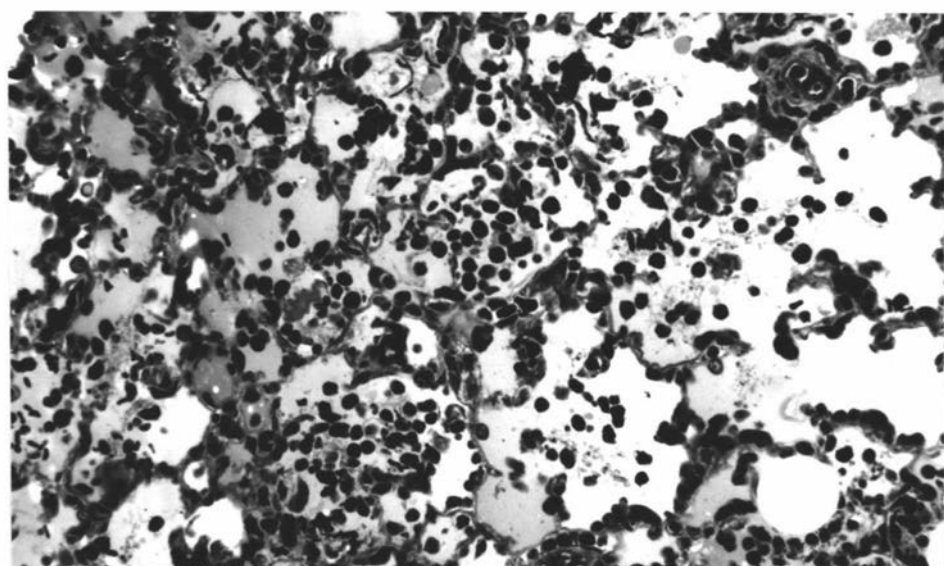
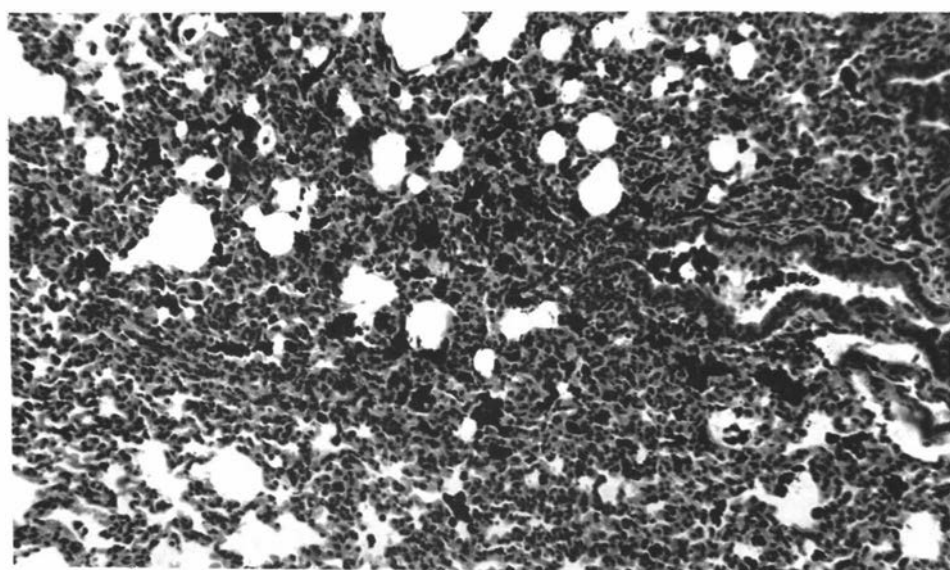
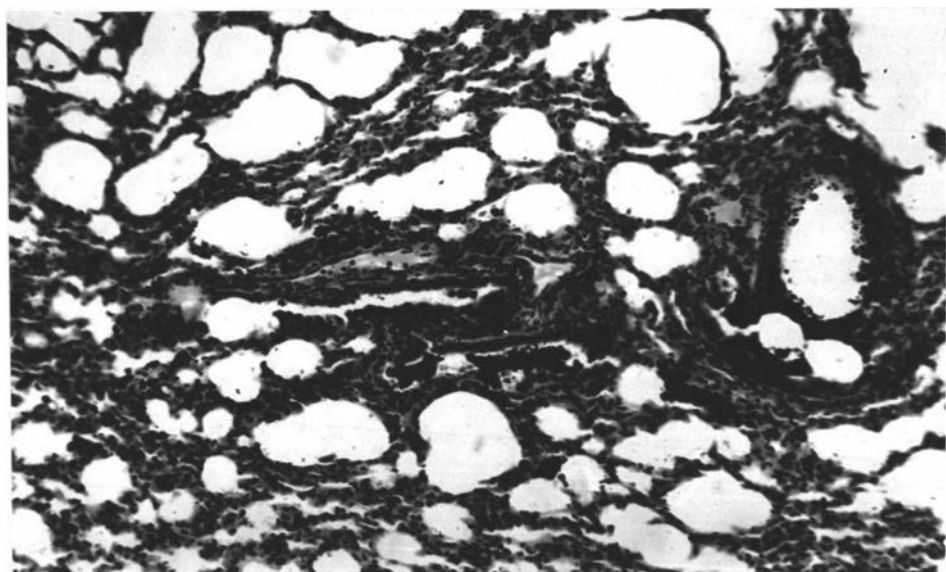
Control animal 1 day after the administration of normal saline and ink. There is only very limited alveolar collapse despite the deposition of ink in bronchioles and alveolar ducts. HE x 100.

Figure 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.

Figure 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.



following page 99

Figure 3.6

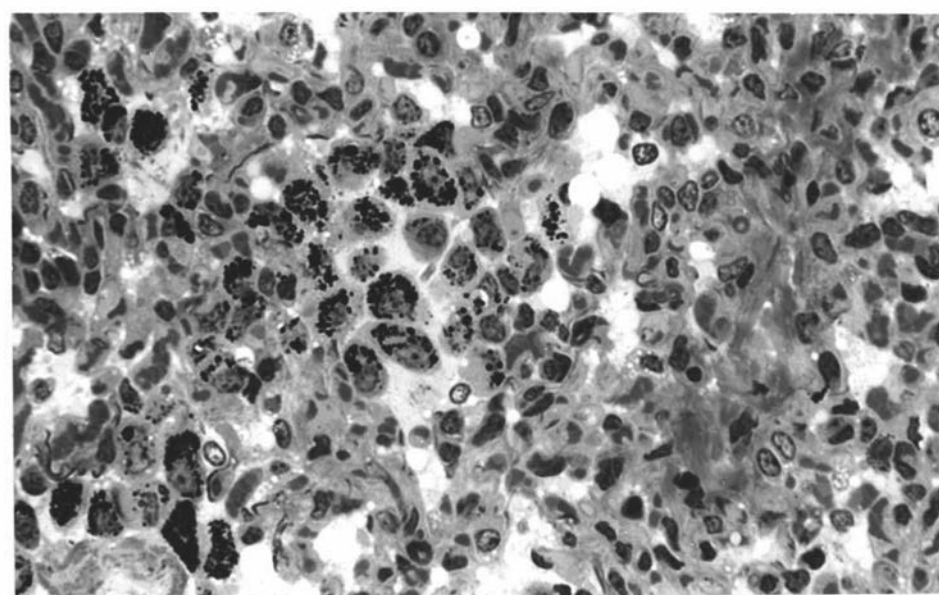
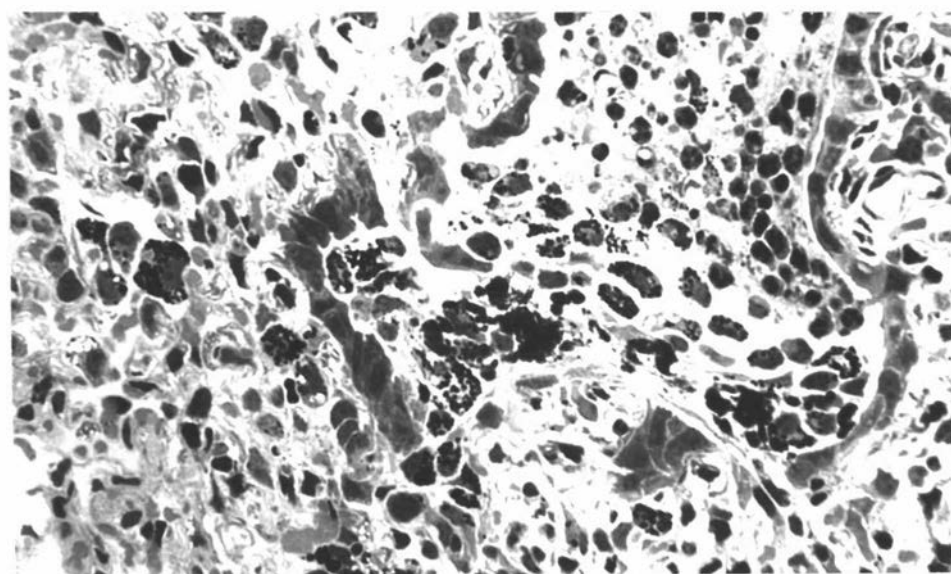
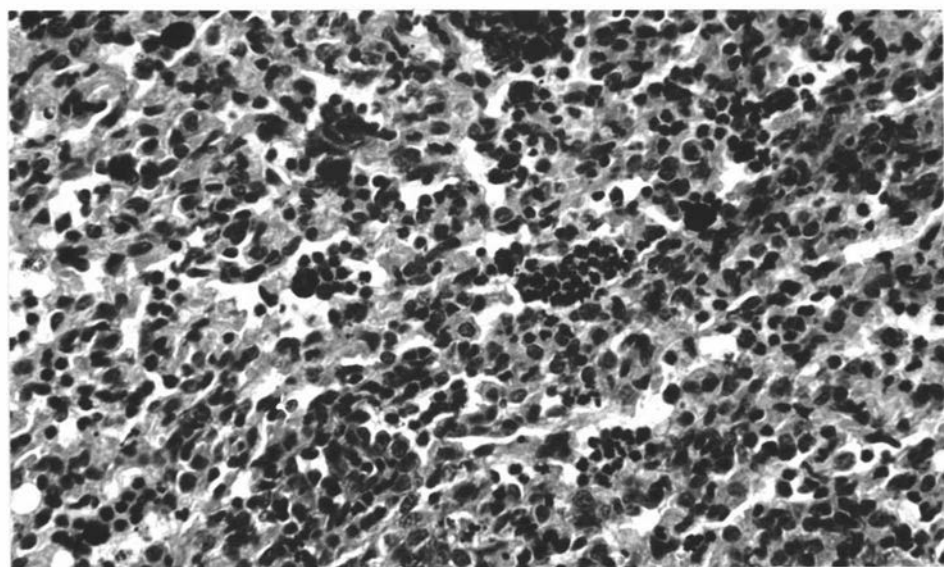
At 12 hours after instillation of dilute nitric acid and ink neutrophil infiltration is still a prominent feature and alveolar collapse is severe. HE x 250.

Figure 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. Eposy resin embedded. TbBf x 400.

Figure 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.



macrophage depots. The first evidence of epithelial hyperplasia was found at 1 day following nitric acid administration when groups of 5 or 6 pale-staining Clara cells could be seen proliferating in the most distal parts of the terminal bronchioles (Figure 3.9). Bronchiolar epithelial hyperplasia increased rapidly thereafter and in the animal killed at 3 days it was severe and had the appearance of extending into surrounding alveolar spaces (Figure 3.10 and 3.11). Type II alveolar epithelial cells were first observed in numbers greater than normal in epoxy-embedded sections taken at 1 day following nitric acid administration. Almost every alveolar space contained a prominent type II alveolar cell and in some cases two type II cells were found adjacent to one another in the same space. These changes became more obvious at 2 days when they were most easily seen in areas immediately surrounding those in which fibrin and cellular exudation occurred (Figure 3.12). Neutrophil participation in the inflammatory response had decreased markedly at 2 days and by 3 days few neutrophils remained in alveolar spaces.

5 to 10 days. Type II cell hyperplasia continued to be a feature of the proliferative response up until 7 days following nitric acid administration. In severely collapsed areas of lung pockets of 2 to 3 vacuolated cells were seen in epoxy-embedded sections which were later identified as type II cells by electron microscopy (Figure 3.13). At 7 days occasional alveoli in areas adjacent to bronchioles were partially epithelialized and contained 4 or more type II cells (Figure 3.14).

Approximately 60% of the remaining carbon pigment had been transported to the interstitial tissues by 7 days after administration. It lay within macrophages which were located around blood vessels and bronchioles. Some macrophages remained sequestered within alveoli surrounded by areas of collapsed alveolar tissue in which interstitial mononuclear cells and fibrous tissue were present (Figure 3.8). Isolated pockets of sequestered carbon-laden macrophages could still be found in alveolar tissue 10 days after administration but by this time 80 to 90% of the remaining carbon was located within macrophages in the peri-bronchiolar or perivascular interstitial tissues.

Figure 3.9

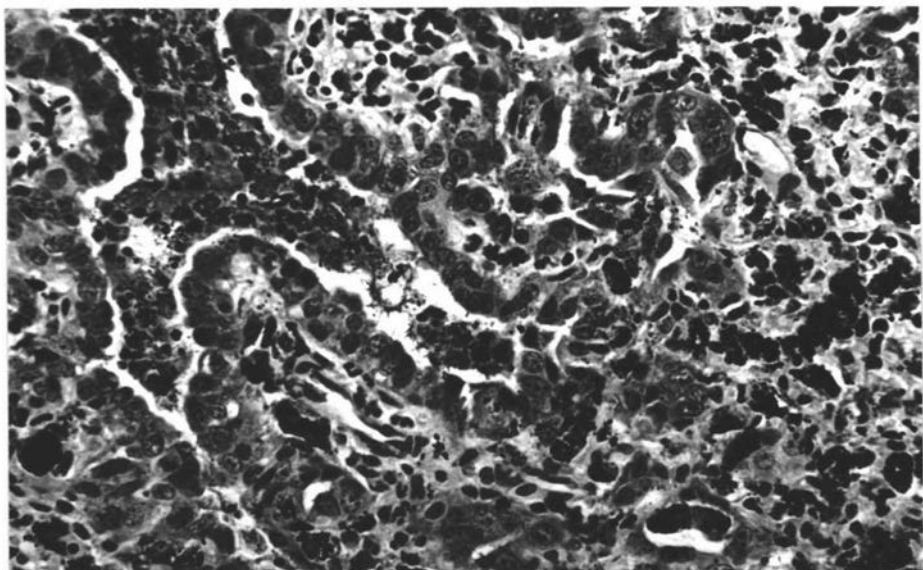
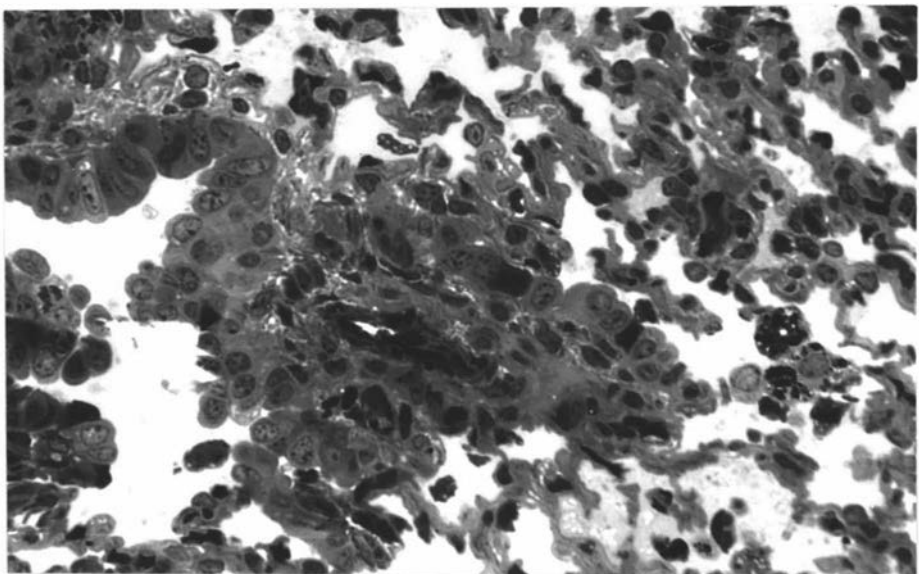
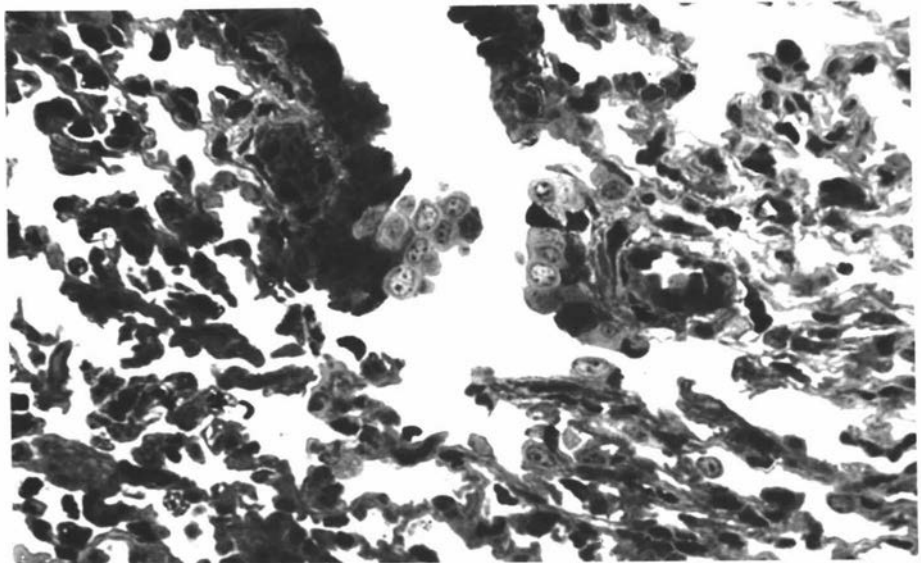
Early epithelial proliferation in the most distal part of a terminal bronchiole 1 day after instillation of dilute nitric acid and ink. The cells involved are pale-staining cuboidal cells resembling Clara cells. Epoxy resin embedded. TbBf x 400.

Figure 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.

Figure 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.



following page 100

Figure 3.12

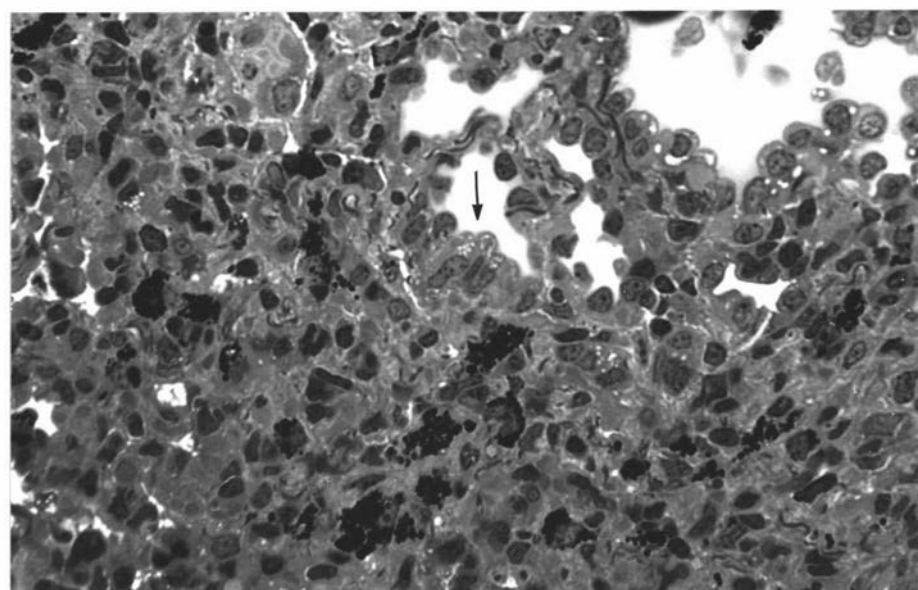
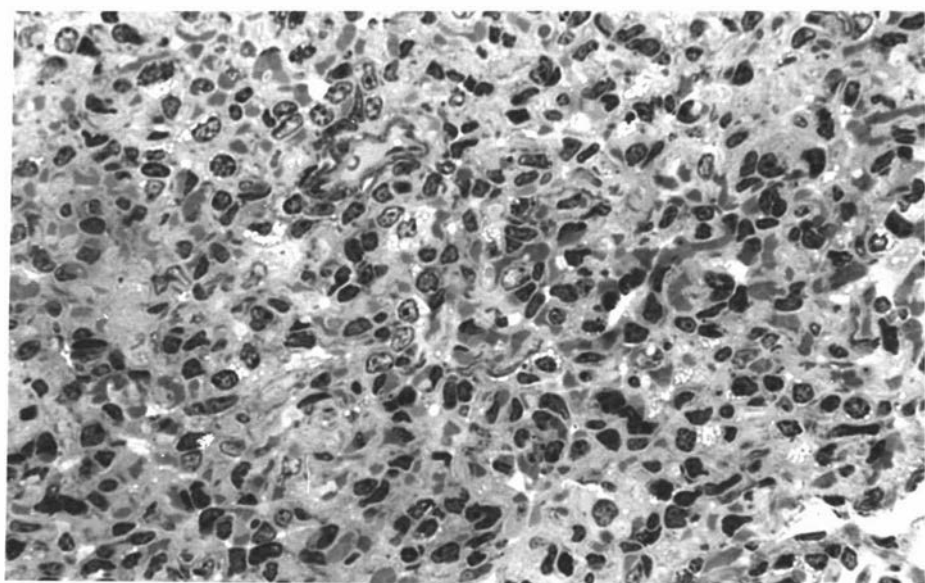
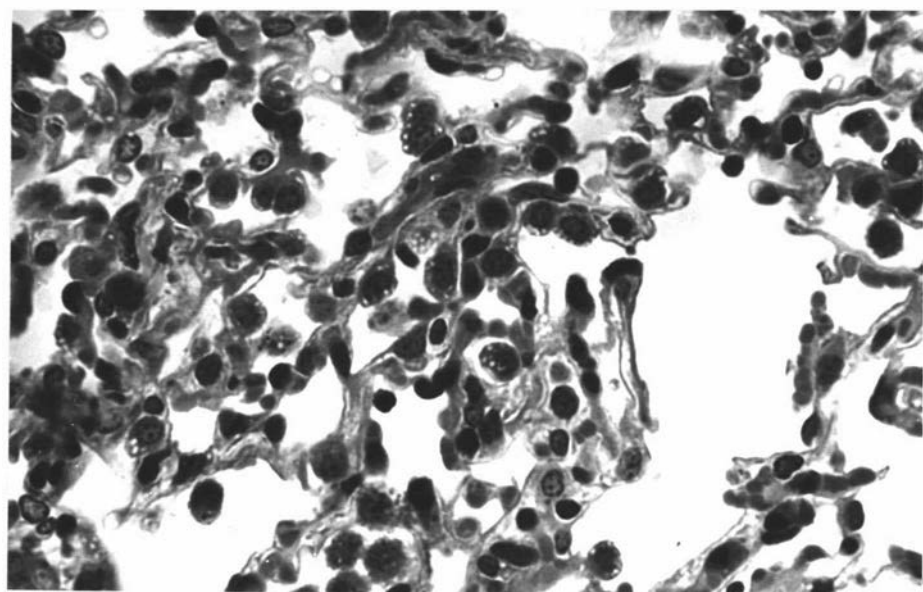
Early alveolar type II cell hyperplasia in an area adjacent to a focus of cellular exudation at 2 days after instillation of dilute nitric acid and ink. Almost every alveolar space contains one or more type II cells. Epoxy resin embedded. TbBf x 400.

Figure 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.

Figure 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.



Although the bronchiolar epithelium was still thickened 10 days after administration the degree of hyperplasia present had diminished considerably. Re-aeration of alveoli was visible within focal areas of the remaining collapsed tissue but the alveolar septa in these areas remained slightly thicker than normal.

Control Animals. At one day after the administration of normal saline and ink, no obvious alveolar collapse, fibrin exudation or epithelial hyperplasia were observed (Figure 3.3). Occasional free carbon particles were seen in terminal bronchioles but the majority of carbon particles lay within macrophages which were located within terminal bronchioles and adjacent alveolar ducts and alveoli. Large carbon-laden alveolar macrophages were still present in many of the alveolar spaces surrounding bronchioles at day 5 but by this time approximately one third of the carbon remaining lay within macrophages in the alveolar interstitium or peribronchiolar and perivascular depots. Occasional collapsed alveoli with mildly thickened alveolar septa were seen in areas adjacent to bronchioles and slight epithelial hyperplasia was observed in those terminal bronchioles where pigment deposition was heavy.

Pulmonary Surfactant Studies

Observations made on the lungs into which dilute nitric acid was instilled showed a gradual decrease in density of stable bubbles formed near areas of carbon deposition from 6 hours after administration up until 3 days (Figure 3.15). By this time there was an almost complete absence of bubbles in the pigmented areas (Figure 3.16). Thereafter bubble distribution was variable and some areas of low bubble density were still apparent in focal areas of the lungs obtained from the animal killed at 10 days.

No obvious reduction in bubble density was seen in the pigmented areas of the control lungs from the animals killed at 1 day or 5 days after the administration of saline and ink.

following page 101

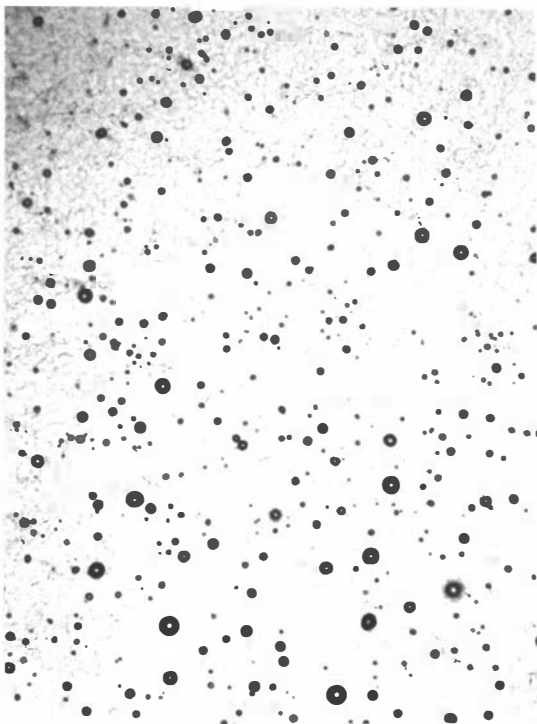
Figure 3.15

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.

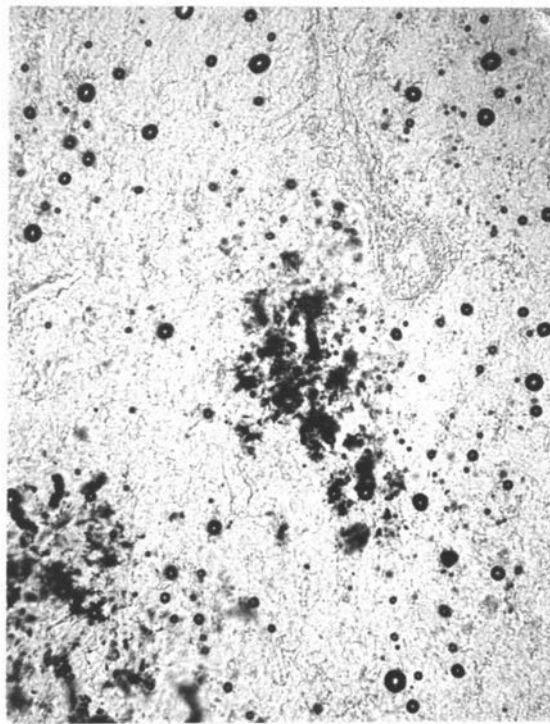
(a) Unaffected area. (b) Affected area in which there is loss of bubbles from areas surrounding the instilled fluid. (Unstained x 100).

Figure 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).



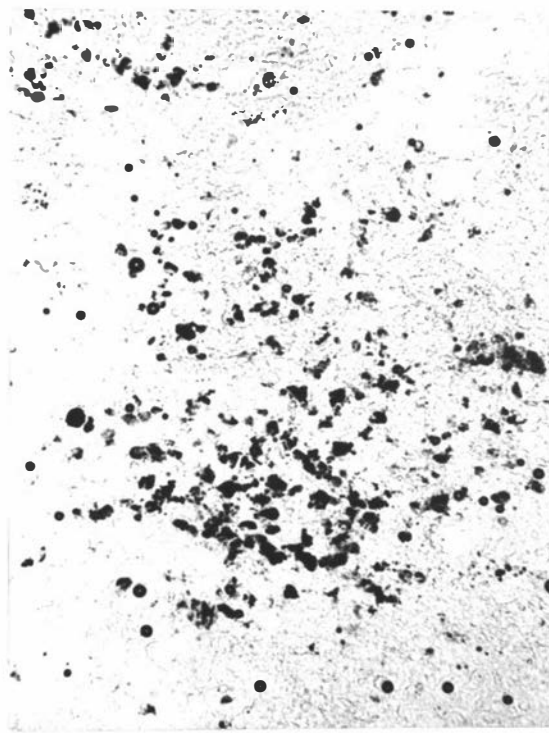
a



b



a



b

Electron Microscopy

Extensive damage to both type I and type II alveolar epithelial cells together with oedema of the alveolar interstitium were the main ultrastructural features in the lungs examined at 2 hours after nitric acid administration. The type I cells exhibited two main kinds of degenerative change. The first type consisted of a diffuse swelling and vesiculation of the cytoplasmic extensions. The vesicles sometimes ballooned out into the alveolar space to form large blebs (Figure 3.17). This type of change usually occurred in areas where damage was less severe and the affected cells always remained firmly attached to the basement membrane. At 6 hours some type I cells in collapsed alveoli appeared diffusely swollen and this swelling now included the cell nuclei which was enlarged up to twice its normal size (Figure 3.18). Numerous small irregular cytoplasmic projections were often present on the cell surface but these were also found on type I cells of normal appearance in collapsed alveoli. Diffuse swelling of type I cells continued to be encountered in all lungs examined up until 2 days after nitric acid administration.

The second type of degenerative change commonly found in type I cells was more severe in nature and involved an increase in density and shrinking of the cell's cytoplasmic extensions. This change was seen in areas where there were marked destructive changes such as oedema and disruption of the interstitial tissues (Figure 3.20) and was often a prelude to complete desquamation (Figure 3.21). Changes of this type were not seen in the lungs obtained after 6 hours following administration.

The degree of damage suffered by type II alveolar epithelial cells was less severe than that seen in type I cells. From 2 hours after administration degenerative changes including swelling of mitochondria, vesiculation of the endoplasmic reticulum, loss of microvilli and the presence of cytoplasmic extensions were commonly observed (Figure 3.19). Despite these and more severe changes such as loss of nuclear membrane and aggregation of chromatin (Figure 3.20) affected cells usually remained firmly attached to the basement membrane and desquamation was only rarely seen. In many areas type II cells showed only minor

Figure 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.

Figure 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.

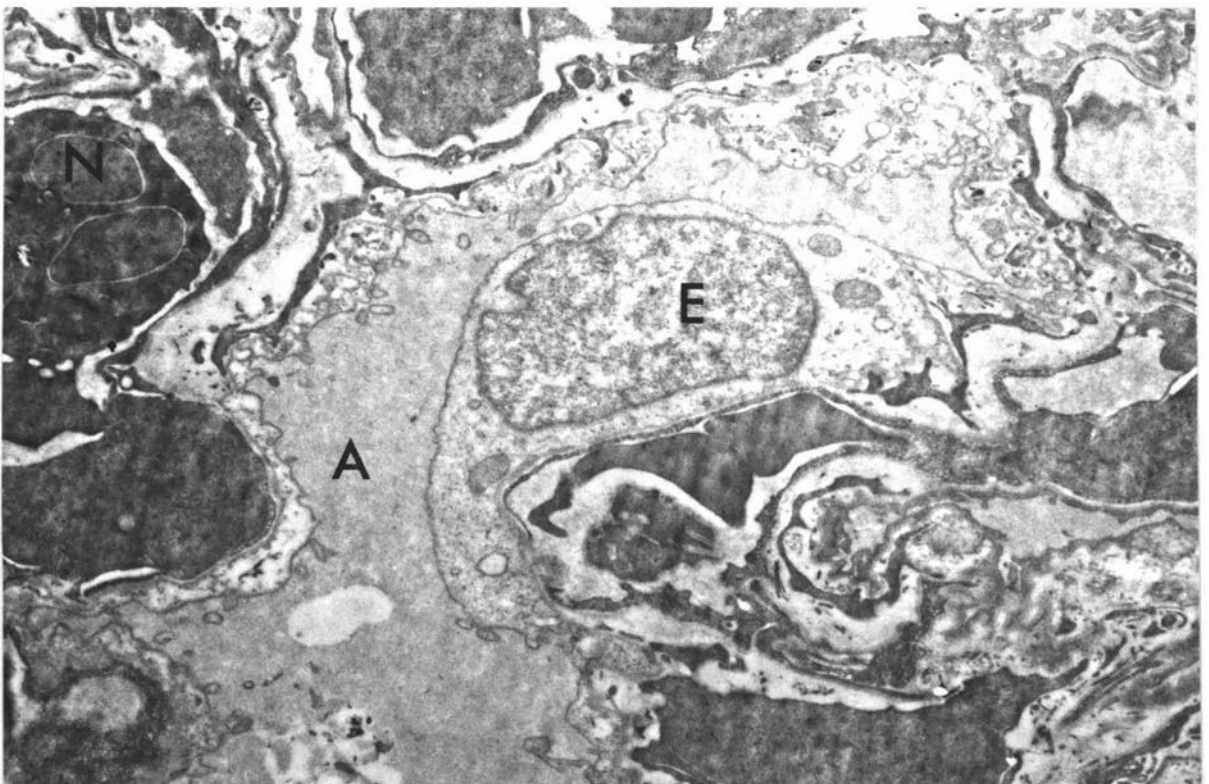
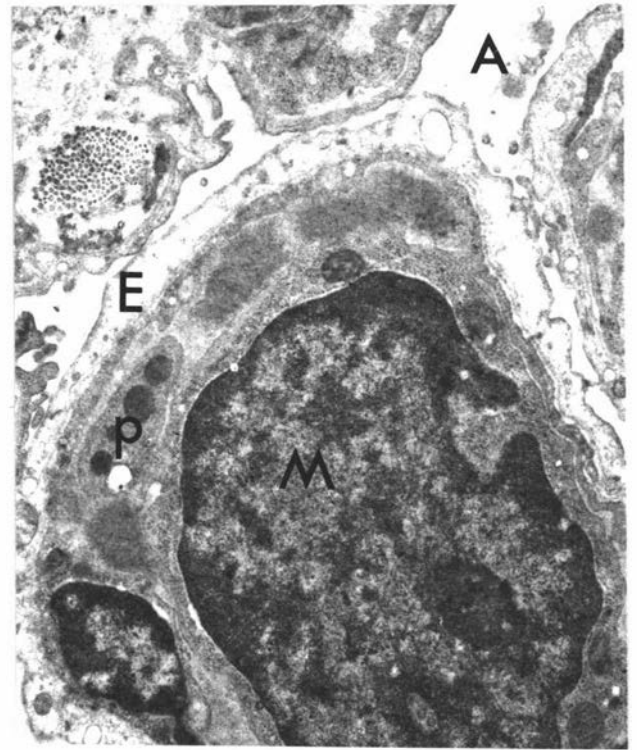
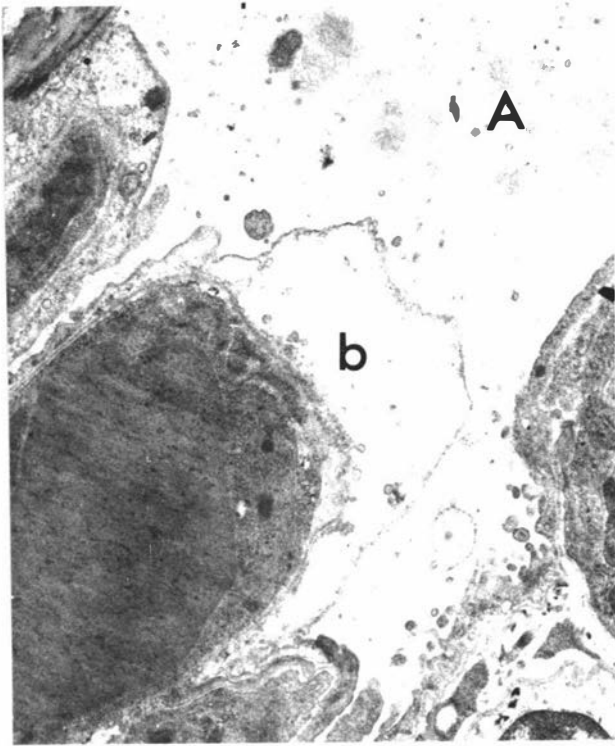


Figure 3.19

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.

Figure 3.20

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.

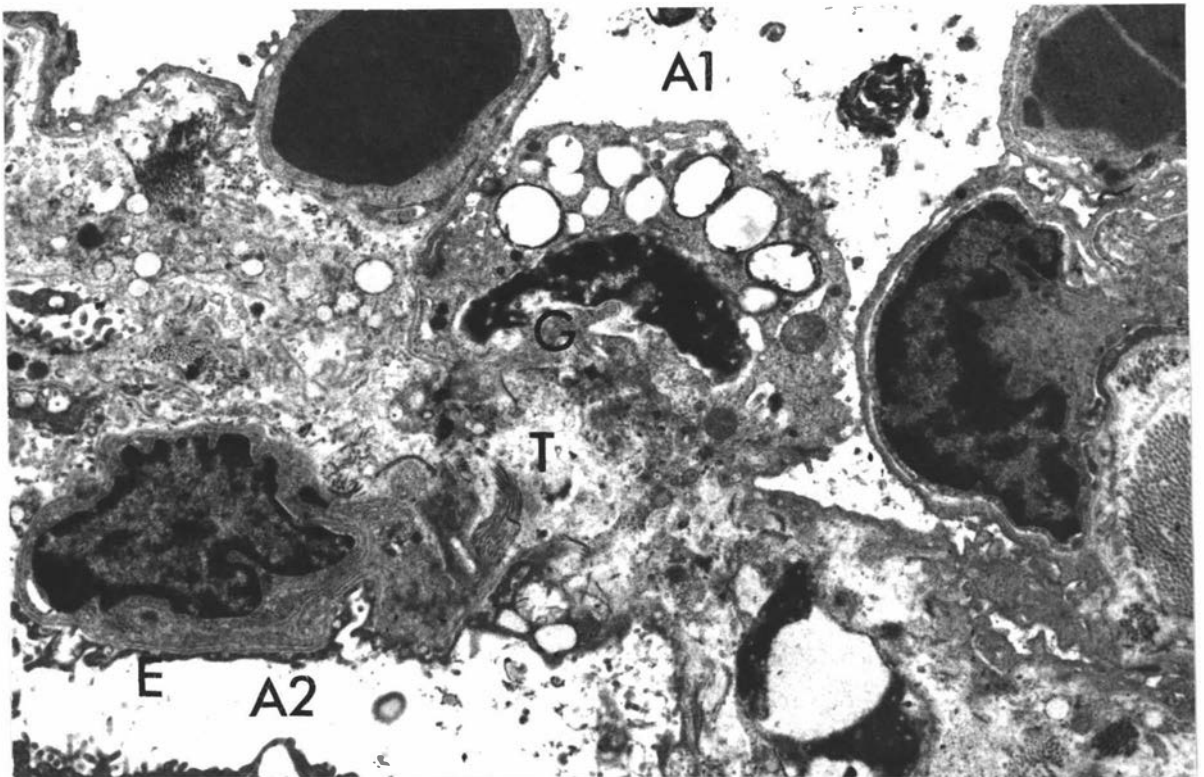
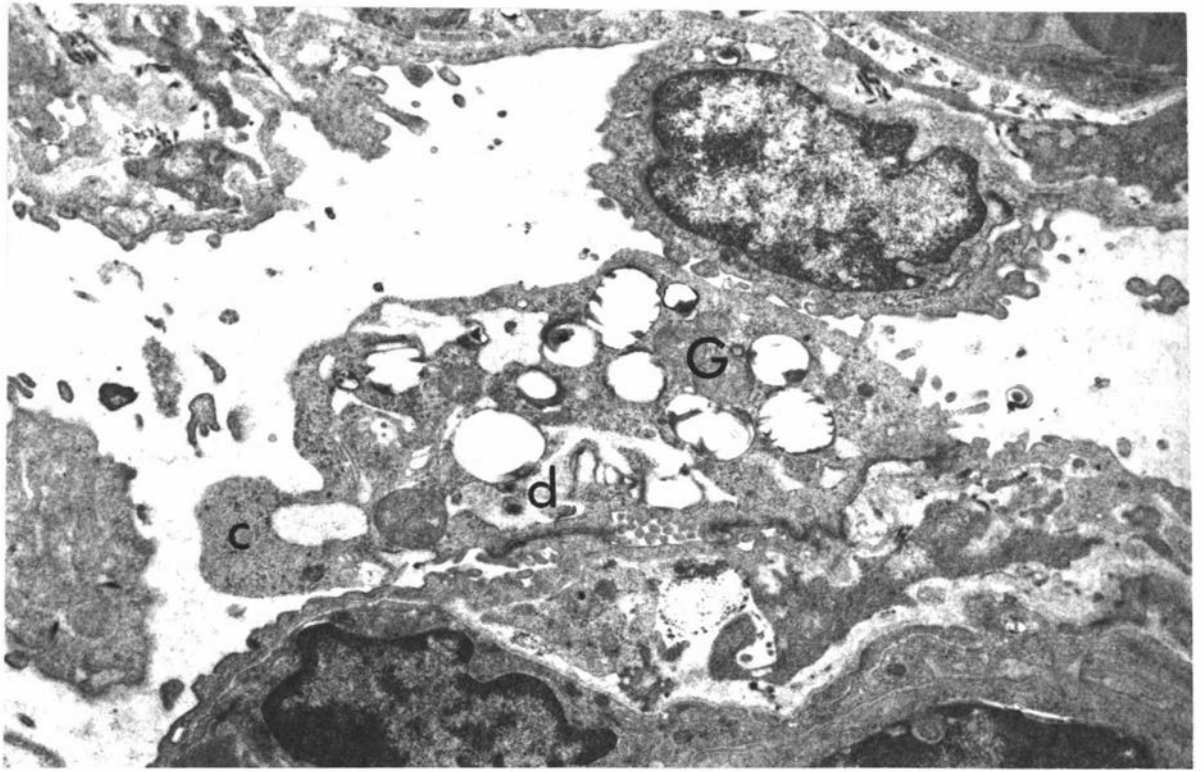
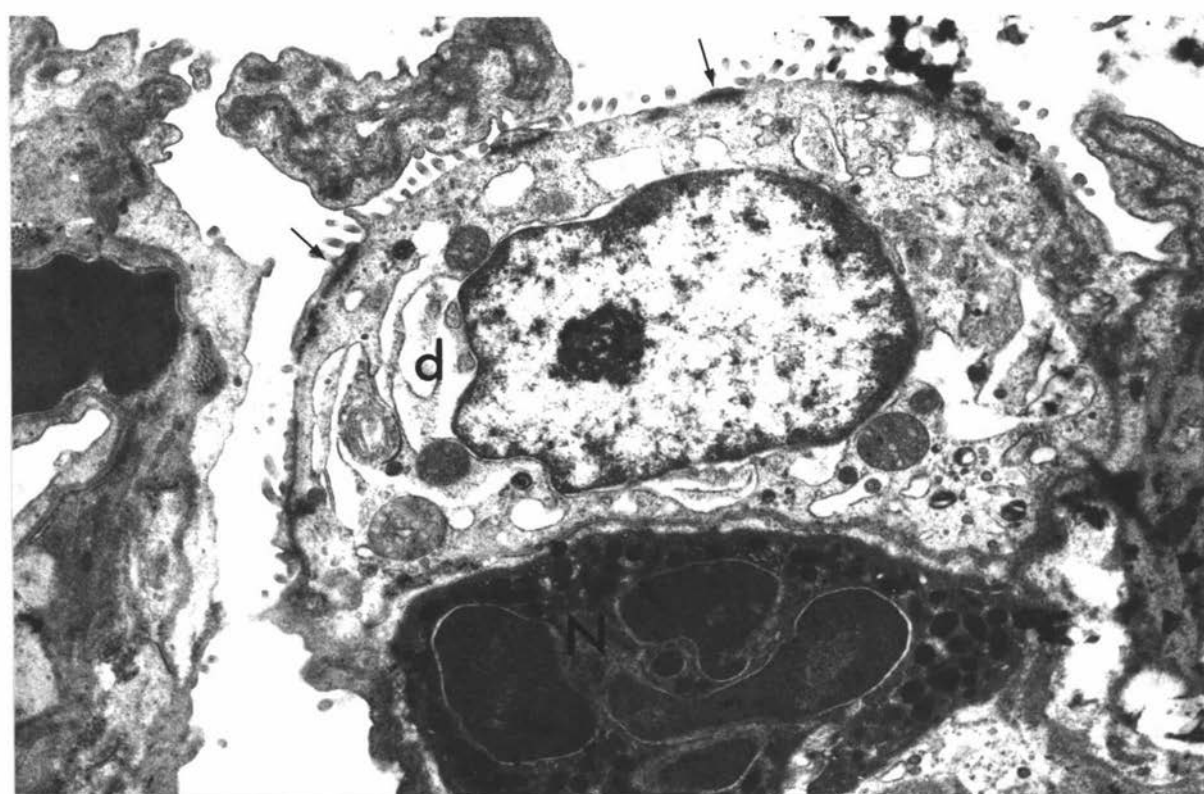
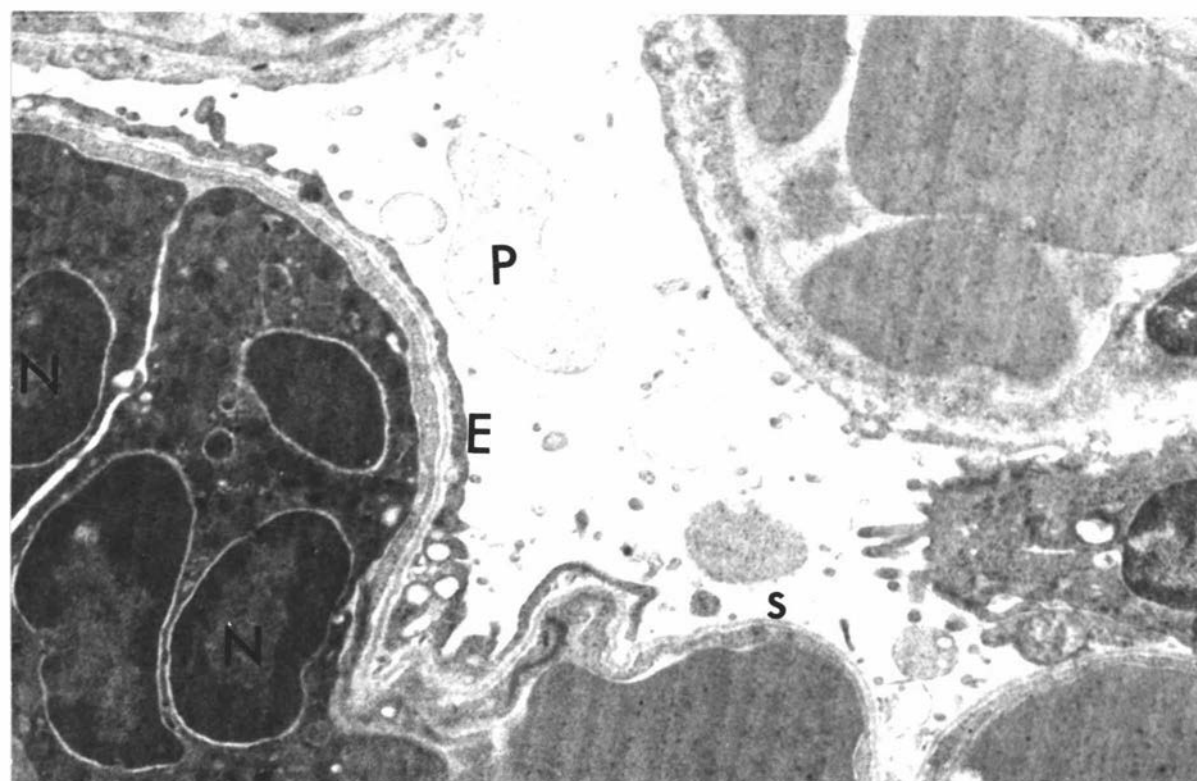


Figure 3.21

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. 13,000.

Figure 3.22

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). 12,000.



structural changes while the surrounding type I epithelial cells were swollen or desquamating.

Injury to the alveolar capillary endothelium was first seen at 6 hours after nitric acid administration. Before this time platelets and neutrophils could often be found closely adherent to endothelial cells (Figure 3.21). The appearance of neutrophils in interstitial spaces (Figure 3.22) and alveoli at 6 hours coincided with the occurrence of endothelial damage. On some occasions neutrophils could be seen migrating through the detached, electron-dense cytoplasmic extensions of damaged endothelial cells. At 12 hours the degree of capillary endothelial injury was less marked but some damage could still be detected up until 2 days after administration in those capillaries in the most severely affected areas.

Proliferation of type II cells was first observed in the lungs examined 1 day after nitric acid administration. It was most pronounced in areas adjacent to those in which destructive changes were proceeding. In such areas two type II cells could sometimes be found lying side by side in the corner of an alveolar space. The proliferating cells had slightly enlarged nuclei with an open chromatin pattern and prominent nucleolus. Their cytoplasmic contents were less dense than normal and their lamellated bodies were more numerous and larger than usual (Figure 3.23). At 2 to 3 days after administration groups of 2 or 3 cells were often found within collapsed alveoli and at times were the only remnants of alveolar epithelium remaining. The most severe type II cell hyperplasia encountered was at 5 days post-instillation when groups of 4 or more cells were found partially lining alveolar spaces adjacent to terminal bronchioles (Figure 3.24).

Division of type I alveolar epithelial cells was also occasionally observed in areas where the destructive changes were less severe. Swollen, bi-nucleate type I cells were found in some instances (Figure 3.25) but the presence of more than one type I cell within a single alveolus was extremely rare.

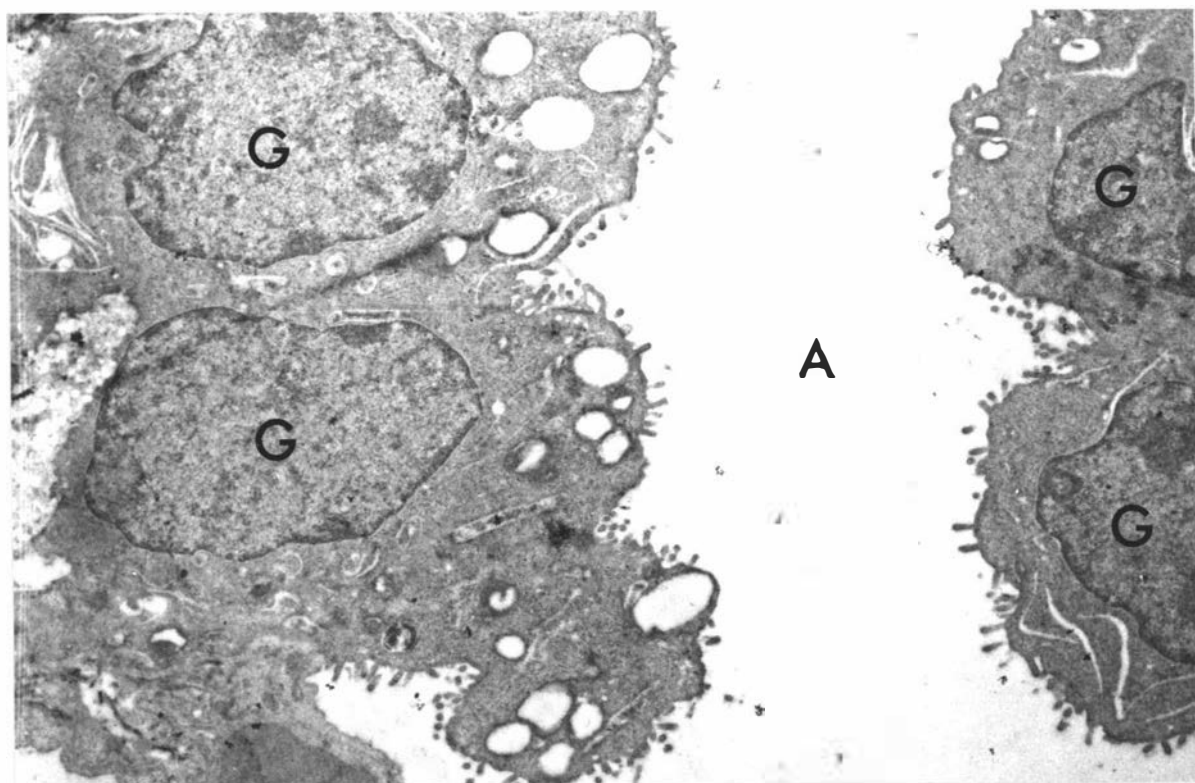
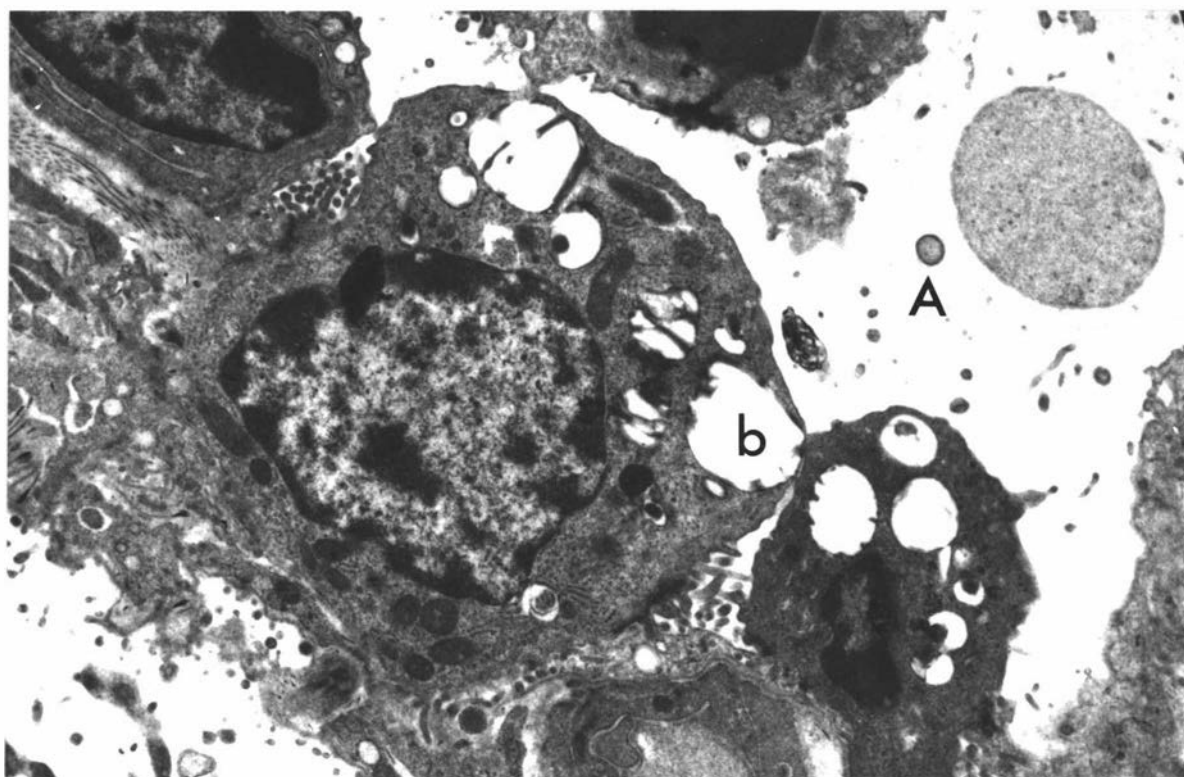
The phagocytosis of carbon particles and necrotic debris was carried out by alveolar macrophages which were active in alveolar spaces

Figure 3.23

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.

Figure 3.24

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.



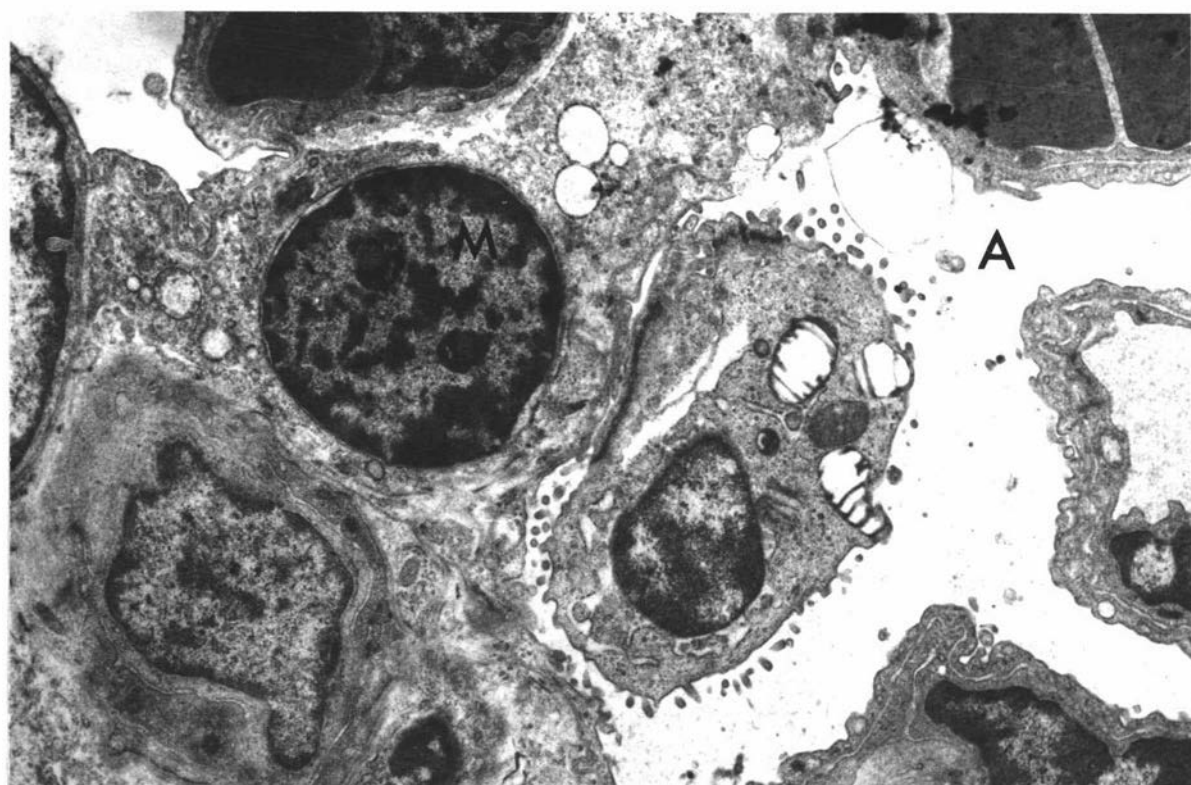
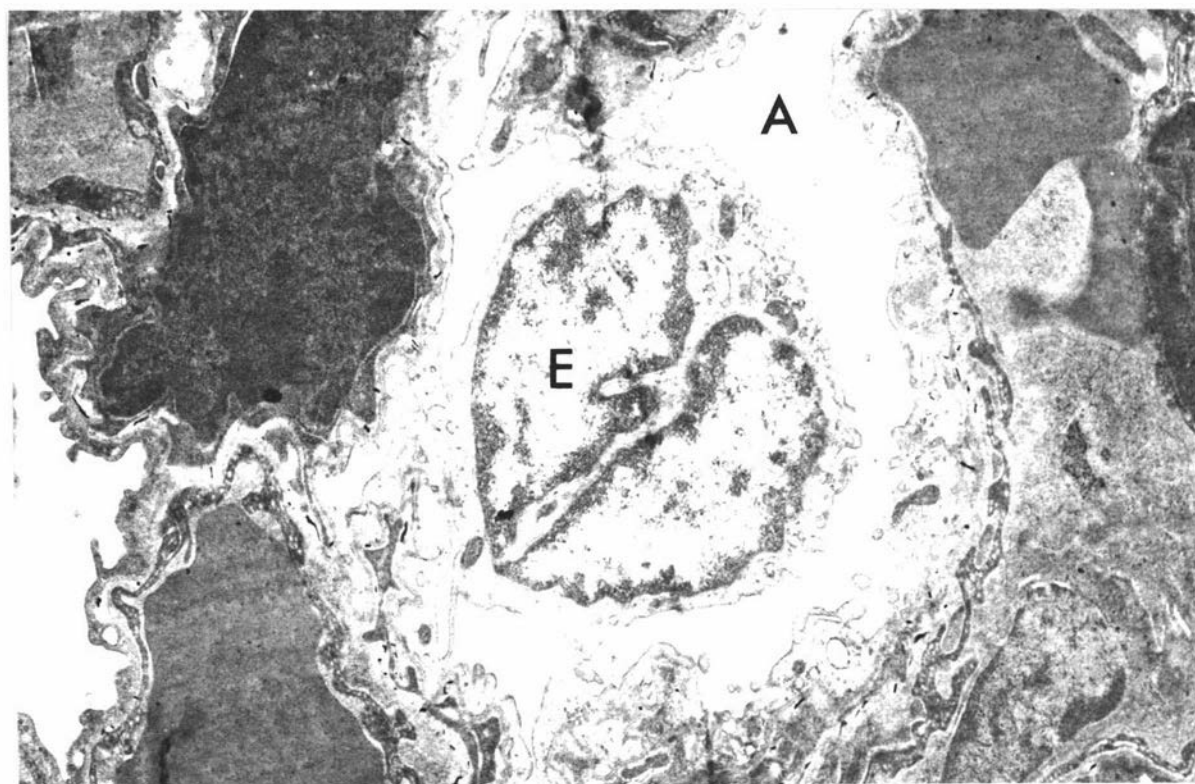
following page 103

Figure 3.25

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.

Figure 3.26

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.



from 3 hours onwards. By 12 hours, two populations of macrophages could be distinguished. The first were large cells which contained many organelles and were usually engorged with carbon particles and cellular debris (Figure 3.27). The second type were smaller cells with a round nucleus and fewer cytoplasmic organelles. They could be found migrating through the interstitium (Figure 3.26) or free in alveolar spaces up until 3 days after administration. At this time occasional cells with lymphocyte morphology were also seen in alveolar spaces (Figure 3.27). By 7 days the remaining macrophages were mainly large carbon-laden cells which often lay sequestered in cavities which had thick collagenous walls (Figure 3.28). Such cavities were presumably the remnants of alveolar spaces which no longer contained alveolar epithelium.

ENDOGENOUS AGENTS: (i) PARAQUAT

Among the chemicals capable of inducing pulmonary damage when administered systemically, Paraquat is outstanding in its ability to injure the alveolar epithelium. This property has rendered it of considerable value in the experimental study of alveolar injury and in particular as a model for the study of respiratory distress (Manktelow, 1967a; Robertson et al., 1971).

The relative susceptibility of the ovine lung to Paraquat poisoning is unknown as no cases of accidental or experimental poisoning have been reported in this species. Other ruminants such as the bovine appear to be at least as susceptible as laboratory mice and rats (Conning et al., 1969) and field cases of poisoning with the related compound, Diquat have been reported in the veterinary literature (Thomas and Amor, 1968). The following experiments were therefore designed to test the value of Paraquat as a systemic agent for inducing pulmonary injury in sheep.

Material and Methods

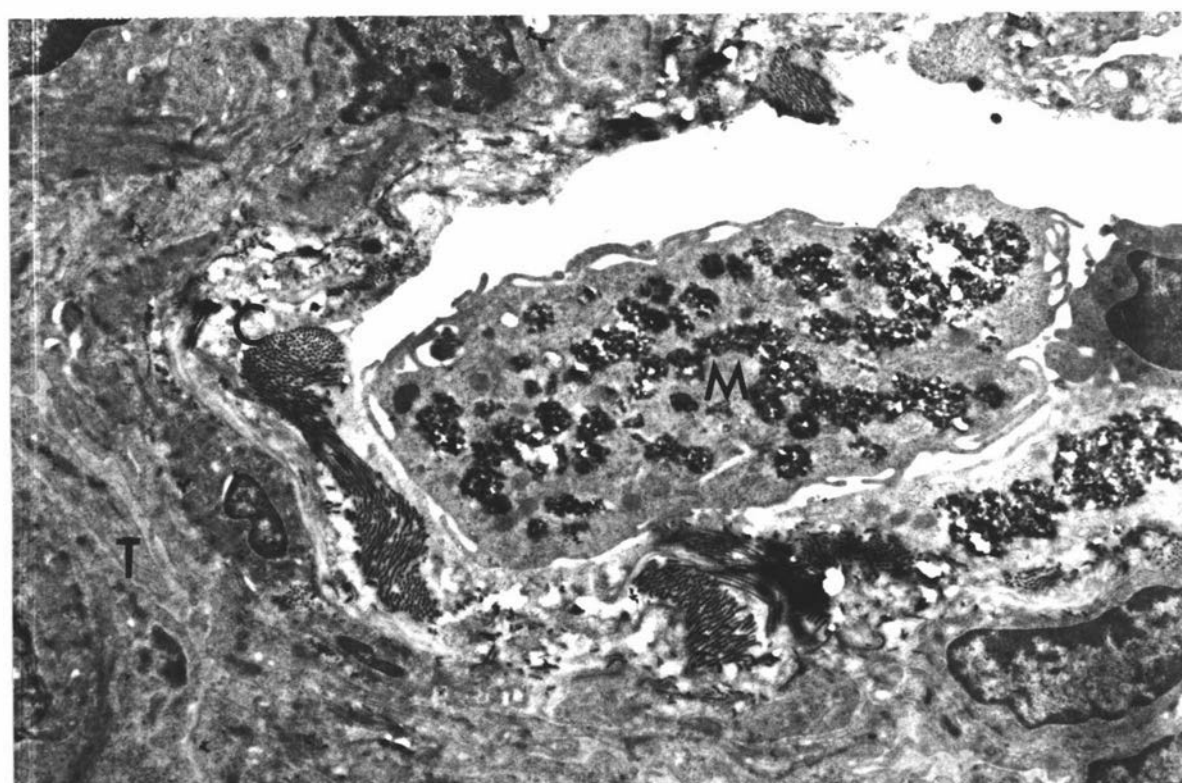
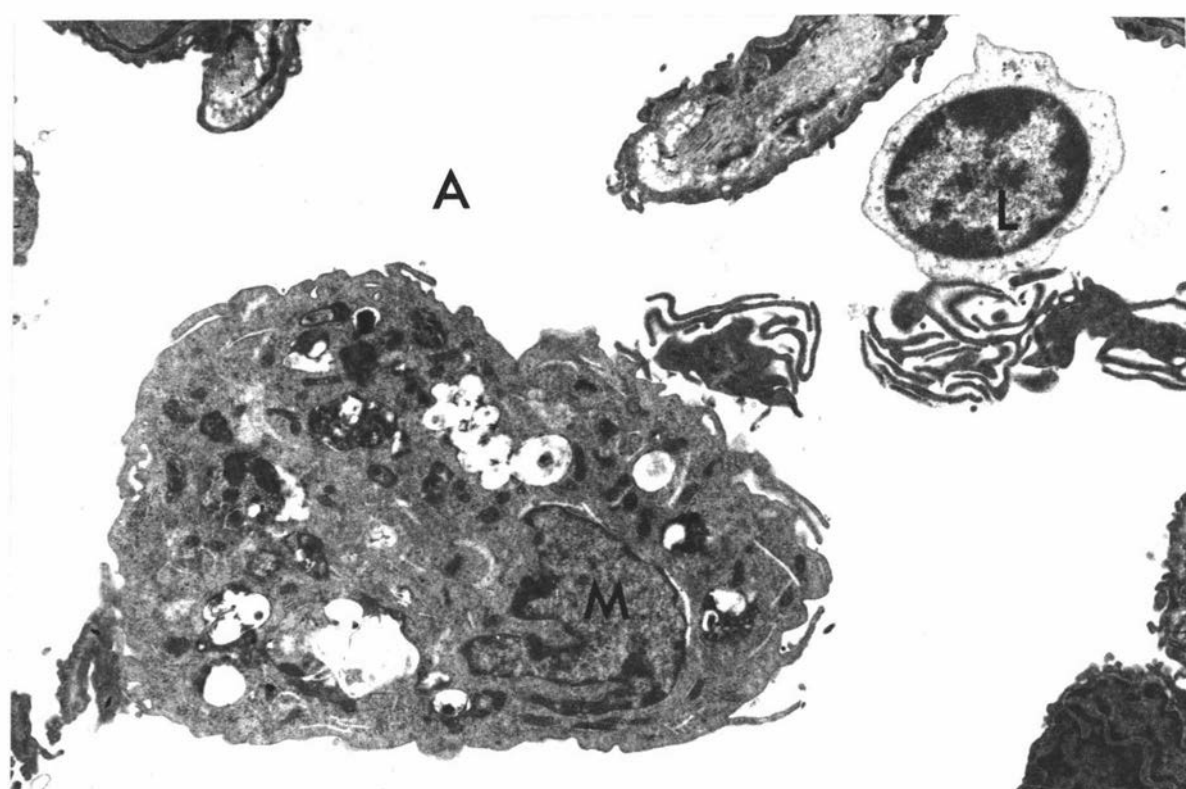
To establish the dose rate at which maximum pulmonary involvement could be expected, Paraquat was administered to 18 sheep of various ages ranging from 2 weeks to 6 years by either intravenous, intra-peritoneal or subcutaneous routes (Table IX). Animals dying during

Figure 3.27

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.

Figure 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.



the course of the experiment were subjected to necropsy as soon as possible after death and sections of lungs, liver and kidneys were fixed in 10% formol-saline and processed for histology as previously outlined. In 4 cases euthanasia by intravenous barbiturate was carried out "in extremis" and on these occasions 1 mm³ blocks of lung tissue were collected in Karnovsky's fixative for subsequent electron microscopy.

On the basis of these initial experiments a dose rate of 6 mg/Kg was used for administration to lambs 2 days prior to the endobronchial inoculation of various bacteria. Twenty-two, 1-month-old, colostrum-fed lambs were divided into 4 even groups according to their body weight. Two of these groups received 6 mg/Kg of Paraquat by subcutaneous injection. Two days after this all groups were inoculated endobronchially with broth cultures of bacteria by the method described earlier. The bacteria used were all pure cultures P. haemolytica, S. aureus or E. coli isolated from naturally occurring cases of sheep pneumonia. Groups 1 and 2 received a mixed inoculum consisting of 1 ml of each of the 3 cultures whereas groups 3 and 4 received a 3 ml inoculum of a single organism. The number of organisms present in each culture before inoculation was estimated by the standard surface colony count technique and are shown in Table X. The animals were observed clinically and body temperatures taken twice daily. Those dying were necropsied as soon as possible after death while those surviving were killed 6 days after inoculation with intravenous barbiturate. The gross lung lesions present were recorded and a range of affected tissues were fixed in 10% formol-saline for subsequent histological examination.

Results

The Toxicity of Paraquat to Sheep

Severe toxic systemic effects were observed clinically in most animals receiving more than 6 mg/Kg intravenously or intraperitoneally and 10 mg/Kg subcutaneously. The animals refused food and water, were reluctant to move and showed an unsteady gait when forced to move. When they became recumbent they showed mild salivation, grinding of

TABLE IX
EFFECTS OF SYSTEMIC PARAQUAT ADMINISTRATION

Sheep No.	Weight Kg	Dose mg/Kg	Survival Time	Gross Lung Lesions
E540	52.7	100 i/P	5 hours	Congestion & haemorrhage
541	52.0	100 i/V	7 hours	Congestion & haemorrhage
659	50.3	25 i/P	5 hours	Congestion & haemorrhage
660	53.1	50 i/P	7 hours	Congestion & haemorrhage
750	12.5	10 i/V	18 hours	Pulmonary oedema, congestion and haemorrhage
1053	59.0	5 i/P	20 hours	Oedema, congestion & ventral collapse
1337A	33.2	4 i/V	3 days ⁽¹⁾	Oedema, congestion & ventral collapse
1337B	31.1	8 i/V	5 days ⁽¹⁾	Oedema, collapse & fibrinous pleural exudate
1494	32.7	9 i/V	4 days	Oedema, congestion and ventral collapse
1497	20.4	8 i/V	1 day	Congestion & haemorrhage
1557	37.8	7 i/V	1 day ⁽¹⁾	Congestion & haemorrhage
1566	7.0	7 i/V	20 hours	Congestion, haemorrhage and ventral collapse
1567	7.2	8 i/V	1 day ⁽¹⁾	Congestion, haemorrhage and ventral collapse
1559	5.5	20 sub/cut	18 hours	Congestion & haemorrhage
1574	4.5	6 i/V	Survived	Nil at euthanasia, 8 days
1577	5.5	6 sub/cut	Survived	Small areas of collapse at euthanasia, 8 days
1578	5.4	10 sub/cut	20 hours	Congestion & haemorrhage
1579	8.2	6 i/V	1 day	Congestion & haemorrhage

(1) Killed "in extremis" and tissues collected for electron microscopy

teeth, rapid respiration and eventual coma.

Table IX shows the survival times and summarises the gross lung lesions. The majority of lungs examined showed severe congestion and oedema. Those from animals dying in less than 24 hours also showed extensive petechial haemorrhages on pleural surfaces and throughout the lung while those from animals surviving for a longer period contained variable dull red areas of ventral collapse. Changes were also frequently seen in other organs. The abdominal viscera showed a slightly muddy discoloration of their serosal surfaces and petechial haemorrhages were often present on the heart. The liver was frequently turgid, pale and blotchy in appearance and both kidneys were invariably pale and swollen.

The lungs of animals dying within 24 hours of Paraquat administration were histologically unremarkable. There was usually moderate to severe congestion, variable intra-alveolar haemorrhage and small amounts of proteinaceous exudate in alveoli. In animals dying later than this the histological changes were more severe. Alveolar macrophages and erythrocytes were often found embedded in thick proteinaceous exudate within alveoli and bronchioles. At the periphery of some lobules this exudate was strongly eosinophilic and fibrin-like in character. In ventral areas of lung there was extensive alveolar collapse and dilatation of alveolar ducts. Many of these dilated ducts were lined with a thick proteinaceous exudate containing hyaline droplets but no strongly PAS-positive hyaline membranes were seen. The alveolar interstitium was infiltrated with small numbers of mononuclear cells and many alveolar blood vessels contained margined mononuclear cells and neutrophils.

Consistent histological changes were also found in the liver and kidney. There was universal cloudy swelling of hepatocytes which was often severe. The kidney showed severe necrosis of proximal tubules in more than 50% of the cases examined regardless of their survival time. In cases where tubular necrosis was not severe there was extensive protein cast formation in proximal tubules and capsular spaces.

TABLE X

EFFECTS OF SYSTEMIC PARAQUAT ADMINISTRATION PLUS ENDOBRONCHIAL BACTERIAL INOCULATION IN LAMBS

Group	Sheep No.	Inoculum	Survival time (post-inoculation)	Organisms recovered at death
Group 1	E87	Mixed bacterial broth	1 day	<u>S. aureus</u> , <u>E. coli</u> .
	88	<u>P. haemolytica</u> 1.1×10^5 orgs.	2 days	" "
	89		3 days	" " , <u>P. haemolytica</u> .
	93	<u>S. aureus</u> 1.1×10^7 "	1 day	" " "
Sub/cut Paraquat 6mg/Kg	99	<u>E. coli</u> 1.5×10^4	1 day	" " "
Group 2	E90		1 day	<u>S. aureus</u> , <u>E. coli</u> .
	91	Mixed bacterial broth	1 day	" "
	92	as above	1 day	" " <u>P. haemolytica</u> .
	94		1 day	" " .
	105		1 day	" " , <u>N. catarrhalis</u> .
Group 3	E102	Autoclaved bacterial broth	Killed day 6	<u>S. aureus</u> , <u>P. haemolytica</u>
	97	" " "	Killed day 6	" "
	95	<u>E. coli</u> 4.5×10^4 orgs.	Killed day 6	" <u>E. coli</u>
	96	<u>S. aureus</u> 3.3×10^7 "	1 day	" "
	107	<u>P. haemolytica</u> 3.3×10^5 "	Killed day 6	" , <u>P. haemolytica</u>
	108	" " "	Killed day 6	" .
Group 4	E101	<u>P. haemolytica</u> 3.3×10^5 "	Killed day 6	No growth
	103	" " "	Killed day 6	<u>S. aureus</u> , <u>P. haemolytica</u>
	98	<u>S. aureus</u> 3.3×10^7 "	1 day	" , <u>E. coli</u> .
	100	" " "	2 days	" .
	104	<u>E. coli</u> 4.5×10^4 "	Killed day 6	No growth
	106	" " "	Killed day 6	<u>E. coli</u> , <u>P. haemolytica</u>

At the ultrastructural level changes were seen in both the alveolar epithelium and interstitial tissues but in most areas the interstitial changes predominated. They consisted of infiltrations of large mononuclear cells and macrophages which could be seen within disrupted interstitial spaces (Figure 3.30). In some areas there was severe interstitial oedema which was often associated with fibroblast proliferation. The proliferating fibroblasts were rounder than normal, contained large amounts of granular endoplasmic reticulum and often entirely filled a portion of the alveolar septum. In the most advanced case collagen proliferation was observed in many areas of the interstitium (Figure 3.32).

Alveolar epithelial change was variable in degree and distribution. In some areas severe vacuolation and ballooning of type I cells was seen (Figure 3.29) while in adjacent alveoli epithelial changes were minimal (Figure 3.30). In the most severely affected areas type II cell degeneration was present but this was not a pronounced feature of the pathological response. Affected type II cells, were rounded, showed loss of microvilli, dilatation of the endoplasmic reticulum and contained numerous, often dilated osmiophilic bodies (Figure 3.31). Mild hyperplasia of type II cells was seen in the most advanced case when groups of 2 or more cells were found in some corners of the more severely damaged alveolar spaces (Figure 3.32).

The Effect of Paraquat Administration on Pulmonary Resistance to the Endobronchial Inoculation of Bacteria

No marked difference was observed in the clinical response to endobronchial bacterial inoculation between the lambs treated parentally with Paraquat and those in untreated groups. All the animals receiving inocula in which Staphylococcus aureus was present showed rapid respiration, and a drop in body temperature when observed 12 hours after inoculation. The survival time of animals in the Paraquat-treated group which received mixed bacterial broth was slightly better than that in the untreated group (Table X) but because of the small numbers of animals involved this difference was not statistically significant ($P > 0.05$). However a substantial difference in the average body temperature between Paraquat-treated and untreated animals was

Figure 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.

Figure 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.

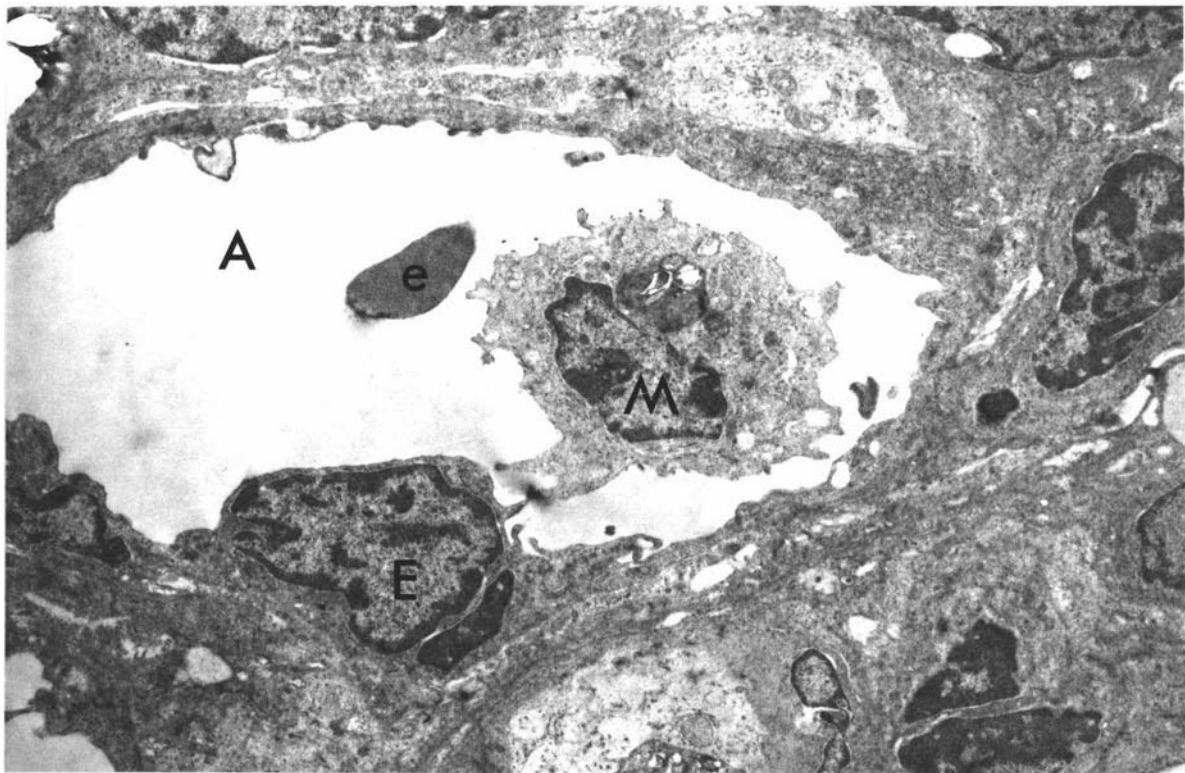
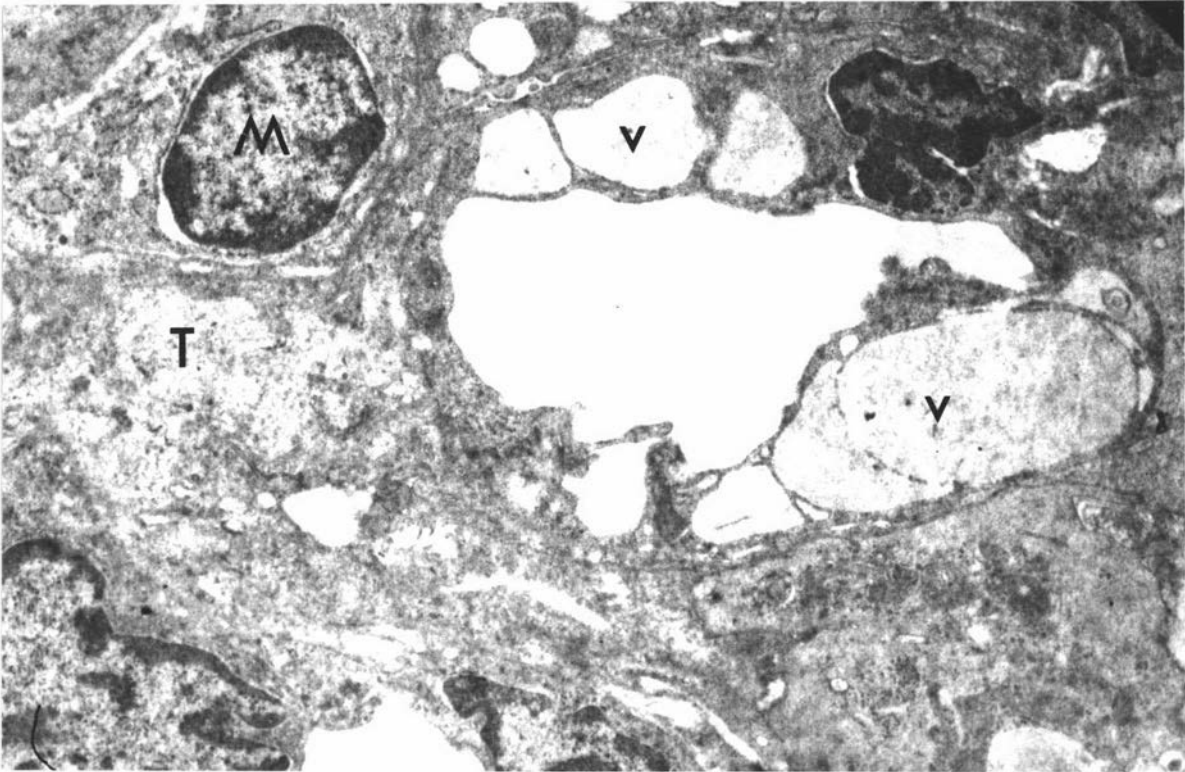
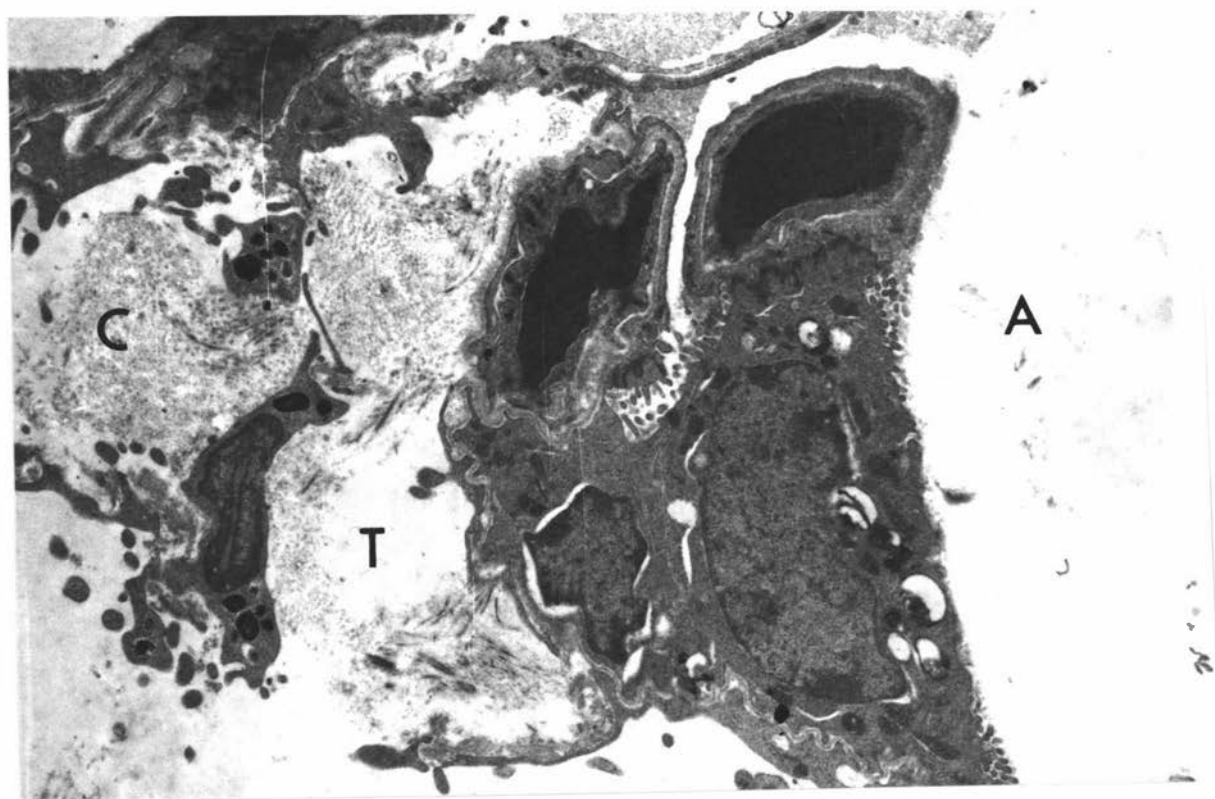
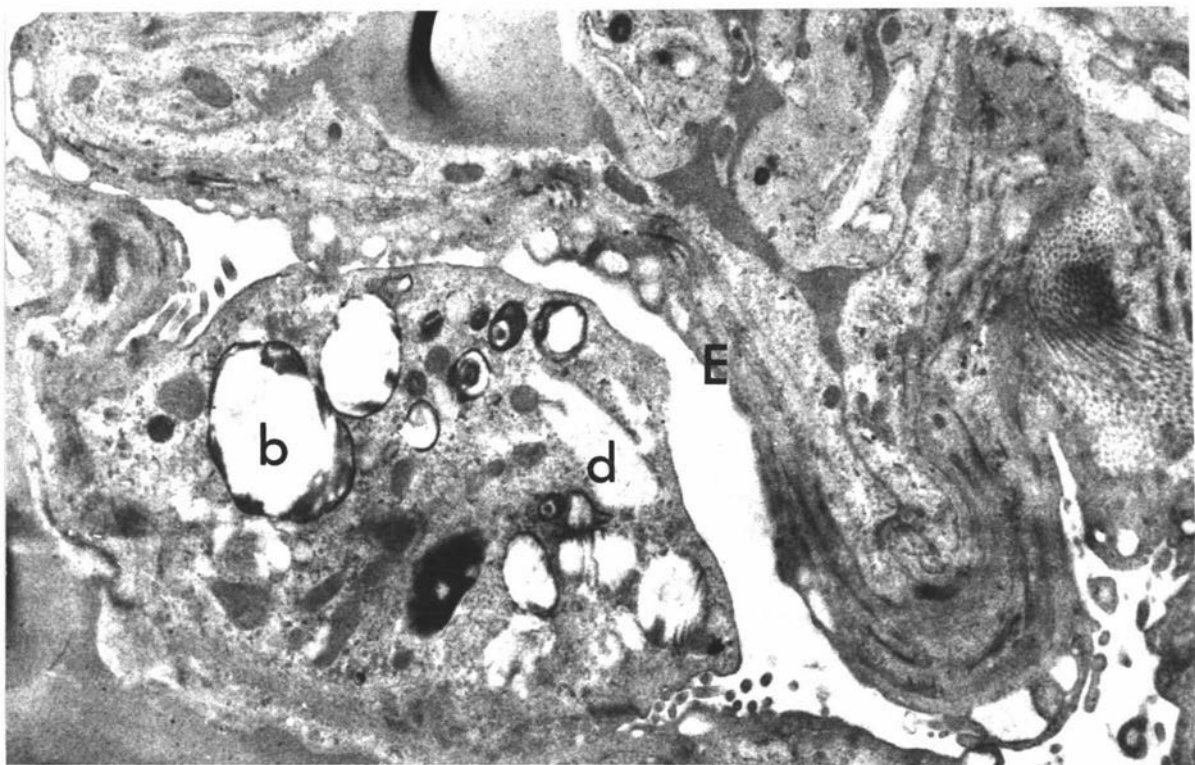


Figure 3.31

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.

Figure 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).



found to occur after Paraquat administration regardless of the type of inoculum received. At 24 hours after treatment the average body temperature of the treated animals was 0.6°C lower than that of the untreated animals and a difference of this magnitude continued in the animals which survived up until euthanasia at day 6 (Appendix 2).

The most severe gross lung lesions evoked were in the 2 groups receiving mixed bacterial broth. These consisted of grey, necrotic central areas surrounded by dark red areas of consolidation which had an irregular distribution (Figure 3.33). Other lungs in these groups showed focal dark-red areas of consolidation which had a lobular pattern of distribution. Surrounding these foci were variable and often severe areas of congestion and oedema. Similar focal areas of consolidation together with severe congestion and oedema were found in those animals in groups 3 and 4 which received inoculations of broth culture containing S. aureus. In the animals which received P. haemolytica and E. coli and were sacrificed at 6 days after inoculation the basic lesion was usually a focal area of brown necrotic lung tissue surrounded by granulation tissue and a variable zone of dark red consolidation. The overlying pleura was often thickened as were the surrounding interlobular septa .

Histological examination of the lungs inoculated with mixed bacterial cultures revealed a similar pattern of response regardless of whether or not Paraquat had been administered. The lesions consisted of a central necrotic area which was invariably located around bronchioles and which extended for a variable distance into the surrounding lung parenchyma. In the most severe cases groups of whole lobules were involved with only islands of living tissue, surrounded by a margin of haemorrhagic exudate and leucocytes remaining. More often the areas of necrosis were bounded midway across a lobule by a zone of densely packed leucocytes with elongated pyknotic nuclei (Figure 3.34). The alveolar spaces within the necrotic area were usually well preserved and contained a fibrinous or haemorrhagic exudate. Colonies of bacteria were often present in bronchioles or alveolar ducts. The alveoli outside the necrotic zones were severely

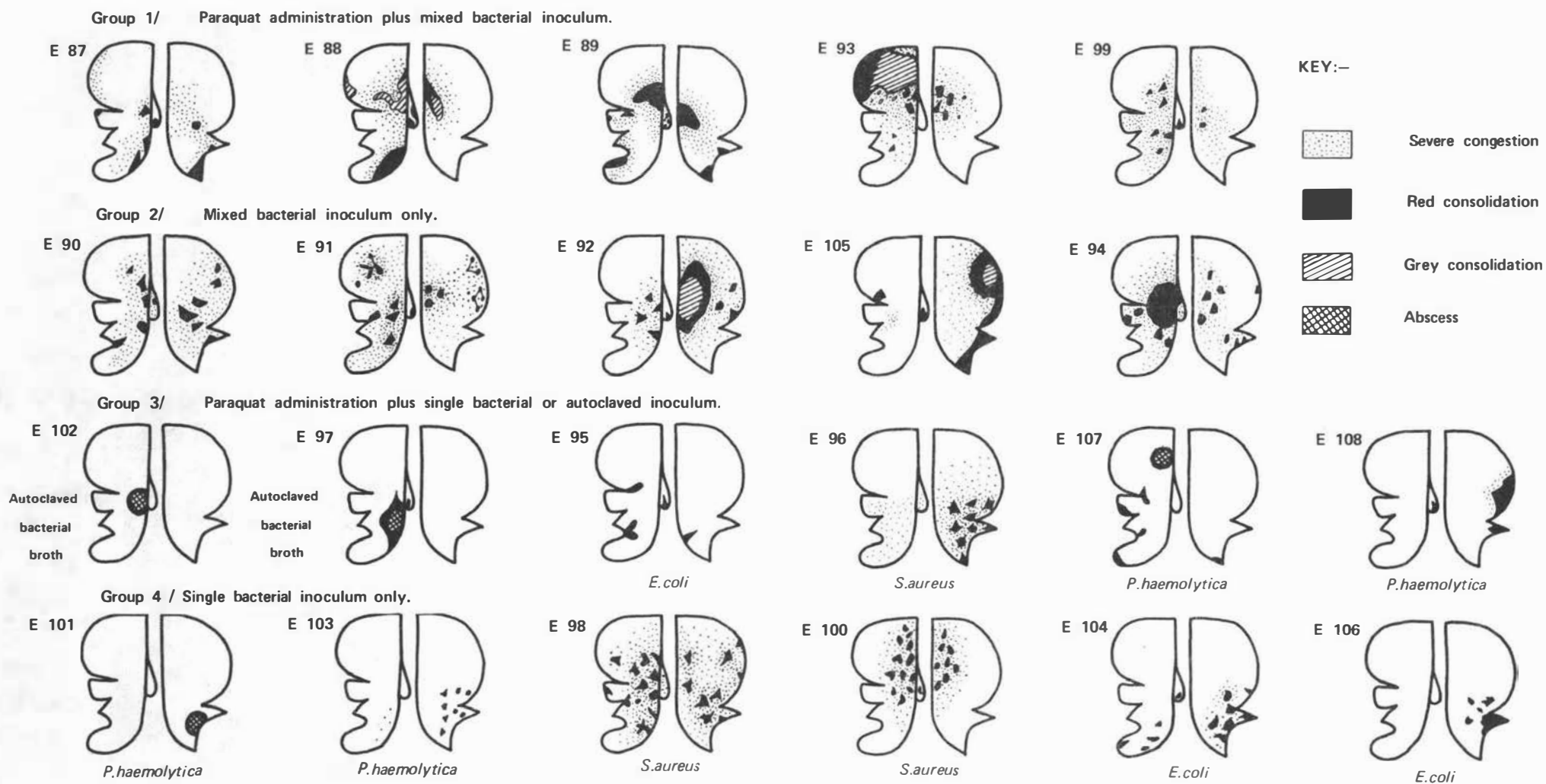


Figure 3.33 Gross lung lesions following systemic paraquat administration and endobronchial bacterial inoculation.

following page 110

Figure 3.34

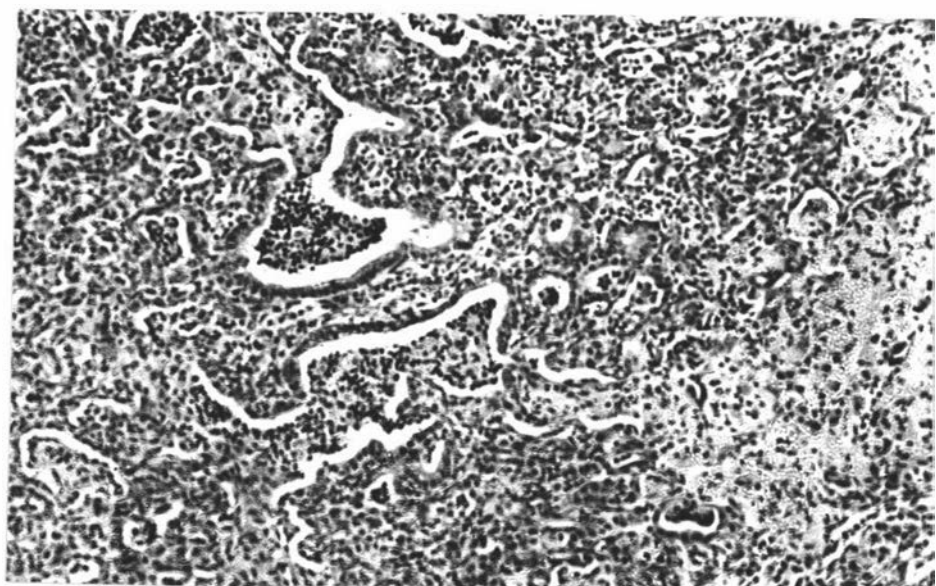
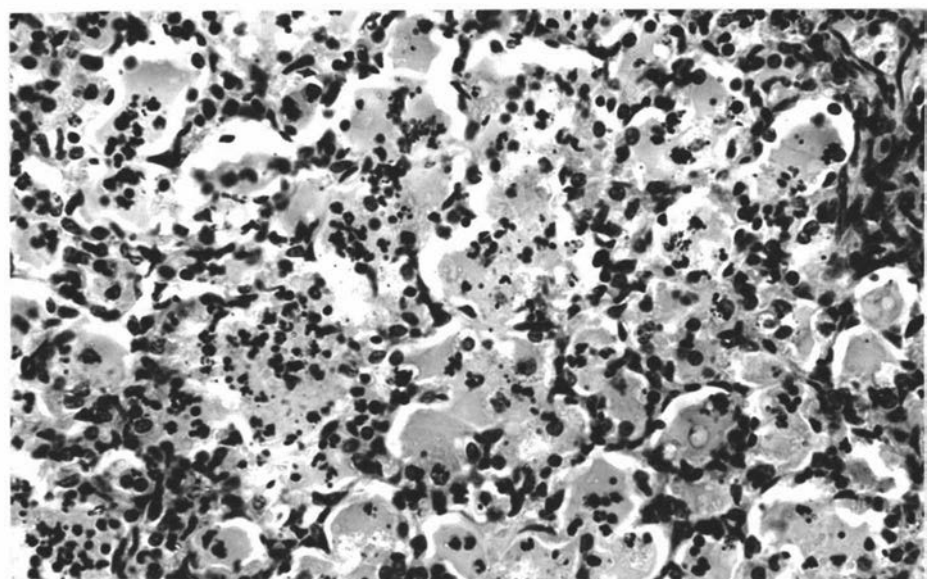
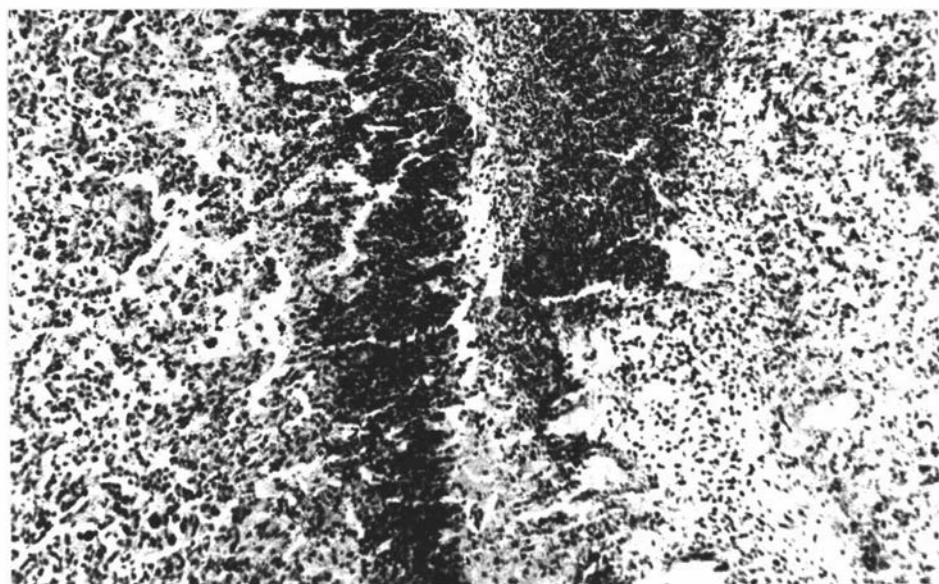
The margin of a necrotic zone from the lung of animal E89 (3 days after the inoculation of bacteria and 5 days after Paraquat administration). There is a dense infiltration of leucocytes many of which are necrotic. HE x 40.

Figure 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.

Figure 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.



congested and contained a thick fibrinous exudate within which lay embedded variable numbers of neutrophils and small macrophages (Figure 3.35). In 4 of the 5 Paraquat-treated lungs the cellular reaction in the non-necrotic areas was slightly more severe than in the untreated group. Greater numbers of mononuclear cells were seen in the alveolar and interlobular interstitial spaces and more macrophages were present in the alveolar exudate.

The microscopic appearance of the lungs inoculated with S. aureus culture showed a similar basic pattern to those which were inoculated with mixed culture. However, the extent of haemorrhagic necrosis, fibrin exudation and oedema was greater with S. aureus than with the mixed culture and the degree of cellular response correspondingly reduced. No difference in the response to S. aureus could be detected between the Paraquat-treated and untreated groups.

The damage induced by P. haemolytica and E. coli was less extensive and because the animals survived longer, more proliferative in nature. Moderate interstitial fibrosis was seen in the alveolar septa surrounding many bronchioles and there was moderate bronchiolar epithelial hyperplasia. Excess numbers of macrophages were frequently present in alveolar spaces in the affected areas and they were more numerous in the paraquat-treated animals than in the untreated group. When abscesses were present they consisted of a central caseating mass surrounded by a zone of granulation tissue in which neutrophils were prominent. This merged externally with a zone of severely epithelialized alveoli which had moderately fibrosed interstitial septa (Figure 3.36). Small lesions of a similar character but with less inflammatory response were induced by the inoculation of autoclaved bacterial broth.

ENDOGENOUS AGENTS: (ii) BUSULPHAN⁽¹⁾

The development of pulmonary complications following Busulphan therapy for chronic myeloid leukaemia in man has led to the recognition

(1) 'Myleran', Burroughs, Wellcome and Co. Limited.

of a clinico-pathological syndrome known as "Busulphan lung". The syndrome was first recognised by Oliner et al. (1961) who described diffuse, bilateral infiltrates which had the appearance of an inflammatory process, in radiographs of the lungs of affected patients. The histopathological lesions (Heard and Cooke, 1968) and ultrastructural changes (Littler et al., 1969) indicate that the disease in man is basically a chemically-induced alveolitis with proliferation of type II cells followed by fibrosis of alveolar walls and intra-alveolar contents.

Because little information is available on the toxicity of the drug in animals the following attempt was made to test its ability to induce pulmonary injury in sheep.

Material and Methods

Two, 6-year-old ewes were dosed orally with Busulphan once daily. Both received an initial dose of 4 mg/day which was increased twice weekly until the onset of clinical symptoms or death. Necropsy was carried out immediately after death and blocks from a range of pulmonary tissues were fixed in 10% formol-saline for light microscopy and Karnovsky's fixative for electron microscopy. Tissues for light microscopy were processed in the usual manner, paraffin-blocked and stained with haematoxylin and eosin and periodic acid-Schiff. For electron microscopy they were processed and stained by the methods previously outlined in Chapter 2.

Results

The first animal died 4 weeks after commencing administration while receiving a daily dose of 36 mg on 3 occasions before death. Few clinical symptoms were exhibited prior to death apart from refusal of food and water, depression and recumbency. At necropsy, the lungs showed mild congestion and oedema and excess serous fluid was present in both the thoracic and abdominal cavities. The liver was shrunken to two thirds normal size and showed severe peripheral fibrosis typical of facial eczema (sporodesmin poisoning). It was therefore concluded that hepatic insufficiency was a contributing factor to the animals susceptibility to Busulphan toxicity.

A dose of 60 mg/day was reached before clinical symptoms became evident in the second animal. These included inappetance, depression, pale mucous membranes and a serous ocular and nasal discharge. Euthanasia by intravenous barbiturate was carried out 8 weeks after the commencement of administration. At necropsy, the lungs were heavier than normal and did not collapse fully on opening the thorax. Excess serous fluid was present in the thorax and abdomen and the liver and kidneys were slightly paler than normal.

The histological appearance of the lungs was similar in both cases. There was a moderate interstitial thickening of alveolar septa which was irregularly distributed throughout the lung. Moderate smooth muscle hypertrophy was also present in both cases and occasional randomly located groups of alveoli were partially collapsed and contained proteinaceous exudate together with free erythrocytes. Electron microscopic observations confirmed that the alveolar interstitial thickening was due to collagen proliferation and fibroblasts were more numerous within the interstitium than usual. Despite these changes the alveolar epithelium and capillary endothelium were largely undamaged.

DISCUSSION

From the observations made in the foregoing study it is apparent that many of the changes induced in the respiratory tract of sheep by chemical and bacterial means are similar to those found in the naturally occurring forms of sheep pneumonia. The changes seen in the natural disease differ mainly in their distribution and greater severity rather than in their essential character.

The use of worm-free experimental sheep in the nitric acid instillation experiments was of particular value as the lungs were free of the proliferative granulomatous and lymphoid areas associated with lungworm infestation, which are commonly seen in pasture-reared animals. This consequently simplified the interpretation of the induced pathological changes. However, the restricted availability of these animals meant that only small numbers could be used and thus little allowance could be made for individual variation in pathological response.

Alveolar collapse was one of the first responses to localised exogenous injury seen in experimental nitric acid instillation. Later the collapse became extensive in the ventral portions of some lungs so that lesions were produced which closely paralleled the gross and microscopic appearance of the early stages of subacute enzootic pneumonia (Figures 3.2 and 3.13). Although alveolar collapse has often been associated with injury by a variety of systemic factors (Henry, 1968; Scarpelli, 1968) its rapid onset in the present study raises the possibility that irritant exogenous agents may also be involved in the early stages of subacute enzootic pneumonia in sheep.

The majority of degenerative changes observed in the alveolar epithelium have been noted previously in the pathological study of the naturally-occurring disease. This suggests that alveolar epithelial injury follows a basic pattern of events which are to some degree independent of the nature of the inducing agent. An exception to this, was the generalised swelling of type I epithelial cells which occurred in dilute nitric acid injury but was not seen in ovine pneumonia. This change has been reported previously in nitric acid injury by Greenberg et al. (1971) who observed plump alveolar cells with extended cytoplasmic processes which covered the surface of damaged type II cells. However, these authors did not report the binucleate forms of swollen type II cells which were seen in the present study.

One of the outstanding features of the reaction to dilute nitric acid was the speed at which both terminal bronchiolar and alveolar epithelium proliferated. To date, insufficient work has been done on the kinetics of type II cell hyperplasia in other species to appreciate the relative significance of these findings. In rabbits the instillation of 5% nitric acid into the trachea evoked alveolar cell hyperplasia that was intensified by prior injection of cortisone acetate (Totten and Pierce, 1964). These epithelial changes were not considered significant prior to 4 days, however by 7 days proliferating alveolar lining cells were quite prominent. An electron microscope study by Faulkner and Esterly (1970) has shown that rabbits lungs, 3 weeks after the injection of Freund's adjuvant, developed increased numbers of granular pneumocytes in which lamellar inclusions were often markedly enlarged and

contained densely packed osmiophilic materia. Similar changes to those seen in the present study were reported by Greenberg et al. (1971) who noted that type II cell hyperplasia was manifest as early as 2 days following dilute nitric acid instillation in rat lungs. These findings are also in agreement with the observations of Bowden and Adamson (1971) who noted focal type II cell proliferation in mice 2 days after exposure to oxygen.

The current experimental findings suggest that type II cells proliferate by the rapid division of existing cells many of which are relatively resistant to injury when compared to their adjacent type I counterparts. Although occasional cuboidal cells, having microvilli but lacking the other features of type II cells were sometimes seen, no clearly transitional forms with morphology intermediate between type II and type I were detected. This does not exclude the possibility that transition may occur when injury is more sustained or during re-epithelialization of denuded alveoli as observed earlier in the study of ovine pneumonia. Neither does it exclude the possibility that Clara cells may differentiate into type II cells in the alveolar duct region under some circumstances.

In the dilute nitric acid experiment two morphologically different populations of macrophages were observed in the alveolar spaces from 12 hours to 3 days after administration. This finding suggests that the smaller, newly-arrived cells were of recent blood monocyte origin whereas the larger, mature cells were derived from a residual population of lung macrophages. Such an explanation would be in line with the concept advanced by Bowden et al. (1969) and Velo and Spector (1973) who have used x-irradiation and bone marrow replacement techniques to study macrophage migration in mice. They suggested that although all macrophages in pulmonary reactions are derived from circulating precursors of bone marrow origin, a residual pool of cells exists in the lung which are capable of rapid division and maturation. How large this pool is and to what extent it can deal with inhaled agents without calling on the bone marrow for reinforcements remains an important problem to be investigated.

Also of interest in this experiment was the sequestration of groups of mature, carbon-laden macrophages within connective tissue lined spaces in the alveolar parenchyma. It seems likely that the retention of long-lived macrophages in this manner may also occur during resolution in chronic pneumonia. If these cells are not able to escape to the main airways or lymphatic system this could also be a means by which bacteria and necrotic debris are sequestered thus prolonging the inflammatory response or leading to abscess formation.

The complete loss of pulmonary surfactant from areas of nitric acid administration was to be expected with the destruction of type II cells and fibrin exudation which occurred. Whether the loss of surfactant was due to mechanical removal in oedema fluids, inactivation by fibrin or leucocyte enzymes, the destruction of type II cells or a combination of these factors is not clear from the present study. However, the irregular distribution of stable bubbles which continued to be observed in affected areas for several days after injury indicates that the return of full pulmonary surfactant activity is gradual despite the occurrence of type II cell hyperplasia.

The pathology of the lung lesion caused by Paraquat in sheep appears to be essentially the same as that described originally in laboratory animals by Clark et al. (1966). These authors reported alveolar congestion, oedema and fibrin exudation together with increasing cellularity of the interstitial tissues including proliferation of fibroblasts as the lesions progressed. Alveolar macrophages were prominent and alveolar and bronchiolar epithelial proliferation occurred in many areas. Although the majority of these changes were seen in the present investigation the degree of alveolar epithelial damage produced in sheep was much less than that observed by Manktelow (1967b) in mice or Vijeyaratnam and Corrin (1971) and Kimbrough and Linder (1973) in rats. Sheep are nevertheless, particularly susceptible to Paraquat poisoning as indicated by the low dose rate needed to produce toxic symptoms, however the present findings suggest that the ovine liver and kidney are at least as equally susceptible to Paraquat injury as the ovine lung. In this respect, the sheep appears to stand midway between the rabbit and the majority of other animal species for

which information on Paraquat toxicity is available. Studies on Paraquat poisoning in the rabbit have failed to produce significant lung lesions since death usually occurred with only atrophy of the thymus and focal degeneration of renal tubules detectable at necropsy (Butler and Kleinerman, 1971).

Attempts to use Paraquat as a systemic toxin to lower pulmonary resistance to bacterial invasion were disappointing. Although not statistically significant, the results obtained suggested that the reverse effect was in fact produced by the administration of Paraquat. A possible explanation for the better survival times obtained in the Paraquat-treated animals, is that the sublethal Paraquat treatment stimulated macrophage activity sufficiently to enable the lungs to cope more effectively with the inoculated bacteria. Further experiments of this nature with lower doses of S. aureus or higher doses of P. haemolytica and larger numbers of experimental animals might well provide more useful information.

The pulmonary response to P. haemolytica, S. aureus and E. coli inoculation was generally similar to that observed by Biberstein et al. (1967) who inoculated adult sheep intratracheally with large doses of these organisms. The greater severity of the lesions obtained with P. haemolytica by Biberstein et al. was probably due to the large number of organisms inoculated which was often in the order of $10,000 \times 10^6$ per animal. These authors found that both P. haemolytica and S. aureus produced lesions composed of elements qualitatively identical to those seen in certain types of naturally-occurring pneumonia. Similarly, the lesions seen in the present investigation fell within the range of pathological response which occurs in acute enzootic pneumonia in New Zealand as described in Chapter 2. However, whereas in the naturally-occurring disease the lesions are predominately in antero-ventral parts of the lungs, in the experimental animals the distribution of lesions was related to the site of inoculation.

Despite reports of busulphan toxicity in man which indicate that alveolar type II cell desquamation and hyperplasia are striking features of the pathological reaction (Littler et al., 1969) the current study in

sheep did not succeed in producing significant alveolar epithelial injury with oral administration. The appearance of toxic symptoms when high doses were administered indicated that some absorption from the gastro-intestinal tract was occurring so it was concluded that the effects of busulphan on sheep lungs are less spectacular than those described in man.

SUMMARY

The experimental induction of pulmonary injury in worm-free lambs was accomplished by the instillation of dilute (1%) nitric acid and India ink through an endobronchial catheter. The development of localised alveolar injury in 12 animals was then studied after euthanasia at different time intervals from 2 hours to 10 days after administration.

Light microscopy showed that alveolar collapse and neutrophil infiltration were among the earliest changes observed. Neutrophils first appeared in alveolar spaces at 6 hours but few remained after 3 days. Alveolar macrophages were present as 2 morphologically different populations. Large macrophages were active from 3 hours onwards and were joined by smaller cells which migrated from interstitial tissues and blood vessels from 12 hours until 3 days after administration. Some alveolar macrophages were apparently long-lived and remained sequestered in connective tissue-lined spaces at 7 and 10 days after injury.

Electron microscopy revealed that alveolar epithelial damage was severe at 2 hours after injury and the majority of changes seen in both type I and type II cells were similar to those encountered in various types of naturally-occurring pneumonia. Proliferation of cells resembling Clara cells and type II alveolar cells was first noted as early as 1 day after administration and partial "epithelialization" of some alveoli adjacent to bronchioles by type II cells was seen at 5 days.

Complete loss of pulmonary surfactant was found to have occurred in areas of nitric acid instillation by 12 hours after administration and thereafter it returned slowly to normal over a period of several days.

The value of Paraquat as a systemic agent for the production of lung damage in sheep was tested by parenteral administration at various dose rates to 18 animals of differing ages. Sheep were found to be particularly susceptible to the drug and maximum pulmonary involvement

occurred at between 6 to 10 mg/kg. The pathology of the lung lesion was essentially the same as in other species although the most severe changes were in the alveolar interstitial tissues. In many instances death appeared to result from liver and kidney injury.

The effects of Paraquat on pulmonary resistance to endobronchial inoculation of P. haemolytica, S. aureus and E. coli were tested in 22 lambs. When Paraquat was administered subcutaneously 2 days prior to inoculation the survival time of the animals was slightly better and the cellular reaction slightly greater in the Paraquat-treated groups than in the untreated groups but these differences were not statistically significant. The lesions produced by mixed bacterial broths and broths containing S. aureus in pure culture were similar in many respects to the lesions seen in acute enzootic pneumonia.

The ability of the drug busulphan to produce systemic pulmonary damage in sheep was tested by oral administration to 2 animals over a 4 to 8 week period. Although collagen proliferation and mild alveolar epithelial damage were produced the value of the drug as a pulmonary toxic agent in sheep appears limited.

PART TWO

THE AETIOLOGY AND TRANSMISSION
OF SHEEP PNEUMONIA

CHAPTER 4

THE MICROBIAL FLORA OF THE NORMAL AND PNEUMONIC OVINE RESPIRATORY TRACT

INTRODUCTION

The ability of certain micro-organisms to inhabit the upper respiratory tract of many mammalian species despite the action of mechanical and immunological defence mechanisms, is now well established (Bosworth and Lovell, 1944; Magwood et al., 1969; Clapper and Meade, 1963; Harris et al., 1969). The role of these organisms in the aetiology of pneumonia has not yet been well defined but studies on the nature and distribution of the bacterial flora in the upper respiratory tract of cattle seem to indicate a close association between the numbers of P. haemolytica carried and the subsequent occurrence of acute pneumonic pasteurellosis (Thomson et al., 1969).

A detailed study of the microbial flora of the ovine respiratory tract has not been reported although Biberstein and Thompson (1966) and Biberstein et al. (1970) have studied the nasal carriage of P. haemolytica in several normal and pneumonic sheep flocks in Scotland.

This chapter presents data collected during several years of investigation into the bacterial and mycoplasma flora of the ovine respiratory tract. It includes a study of normal sheep as well as sheep with various types of pneumonic lesions obtained from abattoir surveys and field sources. In addition, an attempt was made to correlate the numbers of micro-organisms present in a range of subacute and chronic pneumonias with the degree of development of specific histological features within these lesions. No attempts were made to study chlamydia or viral agents as these were considered outside the range of the current investigation.

A SURVEY OF THE BACTERIAL FLORA OF THE NORMAL AND PNEUMONIC OVINE RESPIRATORY TRACT

The objective of this investigation was to compare the bacterial flora of the nasal cavity, trachea and lungs of normal sheep with that of sheep with subacute and chronic forms of pneumonia. Also included was a study of the bacteria present in the various pathological types of pneumonic lesion described in Chapter 2. Quantitative studies of the bacteria isolated were not attempted at this stage. Instead a relatively large number of sheep were sampled so that the frequency of isolation of the various bacteria would give an indication of their relative importance.

Material and Methods

Collection of Specimens

Specimens were collected from lambs aged 5 to 10 months during the December to May period of the years 1969-70 and 1970-71. They were obtained from local meatworks in the Manawatu district at weekly or fortnightly intervals.

On each occasion 10 lungs were selected and the corresponding heads identified and brought to the laboratory. Nasal swabs were obtained by cutting the head sagittally, slightly to one side of the mid-line, with a band saw and removing the nasal septum with bone forceps. A sterile swab was then drawn over the exposed turbinate and inserted in to both the dorsal and ventral nasal meatus. Tracheal swabs were collected by incising the trachea transversely midway along its length and inserting a sterile swab distally as far as the bifurcation of the main bronchi. Samples of lung were collected in sterile petri dishes. From the majority of pneumonic lungs a 3-4 cm area of the right apical lobe was removed with forceps and scissors as this was the area most consistently affected. A slice of pneumonic tissue immediately adjacent to this was routinely collected in 10% formol-saline for subsequent histological examination. Samples of normal lung were collected from the right apical lobe in the same way.

Laboratory Procedures

Nasal and tracheal swabs were plated out initially onto sheep blood agar and McConkey's medium. The surface of all lung samples was heat seared before plating out in a similar manner. Direct gram smears were made on all samples. In the early stages of the survey, plates were incubated both aerobically and with CO₂ added, however after 100 lungs had been processed CO₂ incubation was found to be non-productive and discontinued.

Identification of bacteria was based on the methods outlined by Wilson and Miles (1964) and Cowan and Steel (1965) with the exception of the enterobacteriaceae which were identified by the keys of Ewing (1968). Colonies of suspect Pasteurella sp. failing to show haemolysis on sheep blood agar were subcultured onto horse blood agar and tested with the indole reaction.

Lung samples for histological examination were processed in the usual manner, embedded in paraffin, cut and stained routinely with haematoxylin and eosin.

Results

The Prevalence of Bacterial Species in the Normal and Pneumonic Respiratory Tract

The results summarised in Table XI show a high rate of carriage of several bacteria in the nasal cavity of both normal and pneumonic sheep. Bacteria of this same type were also isolated in large numbers from the trachea and lungs of pneumonic sheep but were only rarely recovered from normal lungs and tracheas.

The predominant organism present in both the normal and pneumonic respiratory tract was Pasteurella haemolytica. This organism was most readily isolated from the nasal cavity of pneumonic sheep although its prevalence here was only slightly higher than that in the nasal cavity of normal sheep. It was recovered in pure aerobic culture from only 20% of all nasal cavities examined but was found in combination with Neisseria catarrhalis in 40% of animals and in combination with other

TABLE XI
THE PREVALENCE OF BACTERIA IN THE RESPIRATORY TRACT OF
NORMAL AND PNEUMONIC SHEEP

Organism		No. of Isolations over 2 seasons (1969-70, 70-71)		
		Nasal Cavity	Trachea	Lung
<u>P. haemolytica</u>	Normal	135 (73%)	9 (5%)	11 (6%)
	Pneumonic	193 (78%)	133 (54%)	144 (59%)
<u>N. catarrhalis</u>	Normal	72 (39%)	2 (1%)	3 (1.6%)
	Pneumonic	114 (46%)	55 (22%)	54 (22%)
<u>E. coli</u>	Normal	22 (12%)	1 (0.5%)	1 (0.5%)
	Pneumonic	31 (13%)	4 (2%)	2 (1%)
<u>S. aureus</u>	Normal	8 (4%)	2 (1%)	2 (1%)
	Pneumonic	23 (9%)	10 (4%)	2 (1%)
<u>Streptococcus sp.</u> (α -haemolytic)	Normal	21 (11%)	0	2 (1%)
	Pneumonic	23 (9%)	0	0
<u>C. pyogenes</u>	Normal	4 (2%)	0	1 (0.5%)
	Pneumonic	2 (1%)	0	0
No Growth	Normal	30 (16%)	169 (92%)	167 (91%)
	Pneumonic	21 (9%)	95 (39%)	83 (34%)

Other bacteria isolated from Nasal cavity

<u>Actinobacillus sp.</u>	6 isolations
Micrococci	4 isolations
<u>Bacillus sp.</u>	3 isolations

Total Examined = 184 Normal sheep
 246 Pneumonic sheep

organisms in a further 14%.

The second most common organism recovered was an organism of the Neisseria sp. which most closely resembled Neisseria catarrhalis (Alley et al., 1970). This organism was isolated most often in combination with P. haemolytica and could be grown in pure culture from only 3% of all nasal cavities examined. N. catarrhalis was also commonly isolated from the pneumonic lung and trachea (Table XI) but was recovered in pure culture from only 6% of pneumonic lungs.

E. coli, Staphylococcus aureus and α -haemolytic streptocci were also frequently isolated from the nasal cavity, however only rarely were these organisms recovered from the trachea or lung. Mixed growths in the nasal cavity were more commonly seen in those animals sampled during the December-January-February period of both seasons. Later during the autumn months, P. haemolytica and N. catarrhalis appeared to dominate the nasal flora of both normal and pneumonic sheep. Only rarely was P. haemolytica or N. catarrhalis isolated from pneumonic lungs and not found to be present concurrently in the nasal cavity (Table XII).

The Prevalence of Bacteria in Different Types of Pneumonic Lesion

For the purpose of this study lungs with pneumonic lesions were divided into 4 categories on the basis of their gross and histological appearance as described in Chapter 2. The prevalence of bacteria in these 4 types of lesion is shown in Table XIII. It can be seen that lesions in the first category in which the main feature was alveolar collapse were much less likely to yield bacteria than lungs in the other 3 groups (No Growth $\chi^2 = 30.5$, $P < 0.001$). The lungs in which the most extensive lesions were present usually showed grey-red consolidation and were placed in the third category. It was from this type of lesion that bacteria were most consistently recovered being present in 83% of the lungs examined.

P. haemolytica was recovered most often from lesions of the second category in which cellular exudation predominated (P. haemolytica $\chi^2 = 11.3$, $P = 0.01$). However the organism was still present in

TABLE XII

COMPARISON OF THE BACTERIAL FLORA OF THE NASAL CAVITY OF PNEUMONIC SHEEP IN WHICH PASTEURELLA HAEMOLYTICA OR NEISSERIA CATARRHALIS WERE ISOLATED WITH THOSE FROM WHICH THERE WAS NO GROWTH

Isolations from Nasal Cavity	Pneumonic Lungs Yielding:-		
	<u>P. haemolytica</u> (144)	<u>N. catarrhalis</u> (54)	No Growth (83)
<u>P. haemolytica</u>	142 (99%)	41 (76%)	62 (75%)
<u>N. catarrhalis</u>	74 (51%)	45 (83%)	36 (43%)
<u>E. coli</u>	14 (10%)	7 (13%)	10 (12%)
<u>S. aureus</u>	10 (8%)	5 (9%)	8 (10%)
α -haemolytic streptococci	13 (9%)	6 (11%)	7 (8%)
<u>Actinobacillus sp.</u>	4 (3%)	1	0
Micrococci	1	0	3 (4%)
<u>Bacillus sp.</u>	1	2 (4%)	0
<u>C. pyogenes</u>	1	0	0
No Growth	0	0	17 (20%)

TABLE XIII

BACTERIAL ISOLATIONS FROM SUBACUTE AND CHRONIC PNEUMONIAS

		Type of Lesion			
		1. Dull Red Consolidation	2. Red-grey Consolidation	3. Grey-red Consolidation	4. Grey Consolidation
<u>P.haemolytica</u>	69-70	13	26	22	7
	70-71	14	23	20	19
	Total	27 (35%)	49 (75%)	42 (64%)	26 (63%)
<u>N.catarrhalis</u>	69-70	4	8	12	1
	70-71	5	6	9	9
	Total	9 (12%)	14 (22%)	21 (33%)	10 (25%)
<u>S.aureus</u>	69-70	1	0	1	0
	70-71	2	0	1	1
	Total	3 (4%)	0	2 (3%)	1
<u>E.coli</u>	69-70	0	0	0	0
	70-71	2	0	0	0
	Total	2 (3%)	0	0	0
<u>C.pyogenes</u>	69-70	0	0	0	0
	70-71	0	0	1	0
	Total	0	0	1	0
No Growth	69-70	15	4	2	3
	70-71	34	8	9	8
	Total	49 (64%)	12 (18%)	11 (17%)	11 (28%)
Total	69-70	101	32	30	10
Examined	70-71	145	33	34	30
		246	77	65	40

the majority of the more advanced lesions in which proliferative changes were the most prominent feature.

Although N. catarrhalis showed a less significant pattern of distribution than P. haemolytica this organism showed a tendency to be present in lesions of a more chronic nature (N. catarrhalis $\chi^2 = 7.3$, $P < 0.1 > 0.05$).

A monthly comparison of isolations was not undertaken although in both seasons, the proportion of type 1 lesions appeared to be greater at the beginning of the season and the proportion of type 3 and 4 lesions greater towards the end of each season. However, the considerable variation in the prevalence of the disease between the different lines of sheep sampled at the meatworks would have necessitated the collection of a very large number of samples in order to make such a comparison valid.

BACTERIAL ISOLATIONS FROM ACUTE PNEUMONIA AND PULMONARY OR PLEURAL ABSCESES

The close association between acute fibrinous pneumonia in sheep and cattle and the Pasteurella sp. has lead to the use of the term "Pasteurella pneumonia" to describe many acute pneumonias in these species (Shirlaw, 1959). Much of the evidence for this association has come from the post-mortem records of veterinary investigation laboratories (Ministry of Agriculture, Fisheries and Food, 1964).

Little information is available on the bacteria present in acute pneumonia and pulmonary or pleural abscesses of sheep in New Zealand although Salisbury (1957) and Downey (1957) noted that Pasteurella haemolytica was almost invariably isolated from cases of acute enzootic pneumonia. The following data was therefore obtained from the post-mortem examinations of field cases of acute and suppurative pneumonic conditions occurring in sheep in the Manawatu district of New Zealand.

Material and Methods

During the period 1969 to 1974 the lungs of 48 sheep of all age groups with acute pneumonia were examined bacteriologically in the

Department of Veterinary Pathology and Public Health, Massey University. In addition, 49 sheep with pleural or pulmonary abscesses from sheep of a variety of ages were examined and cultured bacteriologically.

Samples were collected in sterile petri dishes and direct smears were made before routine aerobic culture was carried out. In the case of purulent or fibrino-purulent lesions, anaerobic culture was also performed. Sections of affected tissue were routinely collected in 10% formol-saline and the nature of the lesions subsequently confirmed by histological examination.

Results

In sheep of all ages with acute pneumonia the most common organism isolated was P. haemolytica (Table XIV). Isolations of N. catarrhalis were largely confined to sheep in the 3 to 12 month age group whereas E. coli appeared to be distributed in the lungs of both young and old sheep. Other organisms were recovered only sporadically and were too few in number to detect any significant pattern of distribution.

The bacterial isolations from sheep with pulmonary or pleural abscesses are presented in Table XV. It can be seen that C. pyogenes was as frequently isolated from these lesions as P. haemolytica. However, C. pyogenes was most often recovered from adult sheep while P. haemolytica was more evenly distributed although neither of these organisms were commonly found in abscesses in young lambs. In contrast, almost all the isolations of S. aureus were made from lesions in young lambs and this organism was not recovered from abscesses in any of the adult sheep examined. Prominent among the other organisms recovered were Spherophorus necrophorus and E. coli both of which were found in suppurative lesions in young and old sheep.

A SURVEY OF THE PREVALENCE OF MYCOPLASMA OVIPNEUMONIAE AND MYCOPLASMA ARGININI IN THE RESPIRATORY TRACT OF NORMAL AND PNEUMONIC SHEEP

Following the isolation and identification of M. ovipneumoniae and M. arginini in the lungs and nasal cavity of sheep in New Zealand (Clarke et al., 1974) there was a need to assess the prevalence of these

TABLE XIV
AGE DISTRIBUTION AND BACTERIAL ISOLATIONS FROM 48 CASES
OF ACUTE PNEUMONIA⁽¹⁾

Bacteria Isolated	Age of Sheep					Total
	0-2 mths	3-11 mths	1-2 years	2-6 years	6 years & over	
<u>P. haemolytica</u>	3	10	6	6	3	28
<u>E. coli</u>	1	2	0	3	3	9
<u>N. catarrhalis</u>	0	6	0	1	0	7
<u>C. pyogenes</u>	0	3	1	0	0	4
<u>S. aureus</u>	2	1	0	0	0	3
α -haemolytic streptococci	0	1	0	2	0	3
No growth	1	1	2	1	0	5
TOTAL EXAMINED	7	16	9	11	5	

(1) Compiled over a 6 year period 1969-74

(The possibility that antibiotic therapy was used in some of these cases cannot be discounted).

TABLE XV

BACTERIAL ISOLATIONS FROM PULMONARY AND PLEURAL ABSCESSSES
IN SHEEP OF 3 DIFFERENT AGE GROUPS ⁽¹⁾

Bacteria Isolated	Age Group			Total
	2 days to 2 months	3 months to 11 months	1 year to 6 years	
<u>P. haemolytica</u>	1	9	7	17
<u>C. pyogenes</u>	1	6	10	17
<u>S. aureus</u>	10	2	0	12
<u>S. necrophorus</u>	3	1	6	8
<u>E. coli</u>	2	1	4	7
α haemolytic streptococci	2	1	2	5
<u>N. catarrhalis</u>	0	2	1	3
<u>B. fragillus</u>	0	1	0	1
<u>Cl. septicum</u>	0	0	1	1
<u>Fusobacterium</u> sp.	0	1	0	1
<u>Mima</u> sp.	0	0	1	1
<u>Proteus</u> sp.	0	1	0	1
<u>Pseudomonas</u> sp.	0	1	0	1
No growth	0	0	1	1
TOTAL EXAMINED	13	17	19	49

(1) Compiled over a 6 year period 1969 to 1974

organisms in the respiratory tract of normal and pneumonic sheep in this country.

A survey of 6 to 9-month-old sheep was undertaken during the autumn of 1973. This initial work was then extended to cover the following autumn so that a quantitative assessment of M. ovipneumoniae present in the lungs could be made in a range of subacute and chronic pneumonias.

Material and Methods

Collection of Specimens

In the initial survey specimens were collected on nine occasions at approximately weekly intervals during March, April and May 1973. All specimens came from a local meatworks which was supplied with sheep from the Manawatu district. On each occasion lung specimens and complete heads were collected from 3 pneumonic and 3 normal sheep and lung specimens alone were taken from 4 sheep with pneumonia. Except where otherwise stated the lungs were removed from the sheep and handled by a meat inspector before they became available for sampling. A subsequent study was undertaken to estimate the titre of M. ovipneumoniae in normal and pneumonic lungs and in this study the lungs were collected on 10 occasions during March, April and May 1974.

The diseased lungs sampled included a variety of subacute and chronic pneumonias and histological sections of tissue immediately adjacent to the area sampled were obtained and processed in the manner described previously in the survey of the bacterial flora. The nasal swabs were also collected by the method previously used in the bacterial flora survey.

Media

The media used for the isolation and propagation of fermenting mycoplasmas (FM4) was the fourth formulation described by Frey et al. (1969). For arginine requiring strains this medium was modified by the addition of 1% arginine hydrochloride and adjusted to pH 7.0. This is referred to as FM4A medium. Solid medium was prepared by the

addition of 1% agar to the liquid medium.

Processing of Specimens

Nasal swabs and lung specimens were normally processed as previously described by Clarke et al. (1974). This involved two passages in fluid medium followed by spotting on solid medium. The resulting colonies were identified by fluorescent antibody methods.

To estimate the titre of M. ovipneumoniae present in sheep lungs, 0.3 gm. of lung was ground with sand using a pestle and mortar and 2.7 ml of FM4 medium was added. This was regarded as a 1:10 dilution of lung. Ten-fold dilutions were then made serially transferring 0.3 ml into 2.7 ml aliquots of FM4 medium up to a final dilution of 10^{-9} . The dilutions from 1:100 upward were then incubated for 10 days, and dilutions showing an unequivocal drop in pH were recorded as positive and the titre was recorded as the highest dilution to show such a change. To confirm that the colour change was due to M. ovipneumoniae, an aliquot of the highest possible dilution was spotted on agar and the colonies were examined after incubation at 37°C for 7 days.

Fluorescent Antibody Methods

To prepare an antigen for antiserum production mycoplasmas were propagated using medium in which swine serum was replaced by horse serum. The mycoplasmas were inoculated into rabbits as described by Clarke et al. (1974). Globulins were precipitated from the immune serum with 50% saturated ammonium sulphate at 0°C and labelled with fluorescein isothiocyanate as described by Clarke et al. (1972). This procedure involved purification of the conjugate by Sephadex and DEAE cellulose chromatography.

Impression smears of mycoplasma colonies were prepared by pressing coverslips on the agar surface. The smears were air dried and fixed in acetone for 10 minutes at room temperature. The smears were then stained for 1 hour at room temperature by the direct technique.

Results

Identification of Isolates by Fluorescent Antibody Methods

At an early stage of the present study it became clear that a rapid and unequivocal method for the identification of mycoplasma colonies was necessary, so that antibody to M. ovipneumoniae (strain 5) and M. arginini (strain 110) and control serum were labelled with fluorescein isothiocyanate and tested for their ability to stain impression preparations of colonies of 10 strains of M. arginini and 6 strains of M. ovipneumoniae which had been identified by gel precipitin tests (Clarke *et al.*, 1974). It was found that in all cases antiserum to either M. arginini or M. ovipneumoniae stained only the homologous organism and control serum stained neither. Subsequently, replicate impression preparations of colonies formed by all the isolates were exposed to the three labelled sera and it was found that all isolates stained with one or the other of the two antisera but not with the control serum. It was concluded that all the mycoplasmas recovered from the respiratory tract in this survey represented isolates of either M. ovipneumoniae or M. arginini and the staining reaction was correlated with colonial morphology: thus all colonies showing "fried egg" morphology stained with antibody to M. arginini whereas all "vacuolated" colonies stained with antibody to M. ovipneumoniae. Some cultures gave mixtures of both colony types (Figure 4.1) and in such cases impression smears stained with both antisera but not with control serum. It should be noted that the formation of centreless colonies refers to medium containing 1% agar since at concentrations significantly lower than this e.g. on 0.75% agar most M. ovipneumoniae colonies became "centred".

Prevalence of Mycoplasmas in the Nasal Cavity

All strains of mycoplasmas recovered from nasal swabs from normal and pneumonic sheep were found to be either M. ovipneumoniae or M. arginini. The recovery rate of both organisms from normal and pneumonic sheep is recorded in Table XVI.

Preliminary Investigation of Mycoplasmas in the Lungs

This study was initially undertaken to compare the isolation rate

following page 135

Figure 4.1

Colonies of Mycoplasma arginini (centred) and Mycoplasma ovipneumoniae (centreless) in oblique transmittted light. x 45

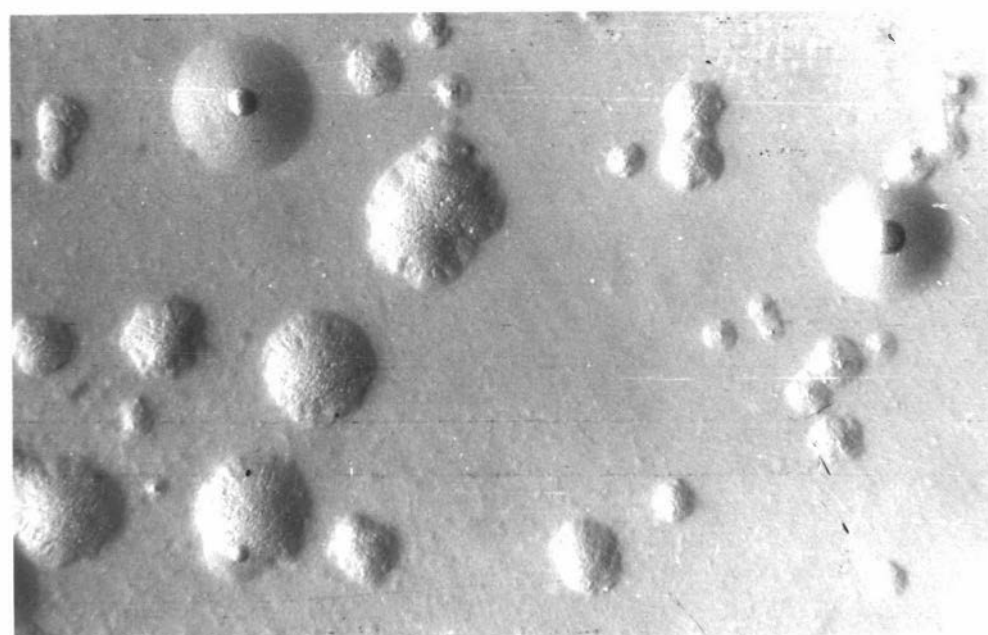


TABLE XVIRECOVERY RATE OF TWO SPECIES OF MYCOPLASMA FROM THE NASAL
CAVITY OF NORMAL AND PNEUMONIC SHEEP

Organism	Recovery Rate	
	Normal Sheep	Pneumonic Sheep
<u>M. ovipneumoniae</u>	13 (48%)	19 (70%)
<u>M. arginini</u>	9 (33%)	14 (52%)
TOTAL EXAMINED	27	27

and identity of mycoplasmas from the lungs of normal and pneumonic sheep. The results after testing 27 normal lungs and 63 pneumonic lungs were as follows: with pneumonic lungs M. ovipneumoniae was recovered from 79% and M. arginini was recovered from 32% of samples whereas with normal lungs M. ovipneumoniae was recovered from 63% and M. arginini from 19%.

No mycoplasma were recovered in this survey except strains of M. ovipneumoniae and M. arginini. However, the recovery rate of organisms from the control lungs and in particular the apparent prevalence of M. ovipneumoniae was unexpectedly high. It seemed possible therefore that cross contamination of lungs had occurred and the most likely time for this to have taken place was when they were palpated by a meat inspector prior to sampling.

To investigate this possibility, 9 normal lungs were sampled immediately after their removal from sheep and the same lungs were re-sampled after they had passed the meat inspector. M. ovipneumoniae was recovered from 3 of the early samples but was detected in 6 of the later samples. It was therefore concluded that cross contamination had occurred.

The problem of interpretation of results because of cross contamination by a small number of superficial micro-organisms may be minimised by estimating the titre of organisms present in the test material. A quantitative estimate of the titre of M. ovipneumoniae in normal and pneumonic lungs was therefore attempted.

Quantitative Study of M. ovipneumoniae in Normal and Pneumonic Lungs

Normal and pneumonic lungs were titrated for M. ovipneumoniae by limit dilution and the results are shown in Figure 4.2. The geometric mean titre of M. ovipneumoniae in pneumonic lung was calculated to be $10^{6.6}$. Since the endpoint was taken as the highest dilution from which M. ovipneumoniae was recovered and each dilution bottle contained 2.7 ml, it was calculated that the mean titre corresponded to approximately 3×10^6 organisms per gm of affected lung.

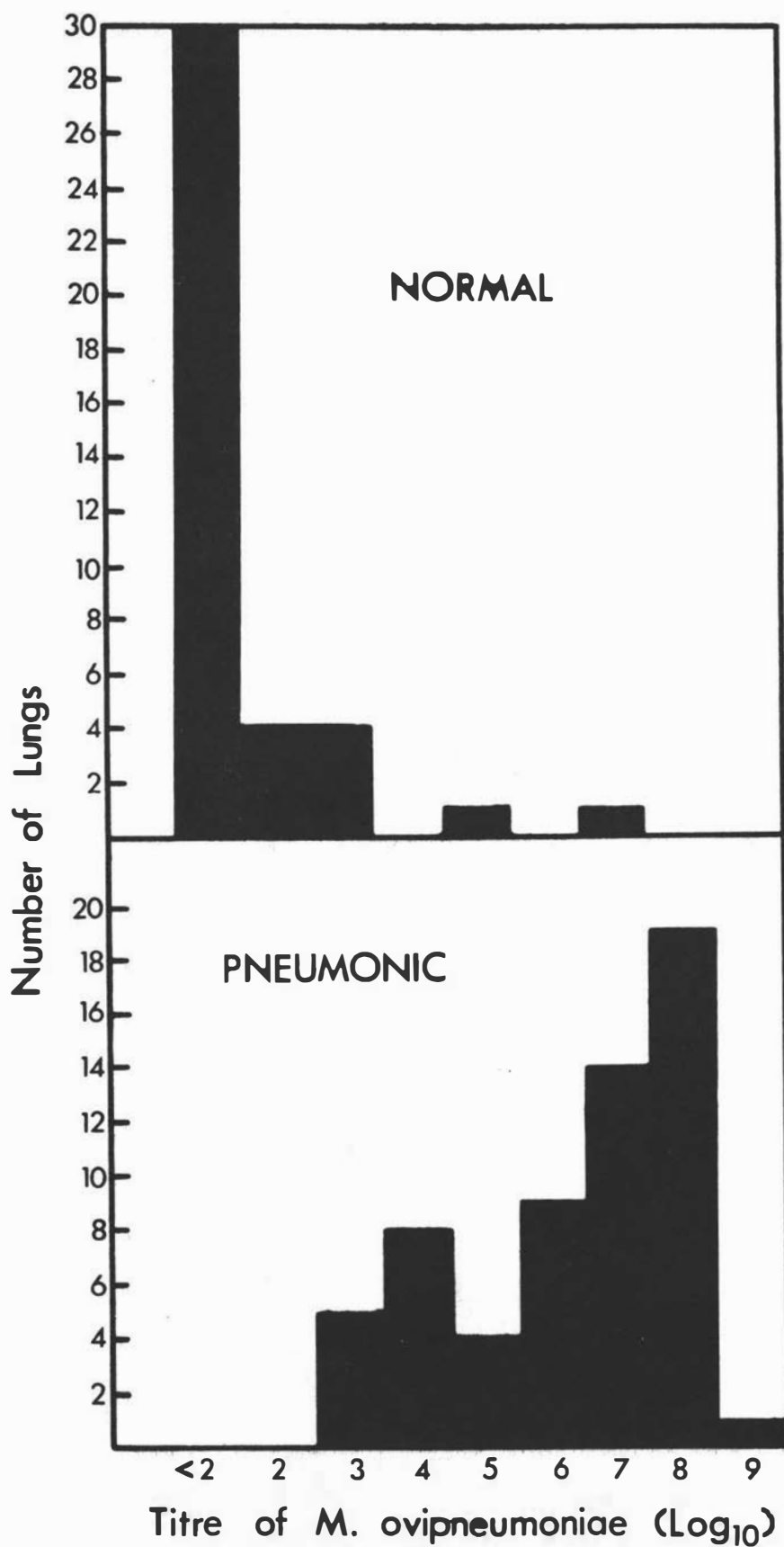


Figure 4.2 Distribution of titres of *M. ovipneumoniae* in normal and pneumonic lungs.

It should be noted that M. ovipneumoniae was recovered from all of the 60 pneumonic lungs tested whereas only 10 of the 40 normal lungs were positive and of these 10, only 2 had a titre of above 10^3 . In about 10% of the titrations of pneumonic lungs but not with normal material, a "prozone-type phenomenon" was detected. In these cases after 3 to 6 days the 10^{-2} dilution and occasionally the 10^{-3} dilution showed no pH change although higher dilutions were positive. In most cases however, the "prozone" disappeared after prolonged incubation. It seems reasonable to suppose that this "prozone" effect was due to the presence of antibody in the lung in sufficient concentration to inhibit mycoplasma growth at the lower dilutions.

The identity of the mycoplasmas present at limiting dilution was checked by plating the dilution on agar and examining the colonies formed following incubation. In all cases the organisms were identified as M. ovipneumoniae.

CORRELATIONS BETWEEN HISTOPATHOLOGICAL FEATURES AND NUMBERS OF M. OVIPNEUMONIAE AND BACTERIA PRESENT IN PNEUMONIC LUNGS

Recent work by Australian investigators has produced evidence that M. ovipneumoniae is associated with a chronic proliferative interstitial pneumonia in sheep in Queensland (Sullivan et al., 1973a). In view of this, and the previous findings that high titres of M. ovipneumoniae are present in pneumonic lungs in New Zealand it was decided to investigate the possible association of certain histopathological features with the numbers of M. ovipneumoniae in pneumonic lungs. At the same time the bacteria present were also quantitated in order that any relationship which existed between bacterial numbers and the type of pathological response which occurred could also be identified.

Material and Methods

This study was performed on the 60 pneumonic lungs which were collected for the previous quantitative survey of M. ovipneumoniae titres. Sections of tissue immediately adjacent to the area of pneumonic lung sampled were fixed in 10% formol-saline, processed in the usual manner, embedded in paraffin and stained with haematoxylin and eosin.

Bacterial colony counts of ground pneumonic lung suspension were made by the standard surface count technique. 0.1 ml of the initial 1:10 dilution was inoculated on to the surface of blood agar plates and 0.1 ml carried over into 1 ml of sterile nutrient broth for the next dilution. The organisms present were identified as P. haemolytica, N. catarrhalis or Staphylococcus sp.

Results

In preliminary attempts to correlate the titres of M. ovipneumoniae with pneumonic lesions the lungs were divided into the 4 categories of subacute and chronic pneumonia described in Chapter 2. The mean titre of M. ovipneumoniae in each of these groups was as follows: type 1 lesions $10^{5.7}$ per gm; type 2, $10^{6.4}$ per gm; type 3, $10^{6.8}$ per gm; type 4, $10^{5.9}$ per gm. The range of titres in each group of pneumonic lungs varied considerably and since the severity of histological lesions in each group also varied it was decided to make a more detailed comparison of the severity of histological lesions with the titres of M. ovipneumoniae and bacteria.

Detailed histological examinations of the lungs were undertaken without prior knowledge of their bacterial and M. ovipneumoniae titres and each pathological feature observed was graded on a 4-point scale of severity. In order to highlight any possible association of numbers of organisms with pathological changes, 4 groups each composed of 7 to 8 lungs, were selected from the 60 lungs examined on the basis of the following criteria:-

Group A	Low	<u>M. ovipneumoniae</u>	titre,	low	bacterial	titre.
Group B	High	"	"	low	"	"
Group C	Low	"	"	high	"	"
Group D	High	"	"	high	"	"

A mean index of severity was then calculated for each histopathological feature in each group and the results of these calculations are shown in Figure 4.3. From this figure it can be seen that large numbers of neutrophils and severe epithelial hyperplasia in both bronchioles and alveoli commonly occurred in lungs in which there were large numbers of both M. ovipneumoniae and bacteria. There was also an inverse

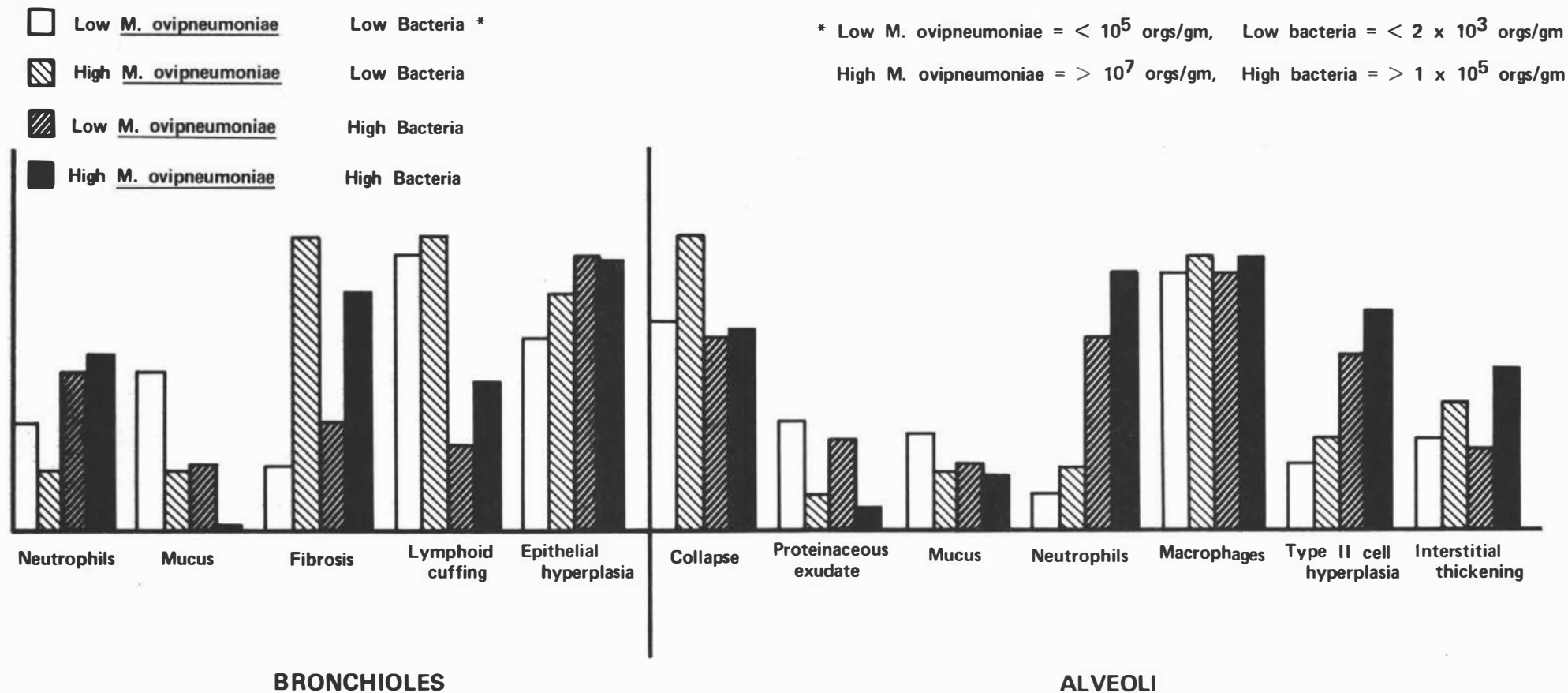


Figure 4.3 Correlation of *M. ovipneumoniae* and bacterial titres with severity of histological lesions

relationship between the degree of lymphoid hyperplasia and the numbers of bacteria present and a similar but less pronounced inverse relationship between the amount of mucus present in both bronchioles and alveoli and numbers of bacteria. In contrast, there was a strong positive association between high numbers of M. ovipneumoniae and the severity of peribronchiolar fibrosis, and a similar but less clear cut association between the numbers of M. ovipneumoniae present and the degree of alveolar interstitial thickening by mononuclear cells and fibroblasts. A further point of interest was the apparent lack of any relationship between the numbers of alveolar macrophages present and the numbers of micro-organisms of all types that were recovered.

The possibility of a relationship existing between the numbers of M. ovipneumoniae and the bacteria present in the lung was also investigated by plotting the titres of these organisms against each other as shown in Figure 4.4. However the distribution of the organisms was random so it was concluded that no obvious relationship existed.

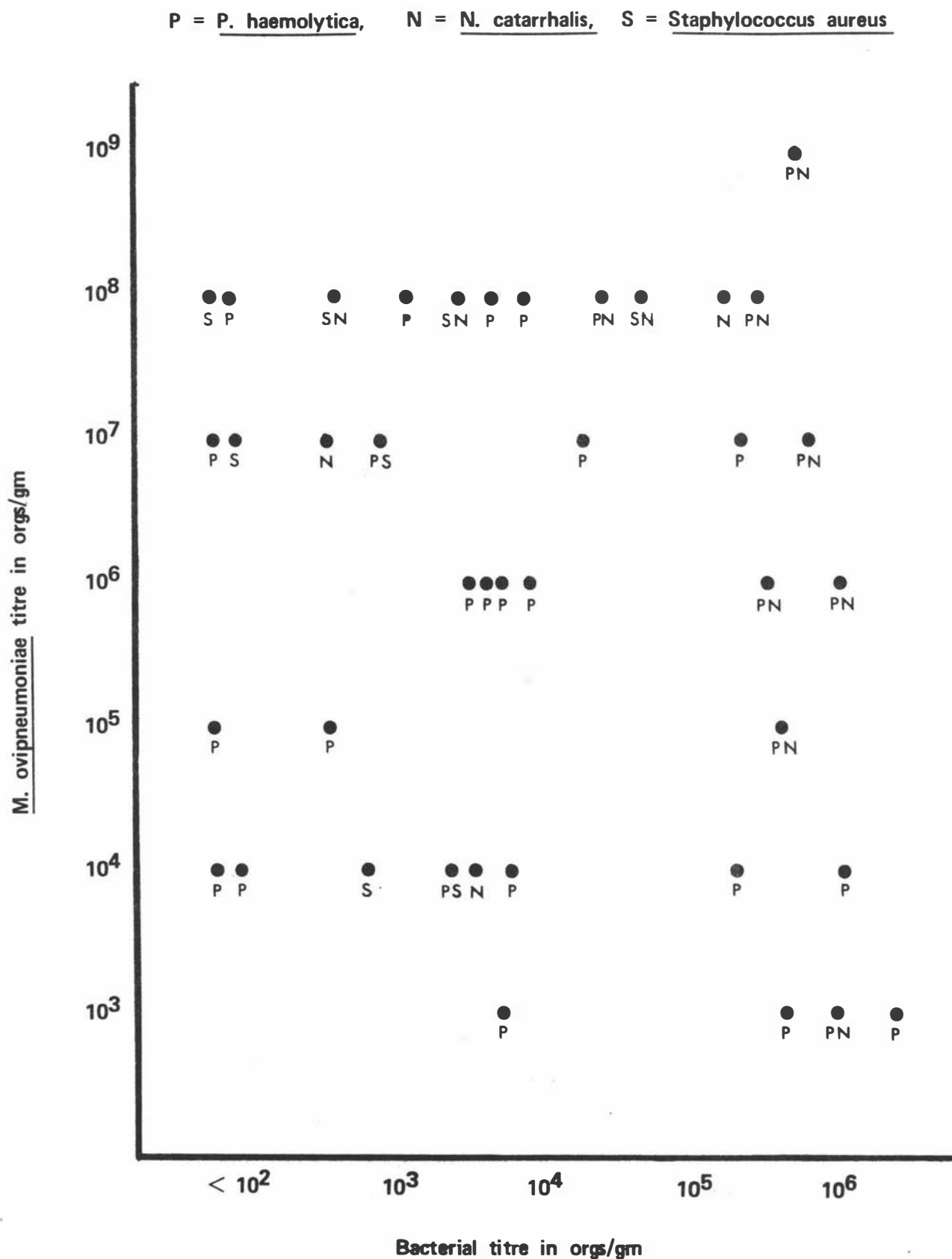


Figure 4.4 The distribution of M. ovipneumoniae and bacterial titres in 40 cases of subacute and chronic pneumonia

DISCUSSION

The bacteriological findings obtained in the survey of normal and pneumonic lambs at slaughter indicate that both groups of animals have a high rate of carriage of several micro-organisms in the nasal cavity. The same types of organisms were present in a similar ratio in the lungs and tracheas from pneumonic sheep that were examined. It seems likely therefore, that bacteria normally resident in the nasal cavity have the ability to establish themselves in the lungs of sheep with pneumonic lesions. Because these organisms were not isolated from all the pneumonias examined it could be inferred that they were not essential for the establishment of the disease. Nevertheless, the high recovery rate of bacteria from the more severe types of pneumonic lesion suggests that they were making an active contribution to the pneumonic process in these animals.

The high rate of recovery of P. haemolytica from the nasal cavity is in keeping with the findings of previous overseas investigators (Boswell and Lovell, 1944; Smith, 1957). The nasal carriage of P. haemolytica in normal flocks has also been investigated by Biberstein and Thompson (1966) who found a relatively lower incidence and wider range of serotypes than in pneumonic flocks. Further work by Biberstein et al. (1970) revealed a bimodal curve of carrier rates for P. haemolytica in several flocks studied over a 12 month period. The first peak occurred in late autumn and the second in late spring to early summer. These peaks coincided closely with the known seasonal pattern of enzootic pneumonia in the region. However, it is not clear from these authors reports whether the enzootic pneumonia mentioned is of the acute "pasteurella" type described by Montgomerie et al. (1938) and Salisbury (1957) or of the subacute and chronic types which were the subject of the present survey.

In New Zealand, Downey (1957) carried out a small survey of lungs from 6 to 8-month-old sheep obtained from a meatworks. The only organism reported to be isolated was P. haemolytica which was cultured from 18 of the 32 pneumonic lungs examined (i.e. 56%). This figure is in keeping with the recovery rate for P. haemolytica from pneumonic lungs of 59% in the present survey.

Isolations of Neisseria sp. from the lungs of sheep have been reported infrequently in the literature (Miller, 1940; Alley et al., 1969; St. George, 1972). In cattle Neisseria catarrhalis is known to occur commonly in the nasal cavity and has been recovered from 46.7% of nasal swabs taken from calves over a 7 month period (Magwood et al., 1969). The present study indicates that this organism is also common in the nasal cavity of sheep in New Zealand where it is most often found in association with P. haemolytica. It also appears to have the ability to colonise the lung in a significant proportion of chronic pneumonias.

Detailed studies of the dynamics of bacterial colonisation of the nasal cavity in cattle have shown a wide variety in the numbers and types of organism present over a 24 hour period (Magwood et al., 1969; Thomson et al., 1969). If this is also the case in sheep it could be argued that prior yarding and transportation could cause cross-colonisation between the nasal cavities of closely confined sheep. Thus samples collected at slaughter would tend to over estimate the field carriage rate of nasal micro-organisms. In this respect it should be noted that similar opportunities for cross-colonisation exist during driving and yarding for many routine sheep husbandry practices.

The recovery of P. haemolytica from a high proportion of the cases of acute pneumonia examined would seem to reinforce the commonly held belief that the organism is usually associated with pneumonias of this type. It is worth noting however, that the organism was not invariably isolated from acute fibrinous or necrotizing pneumonias which suggests that lesions of this type are not specific to the Pasteurella sp. This conclusion is supported by the work of Gourlay et al. (1970) in calves, who found a poor correlation between actual isolation of Pasteurella sp. and the occurrence of histological lesions normally associated with this type of infection.

P. haemolytica was also frequently isolated from pleural and pulmonary abscesses in the present study. A similar observation was made by Downey (1957) when he noted that this organism together with C. pyogenes could nearly always be demonstrated in areas of suppuration associated with anteroventrally distributed pneumonia in lambs and

hoggets. The present finding that C. pyogenes was recovered mainly from suppurative lung lesions in adult sheep, whereas S. aureus was recovered mainly from young lambs, is noteworthy as it raises the possibility that there may be differences in the rate of nasal carriage of micro-organisms in sheep of differing ages. This possibility receives support from a survey of the nasal carriage of coagulase-positive staphylococci in ewes and lambs at weaning carried out in Otago (Ann. Report of Animal Research Stations, N. Z. Department of Agriculture, 1962-63). Investigation of 100 ewes from a clinically normal flock and their lambs at weaning revealed that 30% of ewes and 25% of their lambs were carriers of coagulase-positive staphylococci in the nose. This rate of nasal carriage is considerably higher than the 4 to 9% observed in 5 to 10-month-old lambs in the present study. However, some of this difference may be a reflection of the different methods employed in the collection of samples.

From the survey of the prevalence of M. arginini and M. ovipneumoniae in the respiratory tract of sheep it was concluded that both organisms but especially M. ovipneumoniae, are commonly present in the upper respiratory tract of 6 to 9-month-old sheep in the Manawatu district of New Zealand. All the isolates recovered either gave an increased pH in arginine containing medium; formed centred colonies and stained with antiserum to M. arginini, or gave a decreased pH on FM4 medium; formed centreless colonies and stained with antiserum to M. ovipneumoniae. Thus fermenting mycoplasmas other than M. ovipneumoniae are absent or rare in the respiratory tract of sheep in the Manawatu or alternatively they fail to grow on FM4 medium which is a relatively complex medium designed to propagate fastidious mycoplasmas. It should be noted however that no attempt was made in the present study to recover urea requiring mycoplasmas.

St. George et al. (1971) concluded that M. ovipneumoniae can cause chronic pneumonia in sheep in Australia and as M. arginini is found in many species of animals (Leach, 1970) but is not known to cause lesions in the lower respiratory tract, the present study of lung specimens concentrated on estimating the titre of fermenting mycoplasmas present using FM4 medium. The distribution of M. ovipneumoniae in normal and

pneumonic lung was found to be different both in the proportion of lungs from which M. ovipneumoniae were recovered viz. 100% of pneumonic lungs and 25% of normal lungs and in the geometric mean titre of positives viz. $10^{6.6}$ per gram for pneumonic lung and $10^{3.2}$ per gram for normal lung. Although the high recovery rate and relatively high titre of M. ovipneumoniae in pneumonic lung is consistent with the conclusion that this mycoplasma is the cause of chronic pneumonia it is nevertheless equally possible that it could be an efficient secondary invader of lung damaged by some other agent.

Although the correlations of histopathological lesions with numbers of M. ovipneumoniae and bacteria did not produce evidence of any conclusive relationships some trends were observed which indicated that further studies of this nature may be worthwhile. There appeared to be an association between high titres of M. ovipneumoniae and chronic proliferative changes such as peribronchiolar fibrosis and interstitial thickening with mononuclear cells and fibroblasts. This observation lends some support to the findings of Sullivan et al. (1973a) who have reported that this organism is associated with a chronic proliferative interstitial pneumonia in sheep in Queensland. An additive effect on the intensity of neutrophilic exudation in alveoli and to a lesser extent in bronchioles as well as increased type II cell hyperplasia was produced by combined high titres of both M. ovipneumoniae and bacteria. Roberts et al. (1962) noted a similar effect on neutrophil exudation when investigating the influence of Pasteurella multocida and Mycoplasma hyorhinis on the histopathology of porcine pneumonia but did not observe any changes in type II alveolar epithelial cells.

Peribronchiolar lymphoid hyperplasia was one of the most prominent changes associated with low bacterial titres in lungs with chronic pneumonia. This finding suggests that the proliferation of local aggregations of lymphoid tissue is an effective means of keeping bacteria at a low level since presumably such proliferations are correlated with the immunological response of the lung to antigen. Although "cuffing pneumonia" has in the past been associated with mycoplasma infection, the present finding is in line with the currently accepted concept that this change can be a non-specific response to a

wide range of irritants (Hanichen, 1964; Jericho, 1966). The presence of excess mucus in bronchioles and mucus reflux into alveoli was also associated with low bacterial titres and this could well be explained by the effect of antibacterial IgA or lysozymes within these secretions.

SUMMARY

The nasal cavity, trachea and lungs of 184 normal sheep and 246 sheep aged 5 to 10 months with chronic and subacute pneumonia were examined bacteriologically. Samples were collected at slaughter over a 2 year period during the maximum incidence of pneumonia in the autumn months. The most common organism recovered was P. haemolytica which was present in 78% of nasal cavities of sheep with pneumonia and 73% of nasal cavities of normal sheep. N. catarrhalis was also commonly found in the nasal cavities of both normal and pneumonic sheep and E. coli and S. aureus were less frequently isolated.

Pneumonic lungs were divided into 4 categories according to gross and histological lesions. Lesions characterised by alveolar collapse yielded few bacteria whereas those in which cellular exudate predominated contained P. haemolytica in 75% of cases. P. haemolytica was also recovered from more than 60% of lungs in which severe proliferative changes predominated and N. catarrhalis was recovered from 25 to 33%. No bacteria could be recovered from 91% of normal lungs but P. haemolytica was isolated from 6%.

P. haemolytica was also recovered from the majority of field cases of acute pneumonia in sheep of all ages but was not invariably associated with lesions of this type. The organism was also isolated as often as C. pyogenes from pleural and pulmonary abscesses in sheep and lambs older than 3 months but in lambs younger than this S. aureus was the most common isolate.

The nasal carriage of mycoplasmas by sheep with and without chronic pneumonia was investigated in 6 to 9-month-old lambs sampled at slaughter. It was found that M. ovipneumoniae and M. arginini were ubiquitous in both pneumonic and normal animals, however M. ovipneumoniae was recovered more frequently than M. arginini and both organisms were recovered more frequently from pneumonic than normal animals. No mycoplasmas other than these two species were detected.

The recovery rate and titre of M. ovipneumoniae in normal and pneumonic lungs from 6 to 9-month-old lambs was estimated at slaughter.

All 60 pneumonic lungs tested contained M. ovipneumoniae and had a geometric mean titre between 10^6 and 10^7 organisms per gram of lung. M. ovipneumoniae was recovered from 10 of 40 normal lungs tested and in only 2 cases did the titre exceed 10^3 organisms per gram. It is likely that the low titre of organisms present in the remaining 8 positives represents surface contamination before collection.

An attempt was made to correlate the severity of histopathological lesions with the titre of both M. ovipneumoniae and bacteria in pneumonic lungs. High titres of M. ovipneumoniae were associated with chronic proliferative changes such as peribronchiolar fibrosis and interstitial thickening. The combined effect of large numbers of M. ovipneumoniae and bacteria were associated with neutrophilic exudation and epithelial hyperplasia. However, lymphoid hyperplasia and excess mucus production were associated with low bacterial titres. This suggested that these factors were effective in reducing bacterial numbers presumably by means of local antibody or lysozyme secretion. There was no direct correlation between the numbers of M. ovipneumoniae and bacteria present in pneumonic lungs.

It is concluded that bacteria from the nasal cavity may invade the pneumonic lung and actively contribute to the severity of the lesions in chronic pneumonia but it is unlikely that they are responsible for initiating the disease process. The marked difference in the titre of M. ovipneumoniae between normal and pneumonic sheep suggests that this organism may also have a role on the pathogenesis of chronic pneumonia in sheep in New Zealand. Whether this role is that of a primary pathogen or similarly represents efficient colonization of lung presumably damaged by other agents requires further investigation.

CHAPTER 5

THE EXPERIMENTAL TRANSMISSION OF SHEEP PNEUMONIA

INTRODUCTION

Since the earliest records of pneumonia in sheep it has been assumed that the disease is fundamentally infectious in nature. The evidence for this belief is based mainly on epidemiological findings, such as the occurrence of outbreaks of acute pneumonia following periods in which the sheep were in close contact (Dungal, 1931; Montgomerie et al., 1938). Similarly, the high morbidity of subacute and chronic pneumonia in some flocks but not in others held under similar conditions, favours the notion that infectious agents have an essential role in the development of this form of the disease. This view is supported by the natural development of an effective and permanent immunity to chronic pneumonia in adult sheep in this country.

Experimental transmission of all sheep pneumonias has been difficult and the results often unconvincing. Attempts to produce pneumonia by the inoculation of microbial agents have already been discussed in Chapter 1. In addition to this work both Montgomerie et al. (1938) and Salisbury (1957) attempted unsuccessfully to produce pneumonic lesions by the intratracheal inoculation of various lung suspensions and bacterial cultures. Downey (1957) achieved only partial success with the production of 3 doubtful pneumonias in 22 sheep inoculated with a variety of suspensions derived from pneumonic lung and "infected" chorio-allantoic membranes. Australian workers have reported greater success with the transmission of the proliferative interstitial pneumonia which occurs in sheep in Queensland. St. George et al. (1971) transmitted the disease to caesarian-derived lambs by the intratracheal injection of ground pneumonic lung but the number of animals involved was not mentioned. Sullivan et al. (1973a) produced lesions in 3 out of 4 lambs inoculated intratracheally with lung tissue from a 2-day-old lamb and confirmed the presence of interstitial pneumonia microscopically in all 4 lambs.

Because of the uncertain nature of the disease in New Zealand there was a definite need for information on the transmissibility of both acute

and chronic forms of enzootic pneumonia. The following experiments were undertaken with a view to providing some of the information which was lacking in this area.

THE TRANSMISSION OF ACUTE PNEUMONIA BY ENDOBRONCHIAL INOCULATION

The difficulty of producing pneumonic lesions in sheep has led to the use of a variety of experimental methods by thwarted investigators. These include the inoculation of diseased lung suspensions or various micro-organisms by the intravenous, intrapulmonary, intratracheal or endobronchial routes (Chapter 1). One of the more successful of these routes appears to be the endobronchial route as described by Biberstein et al. (1967). For this reason it was used in the present study in an initial attempt to transmit pneumonia to sheep by the inoculation of either fresh homogenate of acutely pneumonic lung, P. haemolytica suspension or a suspension of a mycoplasma which was later identified as M. arginini.

Material and Methods

The experimental animals were 24 worm-free sheep aged 10 to 12 months. They had been colostrum-fed at birth and reared without contact with other sheep since the age of 1 or 2 days. At the start of the experiment 4 animals were selected randomly and killed. Their lungs were examined at necropsy and found to be free of the blemishes usually seen in sheep of this age. The remaining animals were weighed and both nasal cavities were swabbed following a thorough cleansing of the external nares with 70% alcohol. On culture only 3 animals (15%) were found to be carrying P. haemolytica in the nasal cavity. One of each of these was assigned to the first 3 groups and the remainder of the sheep were divided into 5 even groups according to body weight.

The inoculum used for groups 1 and 2 was prepared from 140 gms of fresh pneumonic lung which was obtained from a field case of acute pneumonia. It was minced with scissors and homogenised in a blender with 40 ml of nutrient broth for $\frac{1}{2}$ minute periods to avoid overheating. The homogenised mixture was then divided into 2 equal parts and homogenised further with either 60 ml of nutrient broth or 60 ml of

Eagle's medium containing 200,000 iu of penicillin and 100,000 μ g of streptomycin per 1,000 ml. The mixtures were then filtered through sieves with a mesh of 250 μ m and the supernatants collected. They were stored at 4°C until inoculation which was carried out on the same day. Immediately prior to inoculation a sample was removed for culture and a standard surface bacterial colony count performed.

A broth culture of a non-glycolytic mycoplasma later identified as M. arginini was used as the inoculum for Group 3. The organism was cultured from pneumonic sheep lung by the method of Hayflick (1965) for the detection of mycoplasmas. The mycoplasma was grown in 150 ml of PPLO broth for 48 hours prior to inoculation and a sample was obtained for a surface colony count on PPLO agar immediately before administration. The animals in Group 4 received a broth culture of P. haemolytica isolated from a sheep lung with acute pneumonia. The organism was grown in nutrient broth overnight and a surface colony count performed immediately before inoculation. The control sheep received either sterile nutrient media or autoclaved lung homogenate as shown in Table XVII.

All animals received 10 mls of inoculum endobronchially by the inoculation method previously outlined in Chapter 3. The animals were observed clinically and rectal temperatures taken twice daily until death. Two of the surviving animals in each group were killed by intravenous barbiturate 6 days after inoculation and the remainder at 10 days. The lungs were examined at necropsy, photographed and selected samples of affected tissue were fixed in 10% formol-saline. Blocks of tissue were paraffin-embedded, cut and stained routinely with haematoxylin and eosin for histological examination.

Results

Clinical Response

The 4 sheep in Group 1 all showed a marked temperature rise of approximately 2°C from 24 to 48 hours after inoculation (Appendix 3). Three of these sheep showed severe coughing and dyspnoea with harsh, moist rales audible on auscultation at 48 hours. Thereafter they

TABLE XVII

ENDOBRONCHIAL INOCULATION OF SHEEP WITH PNEUMONIC LUNG HOMOGENATE, *P. HAEMOLYTICA* OR *M. ARGININI*

Sheep No.	Inoculum	Survival time	Organisms recovered from lung
Group 1	E345 Pneumonic lung homogenate	(<i>P. haemolytica</i> 3 days	<i>P. haemolytica</i> , <i>E. coli</i>
	346	(11 x 10 ⁵ orgs. 2 days	" " <i>N. catarrhalis</i>
	347 in nutrient broth	(<i>N. catarrhalis</i> 5 days	<i>E. coli</i> , "
	348	(29 x 10 ⁵ orgs 2 days	<i>P. haemolytica</i> " <i>E. coli</i>
Group 2	349	Sacrificed 10 days	<i>S. aureus</i>
	350	" 6 days	<i>M. arginini</i>
	351 Pneumonic lung homogenate No growth	" 10 days	No growth
	352 in Eagle's medium (containing penicillin and streptomycin)	" 6 days	<i>E. coli</i> , <i>M. arginini</i>
Group 3	353	Sacrificed 10 days	<i>M. arginini</i>
	354	" 6 days	"
	355 <i>M. arginini</i> 14.6 x 10 ⁶ orgs.	" 6 days	No growth
	356	" 10 days	<i>S. aureus</i>
Group 4	357	Sacrificed 6 days	<i>M. arginini</i>
	358	" 6 days	<i>P. haemolytica</i>
	359 <i>P. haemolytica</i> 18 x 10 ⁷ orgs.	" 10 days	No growth
	360	" 10 days	<i>P. haemolytica</i>
Group 5	361 PPLO broth	Sacrificed 10 days	No growth
	362 Eagle's medium	" 6 days	<i>N. catarrhalis</i>
	363 Nutrient broth	" 10 days	<i>S. aureus</i>
	364 Autoclaved lung homogenate	" 6 days	<i>M. arginini</i>

became recumbent, developed laboured abdominal breathing and cyanosis before death at 60 to 84 hours post-inoculation. The remaining animal showed a high fluctuating temperature which reached 42°C at 96 hours. From 72 to 96 hours it coughed frequently and showed lassitude, anorexia and rapid respiration with moist rales audible on auscultation. This animal's condition deteriorated in the next 24 hours and death occurred 5 days after inoculation.

Few clinical signs were evident in the sheep from the remaining 4 groups. One animal in Group 2 (No. 349) and 2 in Group 3 (Nos. 357 and 359) showed transient temperature rises between 12 and 36 hours post-inoculation. The 2 clinically affected sheep in Group 3 also exhibited loud, harsh rales on thoracic auscultation between 48 and 72 hours and were occasionally noted to cough during this period.

Necropsy Findings

The gross lung lesions that were present at necropsy are illustrated in Figure 5.1. All the animals in Group 1 showed extensive areas of dark red consolidation in the ventral lobes. This consolidation had an irregular distribution but was located mainly in one lung and sometimes predominantly in one lobe (Figure 5.2). In 2 animals (Nos. 346 and 348) the right apical lobe was grey-brown in colour and appeared necrotic when sectioned (Figure 5.3). Both of these cases also had thick strands of fibrin covering the pleural surface of the consolidated areas and excess cloudy yellow-brown fluid was present in the thoracic cavity.

Apart from small areas of emphysema in the apical lobes no significant gross lesions were observed in the lungs from the animals in Groups 2 and 3. In Group 4, 2 of the sheep contained encapsulated necrotic areas of lung in the ventral diaphragmatic lobes. They measured up to 6 cms in diameter and contained necrotic brown haemorrhagic contents (Figure 5.4) from which P. haemolytica was recovered in one case and M. arginini in the other (Table XVII). In addition, small areas of dull red consolidation were present on the periphery of some apical lobes and one lung contained a granulomatous nodule in the left apical lobe. A similar nodule surrounded by areas of emphysema was present in

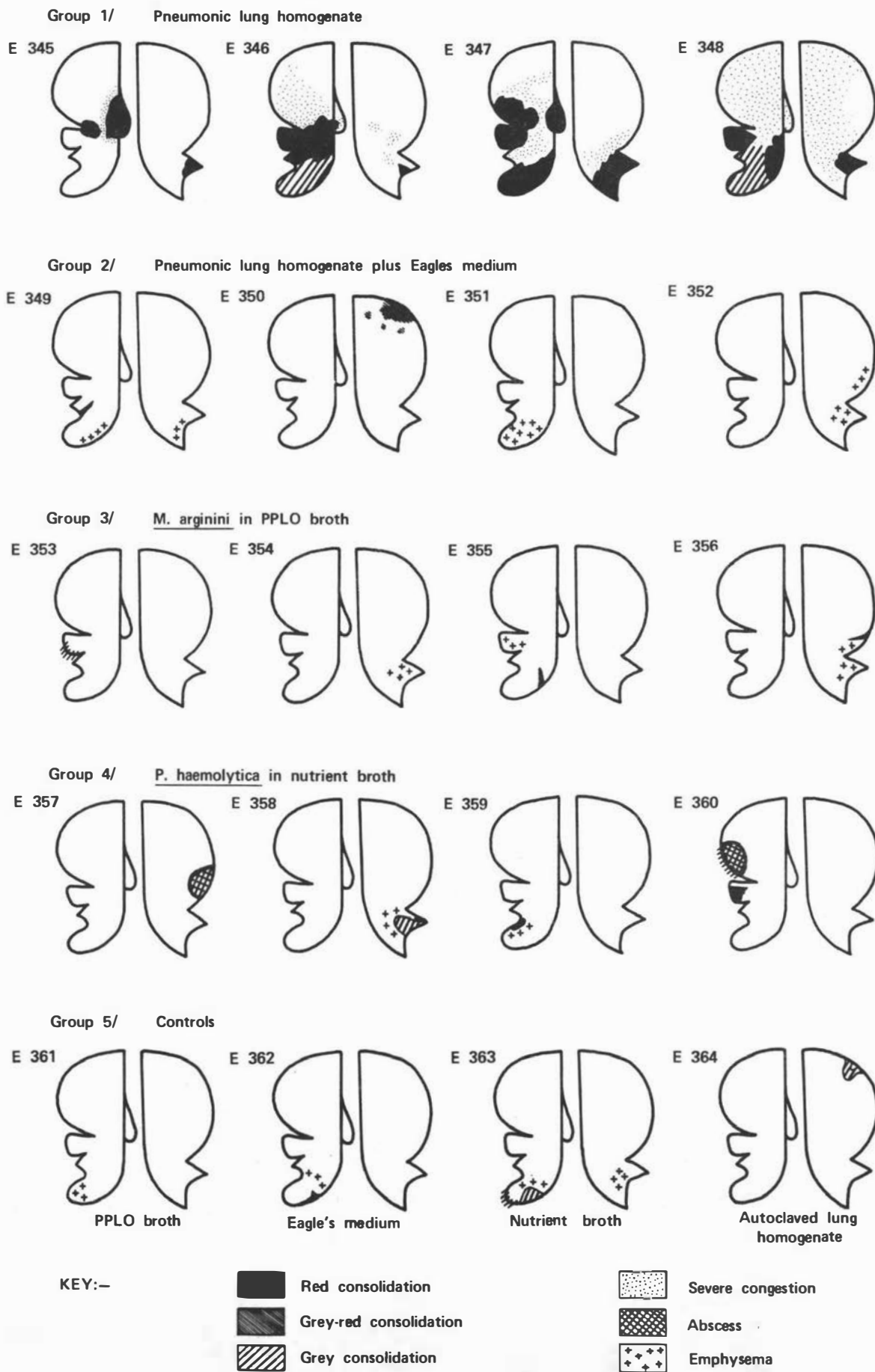


Figure 5.1. Gross lung lesions following endobronchial inoculation of pneumonic lung homogenate,

P. haemolytica or *M. arginini*

following page 152

Figure 5.2

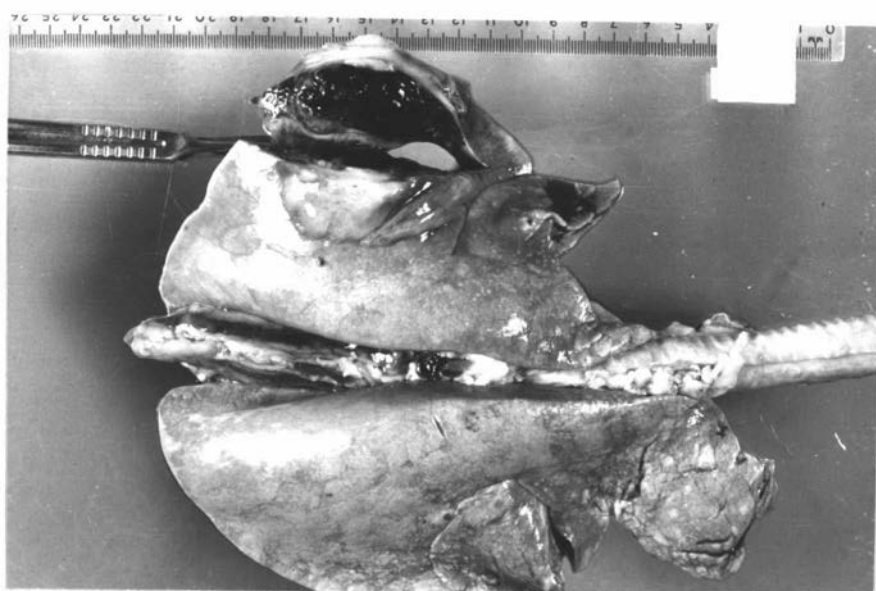
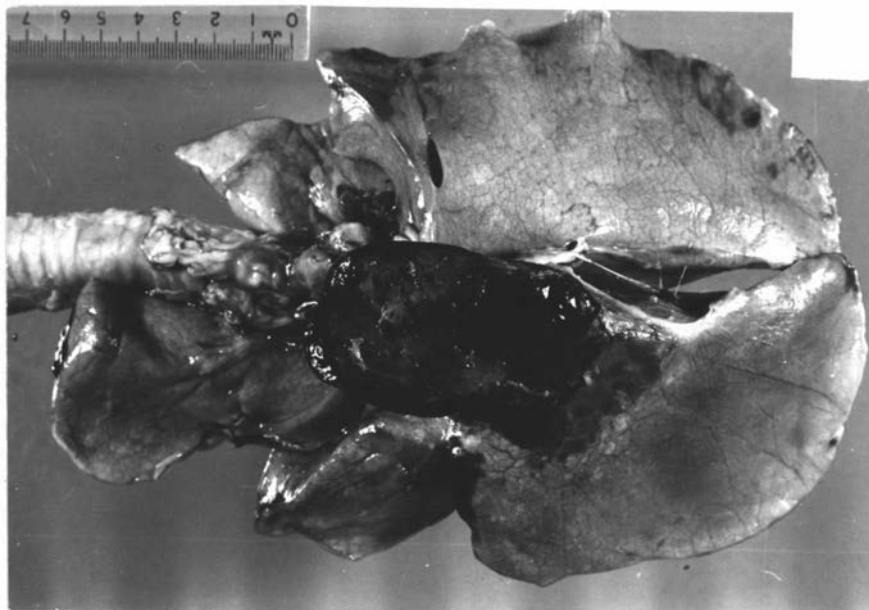
Dark red consolidation of the intermediate and ventral diaphragmatic lobe of the right lung after endobronchial inoculation with lung homogenate derived from a case of acute pneumonia. The surface of the affected area is covered with a fibrinous exudate.

Figure 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.

Figure 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.



the lungs of the control animal which received nutrient broth and a small, grey granulating area of necrosis was seen in the left diaphragmatic lobe of the animal receiving autoclaved lung homogenate.

Light Microscopy

Many of the features seen in the lungs from the animals in Group 1 were typical of the histological appearance of naturally-occurring acute enzootic pneumonia. These features included severe congestion, haemorrhage and oedema with fibrin exudation into some alveolar spaces and dense cellular exudate, often containing colonies of bacteria in other areas. In addition, the lung parenchyma often contained extensive areas of necrosis which were sharply demarcated from the surrounding tissue by zones of infiltrating leucocytes. However, there was considerable variation in both the components and degree of cellular exudate within different parts of each lung. In some areas macrophages, neutrophils, erythrocytes, fibrin and desquamated alveolar epithelium were mixed together to form a necrotic exudate. Often the most peripheral cells contributing to this exudate took on an elongated appearance similar to that seen in "streaming" of cellular exudate in the naturally-occurring disease. In other areas where there was extensive necrosis of whole lobules with thrombosis of blood vessels at the periphery of the lesions, the cellular exudate in the adjacent tissue was primarily neutrophilic in character. The intensity of neutrophil infiltration in these areas was usually far greater than that normally encountered in naturally-occurring cases of acute pneumonia.

In the animals receiving pneumonic lung homogenised in Eagle's medium with antibiotic there was a slight thickening of the alveolar septa adjacent to bronchioles with mononuclear cells or fibrous tissue. There was no histological evidence of lung injury in the animals receiving broth containing M. arginini apart from a small area of granulating pleuritis in one animal (No. 353). The microscopic structure of the abscesses in the 2 animals receiving P. haemolytica broth was similar in both cases. They contained an extensive central area of coagulative necrosis and haemorrhage within which the alveolar structure was still discernable. This was margined by a thick zone of necrotic leucocytes surrounded by a more uneven zone of infiltrating

plasma cells, macrophages and neutrophils. The whole lesion was bounded by a wide zone of granulation tissue diffusely infiltrated with neutrophils. The lungs of the remaining 2 animals receiving P. haemolytica broth showed focal interstitial fibrosis and peribronchial lymphoid hyperplasia.

With the exception of focal alveolar emphysema no histological lesions were seen in the control animals receiving Eagle's medium or PPLO broth. In the animal receiving nutrient broth the lungs contained an area of granulating pleuritis beneath which the alveolar and perivascular interstitial tissue was thickened and alveolar spaces contained moderate numbers of macrophages. The lungs of the animal receiving the autoclaved homogenate of pneumonic lung contained a well defined lobule in which groups of alveolar spaces were filled with a homogenous pink coagulum surrounded by large macrophages and occasional giant cells. The intervening interstitial tissue was thickened and bronchioles in the area showed moderate epithelial hyperplasia. It was assumed that the pink coagulum represented autoclaved inoculum which had become sequestered in alveolar spaces.

SERIAL TRANSMISSION OF CHRONIC PNEUMONIA BY INTRANASAL AEROSOL INOCULATION

The majority of work on the aetiology and transmission of enzootic pneumonia in sheep has until the present time been concentrated on the acute form of the disease and relatively little attention has been paid to the subacute and chronic forms. Recent work in Australia by St. George et al. (1971) and Sullivan et al. (1973a, b) on the transmission of chronic pneumonia in sheep in Queensland has produced favourable results and highlighted the need for similar studies in New Zealand.

As no previous attempts to transmit chronic pneumonia have been reported in this country the following method of aerosol inoculation of pneumonic lung homogenate was developed and tested in lambs in a serial transmission experiment.

Material and Methods

Animals

To find animals most likely to be susceptible to experimental transmission, 8 Romney lambs were chosen from a healthy flock during mid-January, a time of the year when pneumonia was not normally observed in the district. Lateral and antero-posterior radiographs of the thorax of all animals were taken and examined for evidence of lung consolidation. Two of the animals were replaced because slightly suspicious radiographic lesions were observed. Lambs for the second, third and fourth passage were selected from the same flock by the same radiographic procedure. The ewe used for the fourth passage was a healthy non-pregnant ewe aged 6 years. During each passage the experimental animals were housed together in pens until euthanasia.

Preparation of Inoculum

The pneumonic lung homogenate was prepared from 6 naturally-occurring cases of chronic pneumonia in 6-month-old lambs obtained from a local meatworks. The lungs were selected to cover a range of lesions from early dull red peripheral areas of consolidation to severe grey-red consolidation involving several lobes. Portions of pneumonic tissue from each lung were minced and homogenised in nutrient broth as described in the previous section. The homogenate was filtered through 250 μ m sieves and the filtrate was stored at 4°C until required. Inoculation was carried out approximately 6 hours after collection of the pneumonic lung.

The inoculum for the second, third and fourth passages was derived from portions of each of the pneumonic lungs of the animals used in the previous passage. In each case the inoculum was prepared in a similar manner to that used initially and administered on the same day as the animals in the previous passage were sacrificed.

Method of Inoculation

The nozzle of a nebulizing gun⁽¹⁾ was slightly modified by the

(1) "Trigger Teejet", Spraying Systems Co.

addition of a 6 mm diameter metal flange so that it would fit into the external nares of a lamb (Figure 5.5.) The gun was driven by a small air compressor delivering air at approximately 100 kilopascals. The animals were held in a vertical sitting position leaning slightly forward and the nozzle was inserted into one nostril. The aerosol was delivered while the forefingers or thumb were held over the other nostril for short periods of 2 to 4 seconds which coincided with the animals inspiration (Figure 5.6). The aerosol was then stopped for 3 to 5 seconds and the animal allowed to exhale normally. This procedure was repeated constantly for 2 to 3 minutes until 15 to 20 mls of inoculum had been used. Initial trials with this technique on 2 lambs using an inoculum of India ink in normal saline followed by slaughter within 5 minutes of administration showed that the majority of deposition occurred in the nasal cavity. However, a variable but reasonably even distribution of small quantities of ink was also found in the ventral areas of both lungs (Figure 5.7).

After inoculation the animals were observed clinically once daily, rectal temperatures were recorded and lung sounds auscultated. Lateral and antero-posterior radiographs of the thorax of all animals were taken again immediately prior to death. Euthanasia was carried out at 14 or 28 days (Table XVII) by intravenous barbiturate and the lungs were examined grossly, photographed and blocks of affected tissue were fixed in 10% formol-saline or Karnovsky's fixative within 15 minutes of death. Sections for light and electron microscopy were prepared using the same techniques as described previously (Chapter 2).

At the conclusion of the experiment the flock of lambs from which the experimental animals were derived were slaughtered at a local meatworks. The lungs from these animals were collected and brought to the laboratory for examination. The gross lesions present at the time of examination were recorded as slight, when there was consolidation in part of 1 or 2 anterior lobes; moderate, when the consolidation involved the whole of 1 or 2 anterior lobes; or severe, when consolidation involved 3 or more lobes.

following page 156

Figure 5.5

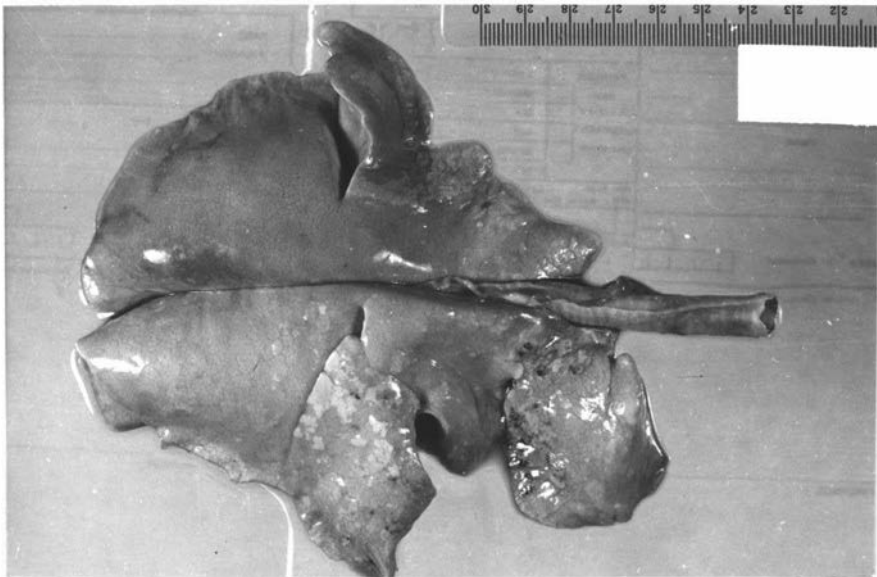
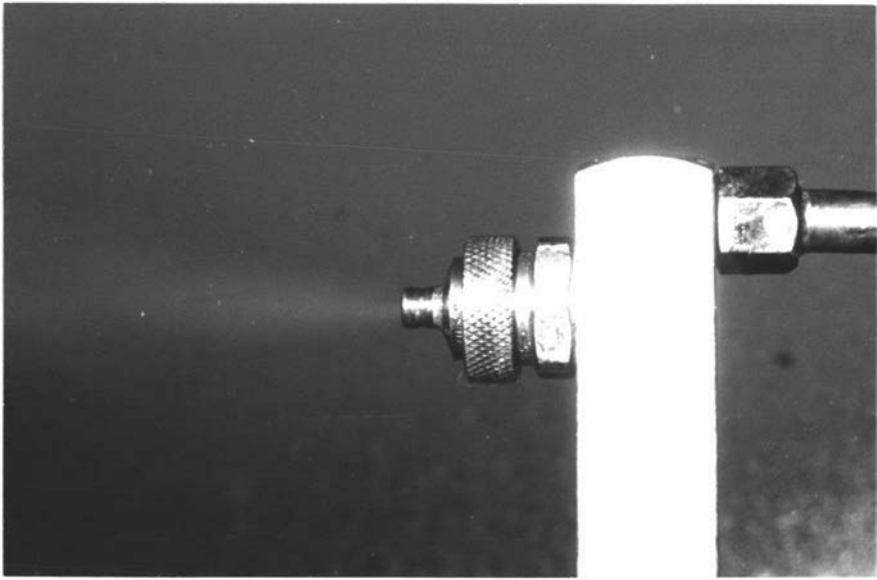
Nozzle of nebulizing gun used for intranasal inoculation.

Figure 5.6

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

Figure 5.7

The deposition of small quantities of ink in the antero-ventral parts of the lungs of a lamb following the trial of the intranasal aerosol inoculation technique.



Results

The clinical signs observed following inoculation and the gross lung lesions present at necropsy are summarised in Table XVIII. Nine of the animals showed clinical evidence of pneumonia as indicated by coughing, temperature rise of several days duration or weight loss. Abnormal lung sounds were audible on auscultation of the thorax in 4 of the animals (Nos. 26, 32, 65 and 108). They consisted mainly of persistent muffled bronchial tones in the anteroventral parts of the thorax together with the variable occurrence of moist rales on expiration. In addition several animals showed a muco-purulent nasal discharge and sneezing for several days after inoculation. Lateral and antero-posterior radiographs taken again immediately before euthanasia revealed areas of increased opacity in the anterior or ventral thoracic cavity in 8 of the 14 animals examined. The area most consistently affected lay between the heart shadow and the thoracic inlet (Figures 5.8 and 5.9).

At necropsy, 10 of the 14 animals showed prominent areas of consolidation in either the apical, cardiac or ventral diaphragmatic lobes of the lungs. These were macroscopically indistinguishable from naturally-occurring cases of chronic enzootic pneumonia. A further 2 animals showed mild dull red areas of consolidation up to 3 cms in width in the apical lobes. No pneumonic lesions were visible in one lamb killed at 28 days or in the ewe killed at 14 days.

Microscopically, the majority of lesions fell within the range of pathological changes previously described in naturally-occurring chronic pneumonia. Some degree of alveolar collapse was seen in all cases and moderate alveolar interstitial thickening with mononuclear cells was seen in many peribronchial areas. Alveolar macrophage infiltration was usually less severe. In 2 cases however, (Nos. 32 and 66) there were very severe neutrophil accumulations together with the exudation of small quantities of proteinaceous fluid into surrounding alveolar spaces. Proliferative changes including bronchiolar epithelial hyperplasia were seen in the majority of cases but were most marked in one animal (No. 54) in which there was extensive peribronchiolar fibrosis, bronchiolar epithelial hyperplasia and alveolar epithelialization.

TABLE XVIII

CLINICAL SIGNS AND GROSS LUNG LESIONS FOLLOWING SERIAL TRANSMISSION OF CHRONIC PNEUMONIA

Sheep No.	Age	No. of days from Inoculation until Killed	Clinical Evidence of Pneumonia	Severity of Lesions	Type of Consolidation	Bacteria Recovered from Lung
1st Passage						
E26	4 months	14 days	Coughing, temp.rise.	+++	Red-grey	<u>P.haemolytica</u> , <u>E.coli</u> , <u>N.catarrhalis</u>
27	"	"	Nil.	++	"	" "
28	"	"	Temp. rise	++	Dull red	" <u>E.coli</u> , <u>S.aureus</u>
29	"	"	Nil	++	Grey-red	" "
32	4 months	28 days	Weight loss, coughing, temp.rise	++++	Grey-red	<u>P.haemolytica</u> , <u>N.catarrhalis</u> , <u>C.pyogenes</u>
54	"	"	Nasal discharge, " "	++++	"	" <u>E.coli</u> , <u>S.aureus</u>
55	"	"	"	-	-	No growth
56	"	"	Coughing	+	Dull red	<u>E. coli</u>
2nd Passage						
E65	5 months	14 days	Coughing, sneezing, temp.rise	+++	Red-grey	<u>P.haemolytica</u> , <u>N.catarrhalis</u> , <u>Strep.</u>
66	"	"	" " "	++++	"	" "
3rd Passage						
E75	5½ months	14 days	Weight loss, coughing, temp.rise	++++	Grey-red	<u>P.haemolytica</u> , <u>C.pyogenes</u>
76	"	"	Nil	+	Dull red	No growth
4th Passage						
E108	6 months	14 days	Coughing, temp.rise	+++	Red-grey	<u>E.coli</u> , <u>C.pyogenes</u>
109	6 years	"	Nil	-	-	No growth

++++ = Consolidation in 4 or more lobes
 +++ = " 3 anterior lobes
 ++ = " 2 " "
 + = " 1 " "

Figure 5.8

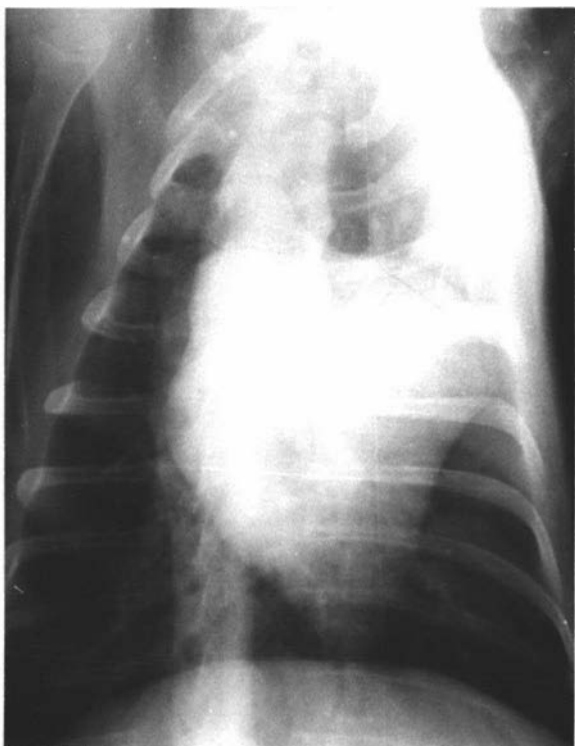
Lateral radiographs of the thorax of sheep No. E65 -

- (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.

Figure 5.9

Antero-posterior radiographs of the thorax of sheep No. E66 -

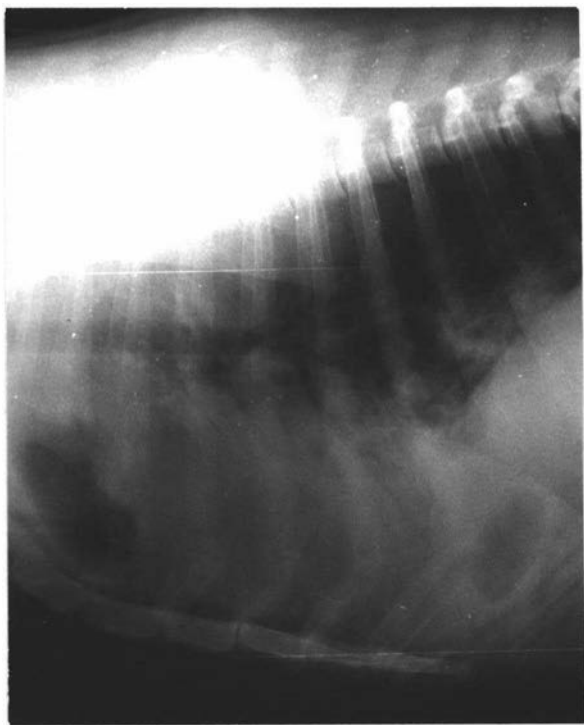
- (a) Before inoculation
- (b) Fourteen days after inoculation. There is an increase in opacity between the heart shadow and the thoracic inlet.



a



b



a



b

The lungs from 4 animals (Nos. 26, 27, 32 and 54) were examined ultrastructurally and the changes observed did not differ significantly from those encountered previously in the naturally-occurring disease.

Gross examination of the lungs of animals in the flock from which the experimental lambs were derived was made at the conclusion of the experiment. Although some pneumonia was present the prevalence of the disease in the residual flock was low and the difference between the 2 groups as shown in Table XIX was highly significant.

THE DEVELOPMENT OF CHRONIC PNEUMONIA: SEQUENTIAL SLAUGHTER FOLLOWING INTRANASAL INOCULATION

Attempts to transmit chronic pneumonia by the technique described in the previous section succeeded in producing lesions in a high proportion of the animals inoculated. In the above experiment animals were sacrificed at either 14 or 28 days after inoculation so there was no opportunity to observe the development of lesions in the immediate post-inoculation period. For this reason the initial transmission studies were extended by the inoculation of a further 12 animals followed by their sequential slaughter at 2 day intervals after inoculation.

Material and Methods

The study was carried out on 7-month-old Romney lambs during February 1974. Sixteen animals were selected randomly from a mob of 28 healthy animals and sent for slaughter at a local meatworks. Their lungs were then collected and examined grossly. Apart from mild lesions associated with Dictyocaulus filaria or Mullerius capillaris and occasional lines of collapse in a small number of animals no pneumonic lesions were found.

The remaining 12 animals were then inoculated with an intranasal aerosol of homogenised pneumonic lung prepared from a range of fresh abattoir cases of chronic pneumonia. The method of preparation and administration was the same as that described in the previous section and inoculation of the experimental group was accomplished within 8 hours of the collection of the pneumonic lung.

TABLE XIX
THE OCCURRENCE OF PNEUMONIC LESIONS IN TRANSMISSION
AND CONTROL GROUPS FOLLOWING SERIAL TRANSMISSION
OF CHRONIC PNEUMONIA

Gross Lesions	Transmission Group	Control Group	Total
Negative	1	28	29
Slight	2	6	8
Moderate	3	5	8
Severe	7	5	12
Total	13	44	57

$$\chi^2 = 15.47, p < 0.01$$

The experimental animals were run together in a small paddock separated by fences from other livestock. Two animals were chosen randomly for euthanasia at 2 day intervals after inoculation. They were killed with intravenous barbiturate, their lungs were examined grossly and then photographed. Blocks of affected tissue were fixed in 10% formol-saline, paraffin embedded and sections stained with haematoxylin and eosin for histological examination.

Results

Necropsy Findings

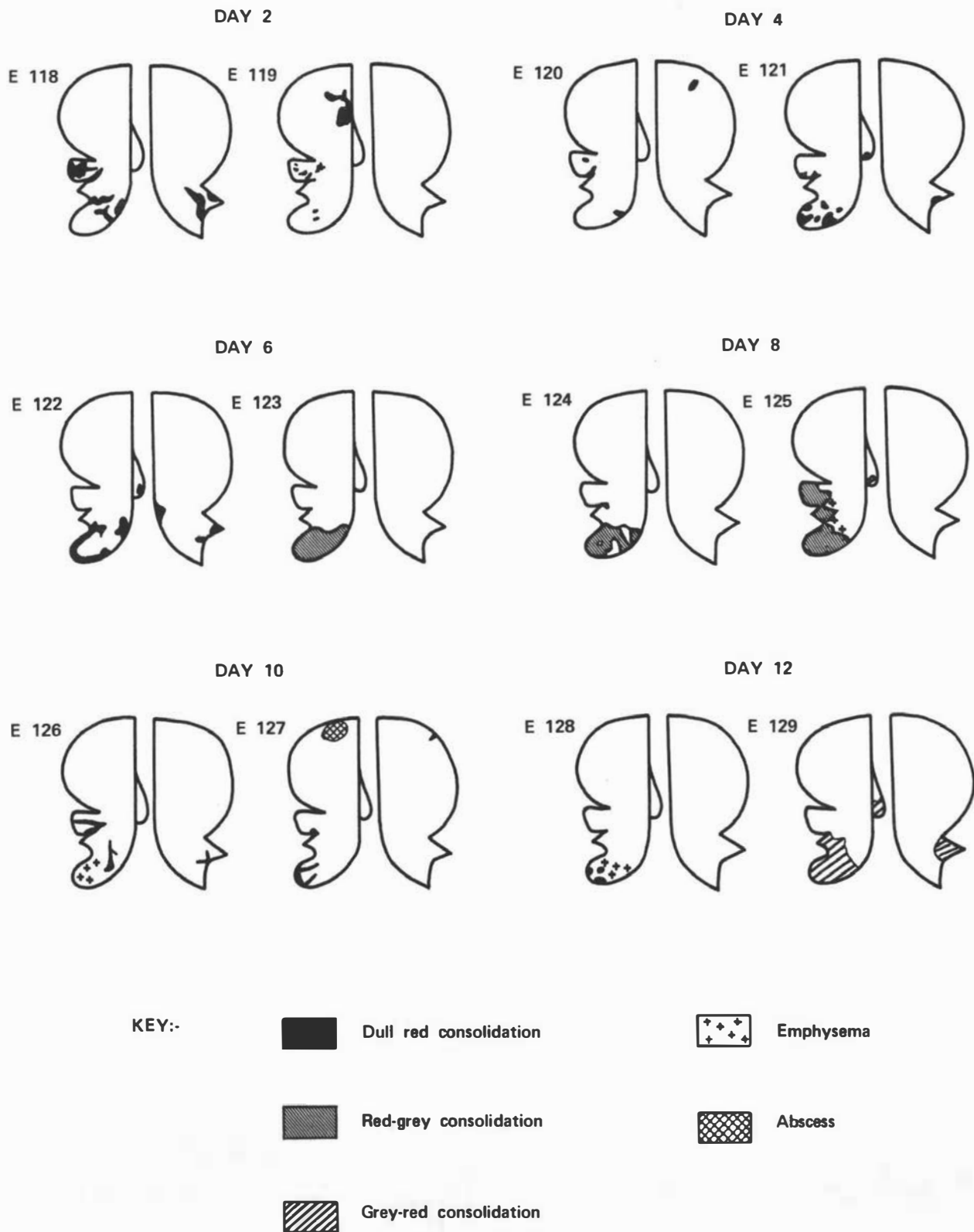
The major gross lesions present in the lungs at necropsy are illustrated in Figure 5.10. Peripheral areas of dull red collapse were visible as early as 2 days after inoculation. In one case several dull red, 3 x 10 mm foci could be seen on the surface and extending into the substance of the right apical and cardiac lobes (Figure 5.11). Similar irregular focal lesions were found in the lungs of the animals killed at 4 days. On the 6th day after inoculation, a 1 to 1.5 cm wide zone of dull red consolidation which was sunken below the surface of the adjacent lung was visible along the margins of the apical lobes in one lamb while the other lamb showed consolidation of the whole anterior part of the right apical lobe.

At 8 days the lungs of both animals contained red-grey areas of consolidation in the anteroventral portions of the right lung (Figure 5.12). The consolidated tissue was moderately firm and slightly turgid although it was level with the surface of the surrounding lung in which small areas of compensatory emphysema were present. The animals sacrificed at day 10 showed only linear bands of collapse in the apical lobes. However, in the lung of one of the animals there were extensive areas of alveolar emphysema in the right apical lobe and the other animal contained a well-encapsulated abscess in the right diaphragmatic lobe. The abscess was 3 cm in diameter and contained red-brown haemorrhagic contents.

The most extensive lesions were seen in one of the lambs killed on day 12. They consisted of areas of grey-red consolidation involving

Figure 5.10

Gross lung lesions obtained at sequential slaughter following the intanasal transmission of chronic pneumonia



following page 161

Figure 5.11

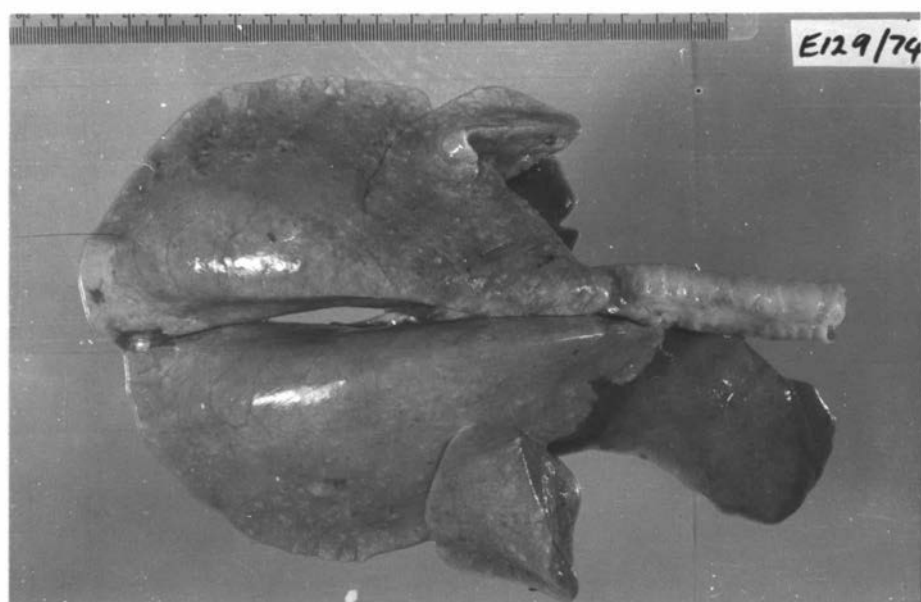
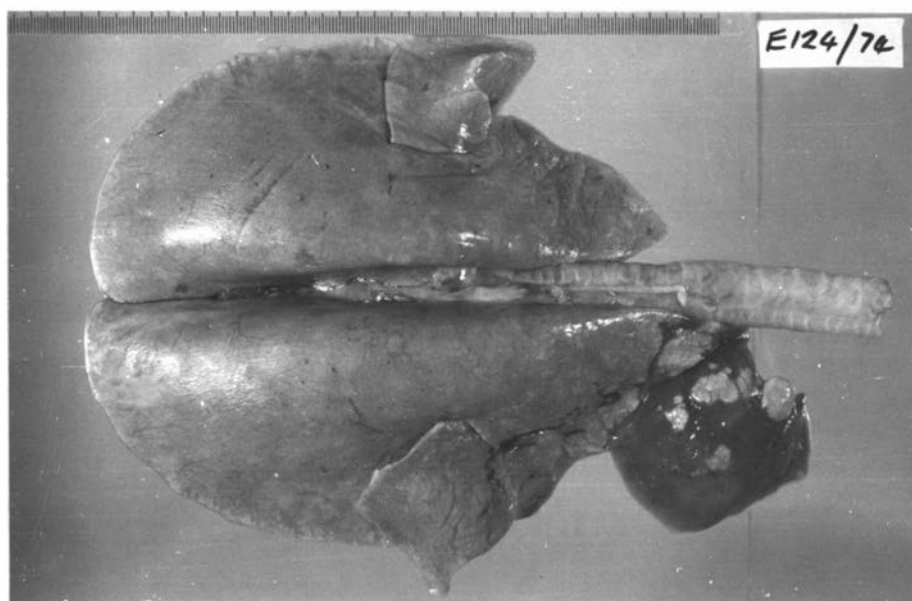
Two days after inoculation with lung homogenate derived from chronic pneumonia. Small dull red depressed foci are visible on the surface of the right apical and cardiac lobes.

Figure 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.

Figure 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.



all of the right apical lobe and ventral portions of the intermediate and left cardiac lobes (Figure 5.13). In the lungs of the other lamb only small foci of peripheral consolidation together with moderately severe alveolar emphysema were found in the right apical lobe.

Light Microscopy

2 to 4 days. The earliest changes seen were focal areas of alveolar collapse around bronchioles. These were most extensive in lobules located towards the periphery of the lung. At 2 days the alveolar walls in the collapsed areas appeared slightly thickened and congested and small numbers of alveolar macrophages were often present in alveolar ducts. In some collapsed areas the bronchiolar epithelium was mildly hyperplastic and small numbers of neutrophils and proteinaceous debris could be seen within the lumen of many terminal airways. At 4 days the changes were similar but slightly more extensive (Figure 5.14). Clumps of neutrophils and small quantities of fibrin were found in some alveolar ducts as well as within the lumen of bronchioles. The collapsed alveolar septa contained small numbers of infiltrating mononuclear cells.

6 to 8 days. By 6 days after inoculation the cellular response in one animal (E123) was severe. Large numbers of neutrophils filled alveolar spaces and bronchioles in many areas and lesser numbers of macrophages were also present. A fibrinoid pink coagulum, more dense than that usually seen in naturally-occurring cases of chronic pneumonia, was found surrounded by macrophages in some alveolar ducts. There was early type II alveolar cell hyperplasia and moderate bronchiolar epithelial hyperplasia with an increase in numbers of mucous cells in the larger bronchioles. In several bronchioles epithelial proliferation extended partially over the surface of fibrinoid material which was adherent to the bronchiolar wall and mildly infiltrated with macrophages (Figure 5.15). In the other animal (E122) neutrophil exudation was less marked but similar focal proliferative changes were seen in the alveolar and bronchiolar epithelium. Interstitial thickening with mononuclear cells was severe in some areas and early accumulations of lymphoid cells were seen in peribronchiolar interstitial areas. At 8 days cellular

Figure 5.14

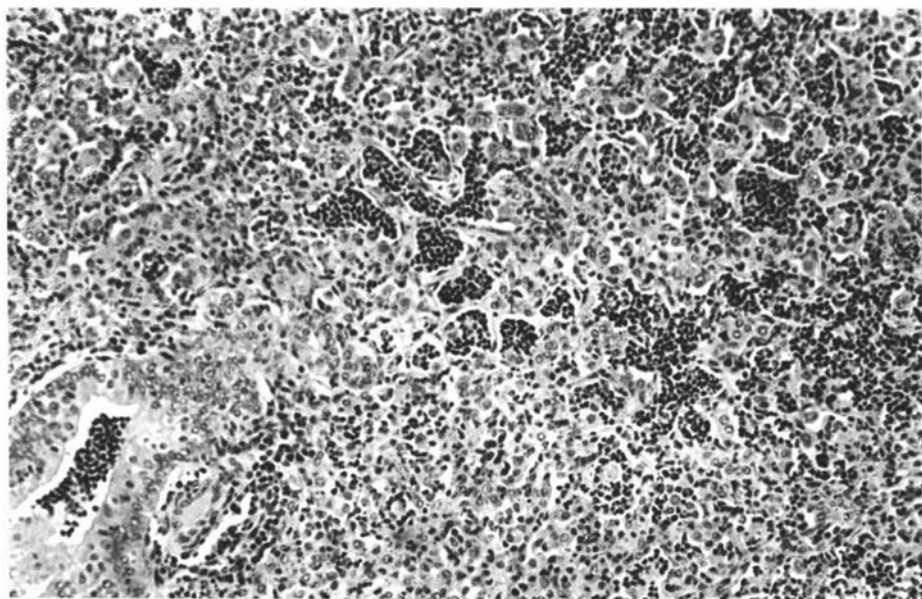
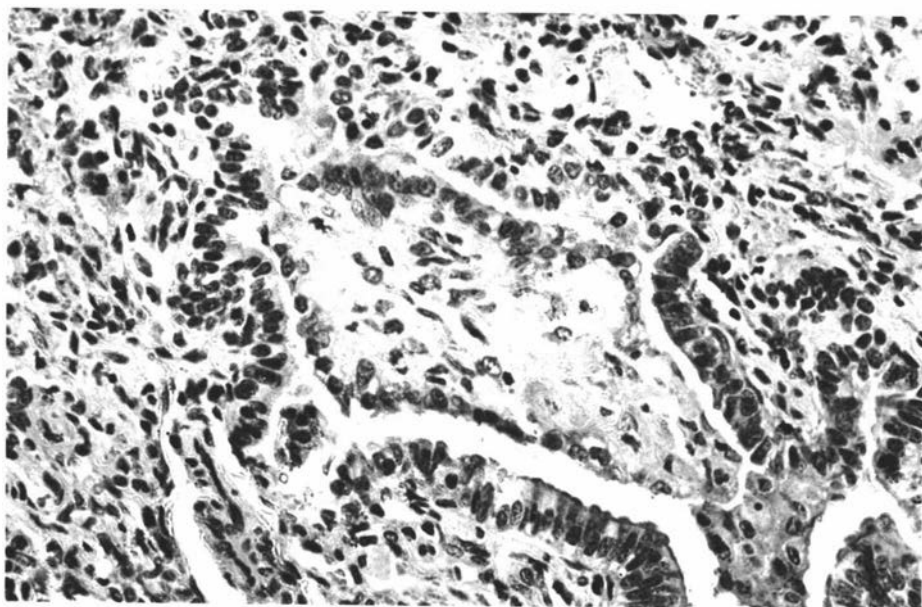
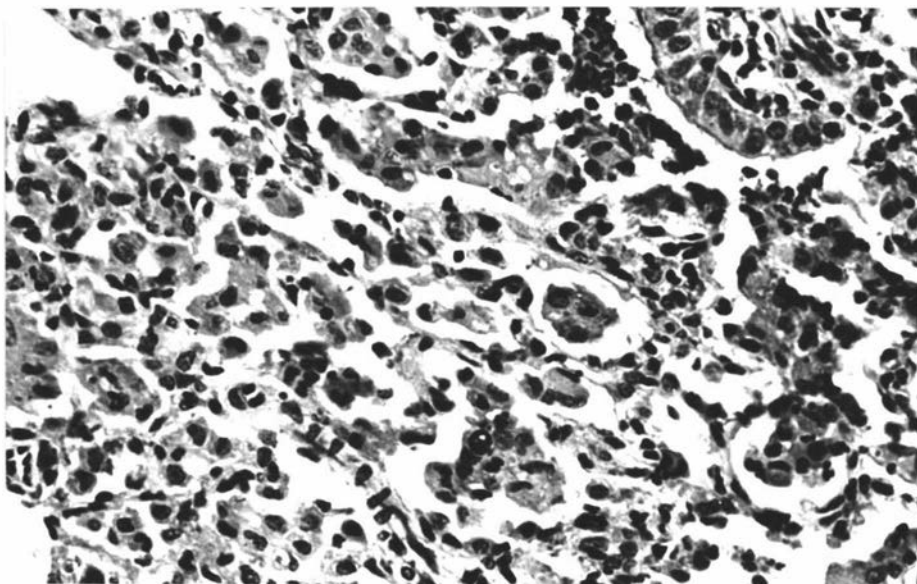
Small numbers of neutrophils mixed with necrotic debris in a terminal bronchiole 4 days after inoculation with homogenate derived from chronic pneumonia. Macrophages are accumulating in surrounding alveolar spaces. HE x 250.

Figure 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.

Figure 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.



infiltration continued to be a major feature of the pathological response in both animals examined. However, bronchiolar epithelial hyperplasia and alveolar type II cell hyperplasia were becoming increasingly prominent. In addition, the smooth muscle tissue around alveolar ducts in one animal (E125) appeared mildly hypertrophied and the peribronchial lymphoid aggregations were now well defined.

10 to 12 days. The animals killed at day 10 showed only mild evidence of alveolar damage as indicated by focal areas of interstitial thickening around some bronchioles. In one animal however (E126), there was moderately severe bronchiolar epithelial hyperplasia and well-developed peribronchiolar lymphoid hyperplasia. Extensive pneumonic lesions of a similar type to those seen earlier were found in one of animals killed at 12 days (E129). The main differences were in the greater accumulation of macrophages in alveolar spaces at this stage although focal infiltrations of neutrophils were still a common finding (Figure 5.16). Proliferative changes were also more prominent and both bronchiolar and alveolar epithelial hyperplasia were widespread but there was no evidence of interstitial fibrosis. Moderately severe peribronchial lymphoid hyperplasia was seen both in this case and in the second animal killed at 12 days. In the other animal however (E128), the only evidence of pulmonary damage was focal alveolar emphysema and thickening of alveolar septa surrounding bronchioles.

THE EFFECT OF CHEMOTHERAPEUTIC AGENTS ON THE TRANSMISSION OF CHRONIC PNEUMONIA

The microbiological data collected in Chapter 4 indicate that both mycoplasmas and bacteria are closely associated with the lesions of chronic pneumonia of sheep in New Zealand. In order to obtain further information on the relative importance of these two classes of micro-organisms in the pathogenesis of pneumonia, the following transmission studies were initiated. Various chemotherapeutic agents with a known specific action against certain groups of micro-organisms were administered to sheep and their efficacy in preventing the development

of chronic pneumonia following intranasal inoculation with homogenised pneumonic lung was observed at necropsy.

Material and Methods

Animals

Forty-eight healthy lambs were selected from a flock of 4 to 5-month-old Romneys early in January 1974. They were divided into 8 even groups on the basis of body weight and 42 animals (Groups 1 to 7) were run together on pasture as one flock. The remaining 6 (Group 8) were kept in a different paddock separated by 2 fences from other livestock. Before inoculation rectal temperatures were taken and blood was collected from all animals. All the animals in Group 7 were radiographed and on examination of the plates no evidence of pneumonia could be detected.

Method of Inoculation

The inoculum was prepared from pneumonic tissue collected from a range of 8 lungs which had severe lesions of subacute or chronic pneumonia. They were obtained from a local meatworks and the inoculum was prepared on the day of collection by essentially the same method as described previously. One hundred millilitres of the pooled inoculum were then autoclaved and re-homogenised before administration to Group 8. The fresh homogenate was administered to the remaining animals approximately 6 hours after collection by the intranasal aerosol method described earlier.

Drug Administration

At the time of inoculation the animals in Groups 1 to 6 were treated with various drugs as shown in Table XX. The first 3 groups received ronidazole⁽¹⁾ orally at 10 mg/Kg, 30 mg/Kg and 100 mg/Kg as a 10 ml fluid suspension administered by means of a McMaster drenching gun. The animals in groups 4, 5 and 6 received the antibiotics tylosin,⁽²⁾

(1) "Ridzol-S" Merck, Sharp and Dohme (N.Z.) Ltd.

(2) "Tylan 200" Elanco Products Ltd.

TABLE XX

DOSE, TYPE AND ROUTE OF ADMINISTRATION OF CHEMOTHERAPEUTIC
AGENTS FOLLOWING INOCULATION

Group	No. of Animals	Inoculum	Drug	Route
1	6	Fresh lung homogenate	Ronidazole 100 mg/Kg	Per os
2	6	Fresh lung homogenate	Ronidazole 30 mg/Kg	Per os
3	6	Fresh lung homogenate	Ronidazole 10 mg/Kg	Per os
4	6	Fresh lung homogenate	Tylosin 8 mg/Kg	I/M
5	6	Fresh lung homogenate	Tetracycline 5 mg/Kg	Sub/cut.
6	6	Fresh lung homogenate	Penicillin 30,000 iu/Kg	I/M
7	6	Fresh lung homogenate	Positive control	-
8	6	Autoclaved lung homogenate	Negative control	-

oxytetracycline⁽³⁾ or penicillin⁽⁴⁾ administered parentally as shown in Table XX. Thereafter the drugs were administered once daily until day 12. On each occasion the sheep were yarded, rectal temperatures taken and lung sounds auscultated before administration. No treatment was given to the animals in Groups 7 or 8. The sheep in Group 8 were always handled separately and had no contact with the other animals.

On the 14th day after inoculation all the animals were weighed, blood samples were collected and the animals were killed by means of intravenous barbiturate. At necropsy, the type and distribution of gross lung lesions were recorded and the lungs photographed. The lungs of all animals were weighed and samples of affected tissue were collected for microbiological and histological examination. When no gross lesions were present, samples of tissue from the right apical lobe were collected for examination. The microbiological methods used were the same as those described previously (Chapter 4) and the histological methods consisted of routine paraffin embedding and staining with haematoxylin and eosin.

Results

Clinical Response

A rise in mean body temperature of $\frac{1}{2}$ to 1°C was seen in all groups with the exception of the negative controls (Group 8), during the first 4 days following inoculation (Appendix 4). Mean body temperatures in Groups 1 (Ronidazole 100 mg/Kg), 3 (Ronidazole 10 mg/Kg), 4 (Tylosin) and 7 (Positive controls) remained slightly elevated until day 11. However, it is possible that hot weather experienced by all these unshorn sheep from the 8th to 11th days may have contributed to the elevated body temperatures during this period.

The clinical signs noted during driving, yarding and on auscultation of the thorax are summarised in Table XXI. In retrospect, it was

(3) "Terramycin Q" Pfizer Ltd.

(4) "Mylipen", Glaxo Laboratories (N.Z.) Ltd.

TABLE XXI

CLINICAL SIGNS FOLLOWING TRANSMISSION OF CHRONIC PNEUMONIA AND ADMINISTRATION OF CHEMOTHERAPEUTIC AGENTS

Sheep No.		Coughing		Nasal	Muffled	Lung Sounds		Moist Rales
		Persistent	Occasional	Discharge		Harsh	Bronchial Tones	
Group 1	E 3		Day 2-3					
Group 2	9		Day 2-5				Day 4-8	
	13	Day 3-14		Day 2-10			Day 8-13	
	15			Day 2-6				
Group 3	17	Day 4-14			Day 10-14		Day 7-9	Day 10-14
	19		Day 1-5					Day 11-12
	20		Day 8-14	Day 4-6				
Group 4	24						Day 4, 8-10	
	26		Day 2-14					
Group 5	27		Day 2-5					
	29		Day 2-7				Day 8, 10-11	
	33		Day 3-6					
Group 6	36			Day 2-5			Day 4, 7-11	
	38		Day 8-14					
Group 7	39						Day 10-11	
	40	Day 8-14		Day 4-10	Day 12-14		Day 5-10	Day 13
	42						Day 10-12	
	43	Day 7-14						Day 11-14
Group 8	50		Day 10-12					

found that the signs which correlated most consistently with the presence of pneumonic lesions at necropsy were:-

- (i) Persistent coughing during exercise.
- (ii) Muffled bronchial tones in the anteroventral thorax.
- (iii) Moist rales audible on inspiration.

A slower than normal respiratory rate was observed in all sheep in Group 1 (Ronidazole 100 mg/Kg) from approximately day 9 until necropsy. During the last week, all sheep in this group noticeably lost weight, became slightly depressed and tended to lag behind the mob when being driven. On day 14 a mean weight loss of 1.67 Kg was recorded for this group whereas the sheep in the remaining groups made mean weight gains of between 1.20 and 3.94 Kg (Table XXII).

Necropsy Findings

Figure 5.17 illustrates the main gross lesions present in the lungs at necropsy. Groups 1 (Ronidazole 100 mg/Kg), 4 (Tylosin) and 8 (Negative controls) were free of lesions in the anterior parts of the lungs. A well-encapsulated abscess (approximately 4 cm in diameter) was present in the left diaphragmatic lobe of bone lamb in the tetracycline-treated group (Group 5) which was otherwise normal.

Extensive areas of consolidation in the anterior lobes were found in 1 lamb in Group 2 (Ronidazole 30 mg/Kg), 2 lambs in Group 3 (Ronidazole 10 mg/Kg) and 2 lambs in Group 7 (Positive controls). In addition, smaller focal areas of consolidation and/or linear collapse were present in 3 lambs in Group 3 (Ronidazole 10 mg/Kg), 1 lamb in Group 6 (Penicillin) and 1 lamb in Group 7 (Positive controls).

Five of the 6 animals in Group 1 (Ronidazole 100 mg/Kg) showed mild ascites as indicated by the presence of 30-50 ml of serous fluid in the peritoneal cavity. In all these animals, the kidneys appeared slightly pale and swollen and in 3 of the 6 lambs the liver was slightly paler than normal.

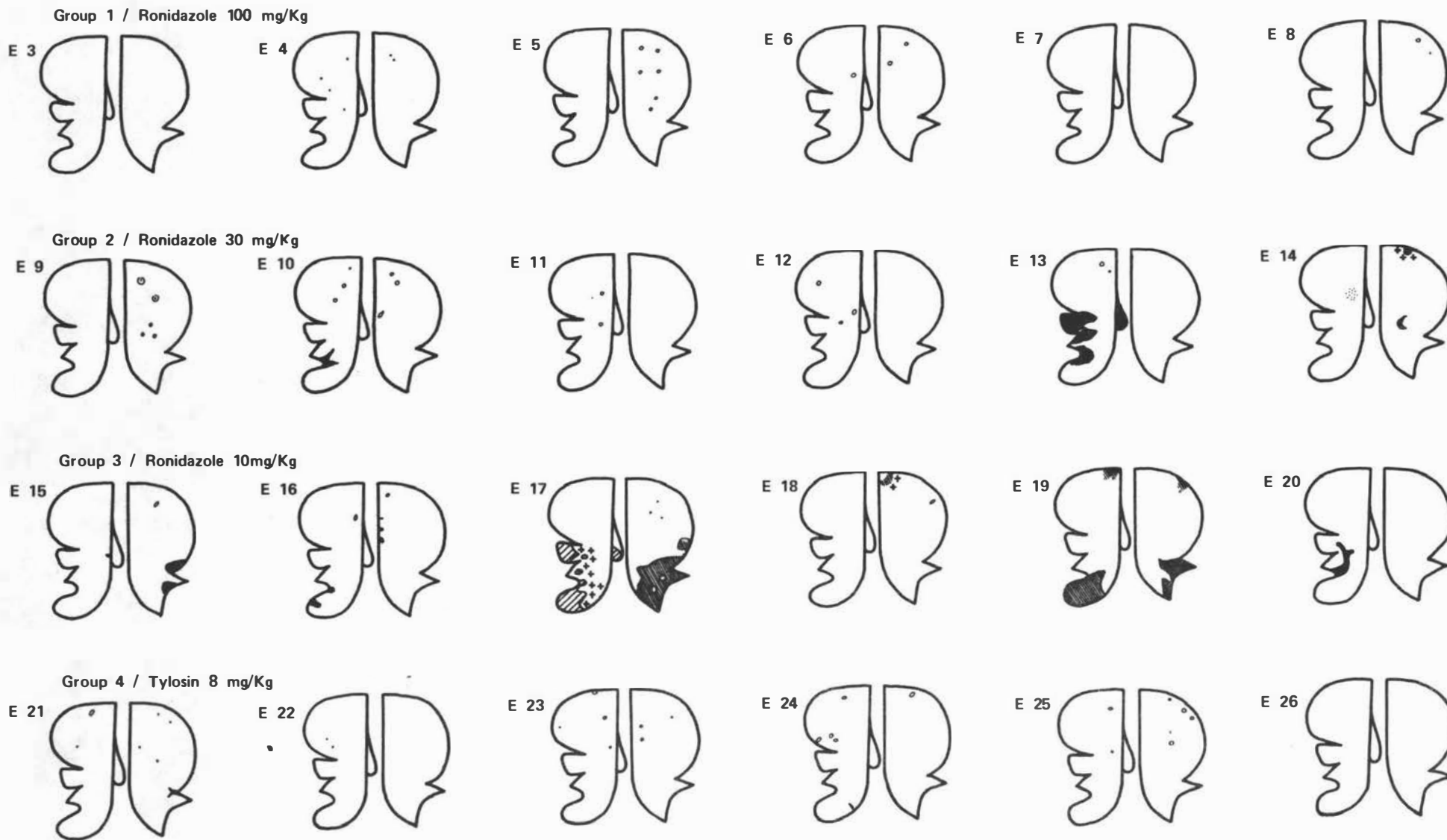


Figure 5.17 Gross lung lesions following the intranasal transmission of chronic pneumonia and administration of various chemotherapeutic agents

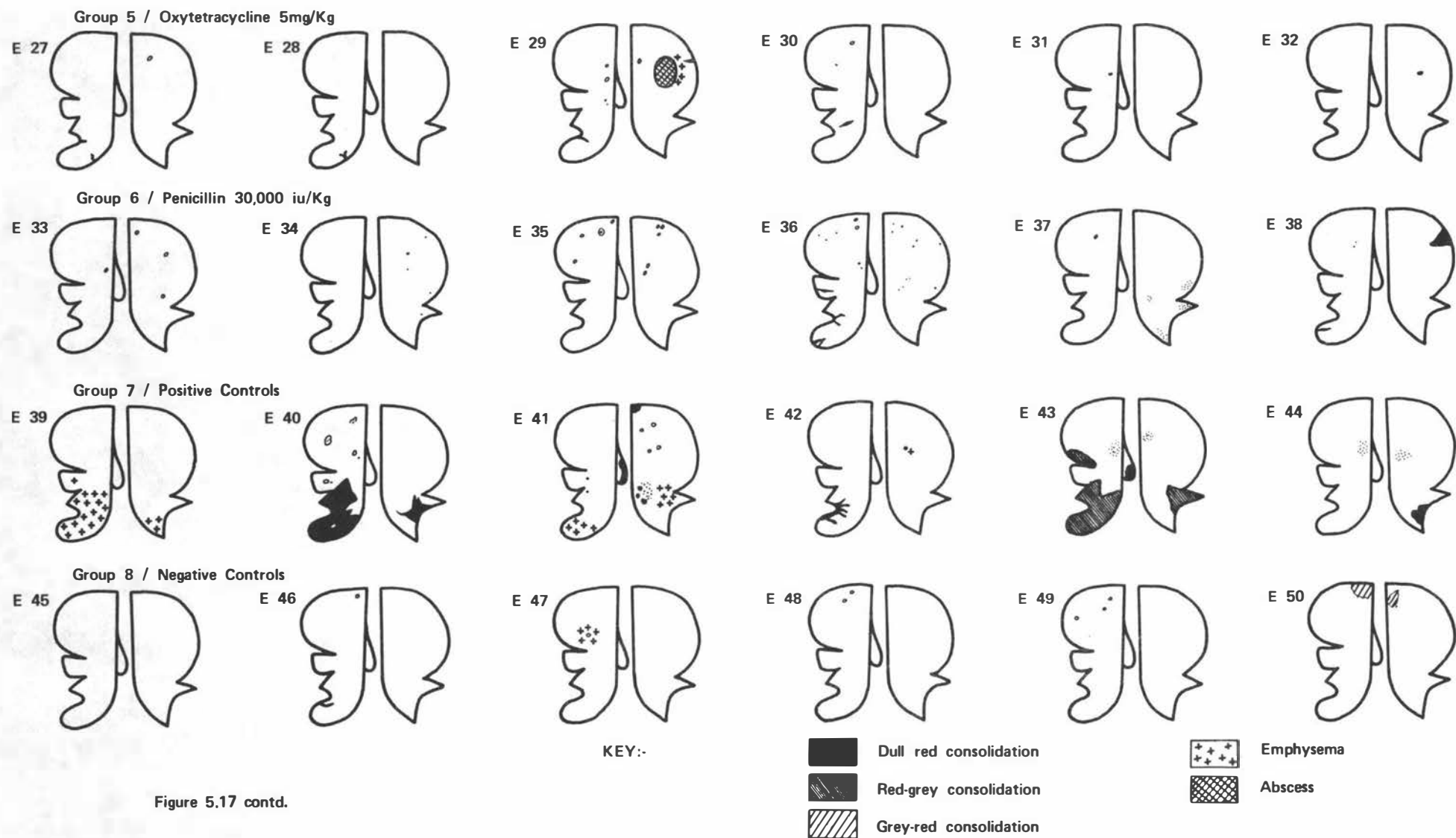


Figure 5.17 contd.

TABLE XXII
MEAN BODY WEIGHTS AND LUNG WEIGHTS OF SHEEP FOLLOWING
TRANSMISSION OF CHRONIC PNEUMONIA AND ADMINISTRATION
OF CHEMOTHERAPEUTIC AGENTS

	Initial Body Weight (Kg)	Final Body Weight (Kg)	Difference	Lung Weight (Kg)
Group 1: Ronidazole 100 mg/Kg	28.03	26.36	-1.67	0.312
Group 2: Ronidazole 30 mg/Kg	27.73	30.45	2.62	0.303
Group 3: Ronidazole 10 mg/Kg	27.58	28.78	1.20	0.361
Group 4: Tylosin	27.27	28.91	1.67	0.277
Group 5: Tetracycline	27.12	31.06	3.94	0.322
Group 6: Penicillin	26.97	30.75	3.78	0.318
Group 7: Positive controls	26.52	28.48	1.96	0.335
Group 8: Negative controls (run separately)	26.52	26.64	0.08	0.312

Light Microscopy

The histological lesions seen in the lungs in which extensive consolidation was present did not differ in any significant detail from naturally-occurring cases of subacute or chronic ovine pneumonia. Focal areas of bronchopneumonia of a lesser severity were found in a proportion of lungs in Group 2 (Ronidazole 30 mg/Kg), 3 (Ronidazole 10 mg/Kg), 5 (Tetracycline) and 7 (Positive controls) (Table XXIII). They consisted of peribronchiolar areas of alveolar spaces. Bronchioles in the surrounding area often contained small clumps of neutrophils embedded in necrotic debris and moderate bronchiolar epithelial hyperplasia was also present. In addition, several other lungs contained focal areas of collapse in which the alveolar septa were mildly thickened with mononuclear cells. In these areas however, there was no evidence of cellular exudation apart from a few macrophages in alveolar spaces.

The majority of lungs from animals in Group 1 (Ronidazole 100 mg/Kg), and half those from animals in Groups 4 (Tylosin), 5 (Tetracycline) and 8 (Negative controls) were remarkably free of microscopic evidence of pulmonary injury. However, all animals in Group 1 showed evidence of drug toxicity in the sections of liver and kidney that were examined. The liver sections showed moderate to severe cloudy swelling of the hepatocytes. In the kidneys there was severe nephrosis manifest by the swelling and degeneration of proximal tubular cells and protein cast formation.

Microbiological Findings

The results of examinations for bacteria and mycoplasmas on all lungs, normal and pneumonic are summarised in Table XXIV. The most common organism recovered was M. ovipneumoniae which was isolated from the lungs of 4 of the 6 animals in Groups 3 (Ronidazole 10 mg/Kg), 6 (Penicillin) and 7 (Positive controls) but could not be recovered from the lungs of any animals in Groups 1 (Ronidazole 100 mg/Kg) or 8 (Negative controls).

TABLE XXIII

SUMMARY OF HISTOPATHOLOGICAL FINDINGS FOLLOWING CHRONIC PNEUMONIA
TRANSMISSION AND ADMINISTRATION OF CHEMOTHERAPEUTIC AGENTS

	No signifi- cant findings	Focal alveolar collapse, interstitial thickening, etc.	Focal broncho- pneumonia	Extensive broncho- pneumonia	Total examined
Group 1					
Ronidazole 100 mg/Kg	5	1	0	0	6
Group 2					
Ronidazole 30 mg/Kg	1	2	2	1	6
Group 3					
Ronidazole 10mg/Kg	0	1	2	3	6
Group 4					
Tylosin	3	3	0	0	6
Group 5					
Tetracycline	3	2	1	0	6
Group 6					
Penicillin	2	4	0	0	6
Group 7					
Positive controls	0	2	2	2	6
Group 8					
Negative controls	3	3	0	0	6

TABLE XXIV

SUMMARY OF MICROBIOLOGICAL FINDINGS FOLLOWING TRANSMISSION OF CHRONIC PNEUMONIA AND ADMINISTRATION
OF CHEMOTHERAPEUTIC AGENTS

Organism	1. Ronidazole 100 mg/Kg	2. Ronidazole 30 mg/Kg	3. Ronidazole 10 mg/Kg	4. Tylosin	5. Tetracycline	6. Penicillin	7. Positive Controls	8. Negative Controls
<u>Past.haemolytica</u>	0	0	1	0	0	1	2	0
<u>N.catarrhalis</u>	0	1	0	0	0	0	1	0
<u>Staph. aureus</u>	0	0	0	0	1	0	0	0
<u>α-haemolytic Streptococci</u>	0	0	0	0	0	1	0	0
<u>E.coli</u>	0	0	0	1	0	0	0	0
<u>Mycoplasma ovipneumoniae</u>	0	2	4	2	0	4	4	1
Total animals in each group	6	6	6	6	6	6	6	6

THE EFFECT OF PNEUMONIA TRANSMISSION ON WEIGHT GAIN

The rate of weight gain in young lambs not only provides a measure of the economic success of fat lamb production but also closely reflects the health of the lamb flock. It might be expected that a chronic pathological process which on occasions may involve up to 50% of the lung capacity, would result in sub-optimal growth rate in affected animals at some stage during the course of the disease.

Convincing evidence on the depressant effect of chronic respiratory disease on food conversion efficiency and growth rate is available in pigs (Betts and Beveridge, 1953) but comparable data indicating a similar relationship in lambs is lacking at the present time. The possibility of inducing pneumonia in lambs immediately before slaughter provided an opportunity to collect information on the relationship between chronic pneumonia and weight gain.

Material and Methods

Two groups of 24 Southdown x Romney lambs, aged 5 to 6 months were chosen from a flock by stratified random selection to give groups of similar liveweight distribution. On January 25, 1974 all animals were tagged, weighed and blood was collected. Group 1 were then inoculated with an intranasal aerosol of pneumonic lung homogenate prepared by the method described earlier in this chapter, from a range of subacute and chronic pneumonias obtained from a local meatworks. Because of the difficulties of handling a large number of animals on one day, the inoculum was prepared on the previous day and held overnight at 4°C. The inoculum was then administered at approximately 26 to 30 hours after collection by the method described previously. Group 2 were untreated controls which were run together on pasture with Group 1.

An interim weighing of all animals was made on February 4 and a final weighing and blood collection on February 19. The animals were slaughtered the following day and the lungs individually identified and examined grossly for evidence of pneumonia.

Results

Liveweight details are given in Table XXV. The difference in

TABLE XXV

THE EFFECT OF PNEUMONIA TRANSMISSION OF WEIGHT GAIN

	Initial Mean Liveweight (lbs) January 25	Interim Weight Gain (lbs) February 4	Final Weight Gain (lbs) February 19
Group 1 (Inoculated)	63.67	+1.16	+4.42
Group 2 (Control)	63.75	+6.21	+ 8.75

Analysis of Variance on Final Weight Gain

	df	s.s.	m.s.	F
Between Groups	1	225.34	225.34	27.68 (p<0.005)
Within Groups	46	374.33	8.14	
Total	47	599.67		

weight gain between the 2 groups of 4.33 lbs, over the period January 25 to February 19 is highly significant.

Correlation of individual weight gains with the presence of pneumonic lesions at slaughter is made in Figure 5.18. Unfortunately the incidence of severe pneumonic lesions was low (17% in Group 1 and 4% in Group 2). However, it can be seen that despite a significant reduction in weight gain in the inoculated group there was no correlation between the presence of pneumonic lesions and weight gain in either the inoculated or control groups.

MYCOPLASMA OVIPNEUMONIAE INOCULATION TRIALS

The isolation of M. ovipneumoniae from the respiratory tract of sheep in New Zealand (Clarke et al., 1974) and the subsequent finding of its close association with the lesions of subacute and chronic pneumonia (Chapter 4) suggests that this organism is either a primary pathogen or an extremely efficient coloniser of ovine lung damaged by other agents. Although the work of Australian investigators (St. George et al., 1971; Carmicheal et al., 1972; Sullivan et al., 1973a, b) with chronic pneumonia of sheep in Queensland supports the former view, evidence that M. ovipneumoniae is a primary cause of chronic pneumonia in the New Zealand situation can only be obtained by appropriate animal inoculation experiments.

The following inoculation trials were therefore undertaken in an attempt to provide some of the information on the pathogenicity of M. ovipneumoniae which is lacking at the present time.

Material and Methods

Pilot Trial

Two caesarian-delivered, colostrum-deprived, Romney lambs, aged 8 weeks, were inoculated intranasally with broth cultures of M. ovipneumoniae. One received an attenuated strain of the organism (Strain 5) which had been extensively subcultured while the other received a fresh isolate (50 pL). Five millilitres of each broth culture were instilled into one nostril and a further 5 to 10 mls were

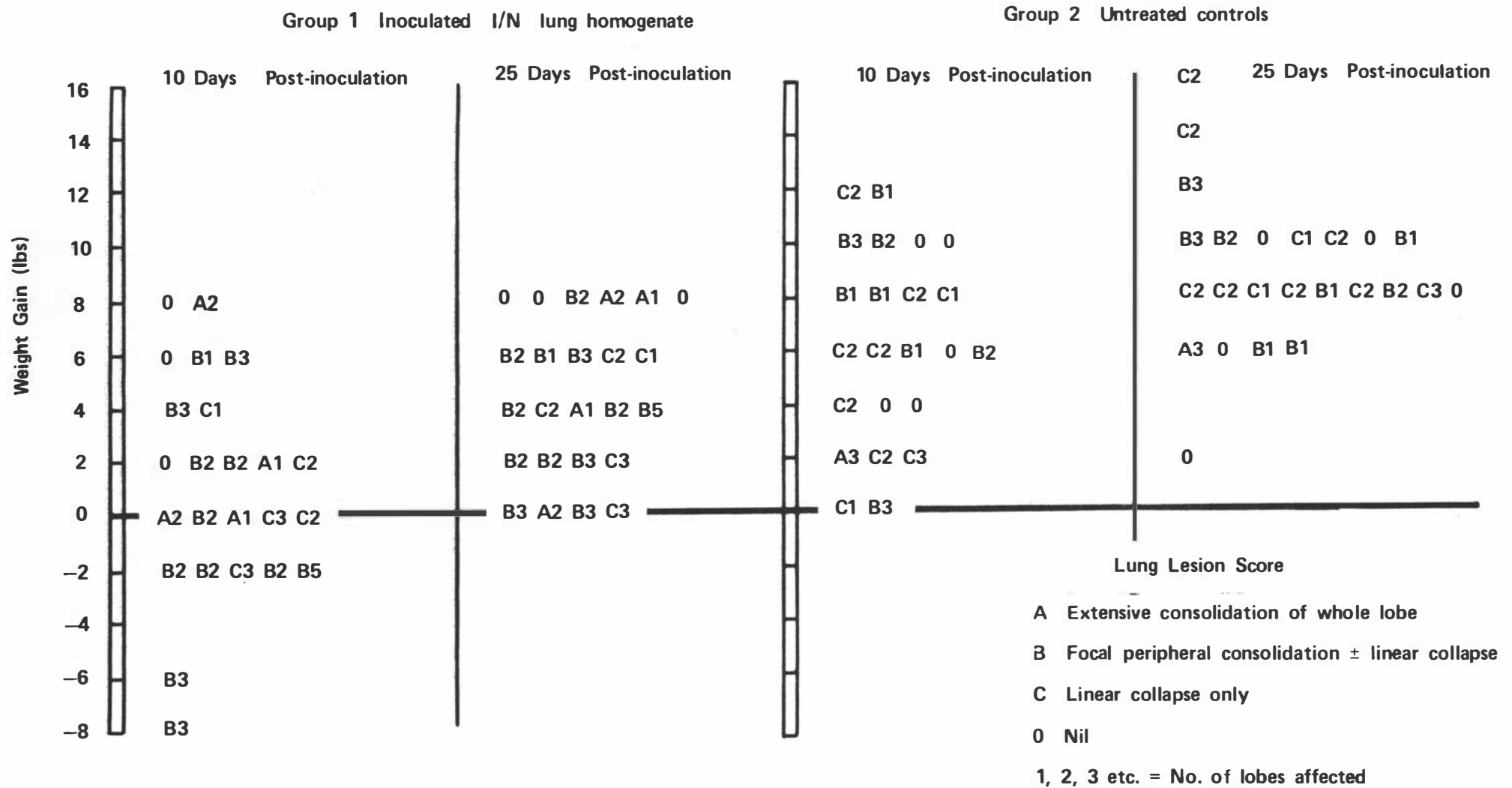


Figure 5.18

Correlation between weight gain and severity of pneumonic lesions.

administered by intranasal aerosol as described previously in this chapter. The titre of organisms immediately before inoculation was estimated by a surface colony count. Strain 5 was found to contain 1×10^9 organisms/ml and 50 pL contained $1 \times 10^{8.5}$ organisms/ml.

Following inoculation the animals were observed twice daily, their lung sounds were auscultated and rectal temperatures recorded. Euthanasia by intravenous barbiturate was carried out on the 12th day after inoculation. At necropsy, the gross lung lesions were recorded, photographed and samples were collected for subsequent histological and microbiological examination. The histological and microbiological methods were the same as those previously described.

Inoculation of Housed Lambs

The animals used were 20, 5-month-old, Romney x Perendale lambs which were colostrum-fed at birth but housed without contact with other stock or internal parasites since 1 to 2 days of age. At the commencement of the trial 2 animals were selected at random and killed. At necropsy, their lungs were found to be free of any evidence of respiratory disease. Bilateral nasal swabs were collected from the remaining animals and on culture no M. ovipneumoniae could be recovered.

The lambs were then divided into 2 even groups on the basis of body weight. Group 1 were inoculated with a broth culture of M. ovipneumoniae by the same technique as that used in the pilot trial. The culture was prepared from the Strain 5 isolate the titre of which was estimated immediately before inoculation as $1 \times 10^{7.5}$ organism/ml. The animals in Group 2 were inoculated intranasally with pneumonic lung homogenate prepared and administered by the same methods described in the previous section. The 2 groups were kept in separate pens without contact with one another and the animals were examined once daily when clinical signs were observed, lung sounds auscultated and rectal temperatures recorded. On days 2, 4, 6, 10, 14, 20, 30, and 40 following inoculation 1 lamb from each group was randomly selected for euthanasia by intravenous barbiturate. Because 1 animal in Group 2 died on day 5 the remaining extra animal in Group 2 was sacrificed 25 days after inoculation. The gross lung lesions were recorded at

necropsy and lung samples from all animals were taken for histological and microbiological examination as previously described.

Results

Table XXVI summarises the clinical signs seen following inoculation and the gross and microscopic lesions observed at necropsy.

Pilot Trial

Both animals showed a temperature rise of between $\frac{1}{2}$ and 1°C from the 3rd to the 5th day after inoculation. During this period the animal receiving the 50 pL strain (E275) coughed occasionally and harsh bronchial tones were audible on auscultation of the chest. At necropsy, the lungs of the animal receiving Strain 5 (E274) contained areas of dull red consolidation in the anterior right apical and left ventral diaphragmatic lobes (Figure 5.19). Microscopically, these consisted of lesions of recent bronchopneumonia in which there was alveolar collapse and focal infiltration of neutrophils into alveolar spaces and bronchioles. Early epithelial hyperplasia and peribronchial lymphoid aggregations were also seen and the general appearance of the lesions was typical of early chronic enzootic pneumonia. In the second animal (E275) no areas of consolidation were present but the ventral areas of both lungs showed a diffuse blotchy discoloration (Figure 5.20) and the cut surface in these areas had a darker than normal appearance around bronchioles. Histologically, mild irritation of alveoli was evident in these areas as there were slightly increased numbers of macrophages and occasional neutrophils in alveolar spaces. On microbiological examination M. ovipneumoniae was recovered from the lung of E274 and the nasal cavity of E275.

Inoculation of Housed Lambs

Apart from a slight temperature rise on the 2nd and 3rd days after inoculation in 2 of the animals, none of the animals in Group 1 showed clinical evidence of either pneumonia or macroscopic lung lesions at necropsy. On histological examination, evidence of mild pulmonary irritation was seen in the lungs of the lambs killed on days 2 to 10 after inoculation. A slight excess in numbers of alveolar macrophages

TABLE XXVI

CLINICAL SIGNS AND LUNG LESIONS FOLLOWING MYCOPLASMA OVIPNEUMONIAE INOCULATION

Sheep No.	Age	Inoculum	No. of Days from Inoculation until Killed	Clinical Evidence of Pneumonia	Severity of Lesions	Type of Lesion
Pilot Trial	E274	<u>M.ovipneumoniae</u> 1 x 10 ⁹ orgs/ml	12 days	Temp. rise	++	Dull red consolidation
	275		"	Temp.rise,coughing	++++	Ventral discoloration
Group 1	E 58	<u>M.ovipneumoniae</u> 1 x 10 ^{7.5} orgs/ml	2 days	Nil	-	Nil
	60		4 days	Nil	-	"
	63		6 days	Temp.rise	-	"
	65		10 days	Nil	-	"
	66		14 days	Temp.rise	-	"
	68		20 days	Nil	-	"
	70		25 days	Nil	-	"
	71		30 days	Nil	-	"
Group 2	73		40 days	Nil	-	"
	E 57	Pneumonic Lung Homogenate	2 days	Temp. rise	-	Nil
	59		4 days	Nil	-	"
	61		5 days died	Temp.rise,coughing	++++	Dark red consolidation
	62		6 days	Temp.rise	-	Nil
	64		10 days	Nil	-	"
	67		14 days	Coughing	+++	Red-grey consolidation
	69		20 days	Nil	-	Nil
	72		30 days	Nil	±	Linear bands of collapse
	74		40 days	Nil	-	Nil

++++ = Lesions in 4 or more lobes

+++ = " " 3 anterior lobes

++ = " " 2 " "

± = " in parts of 1 anterior lobe

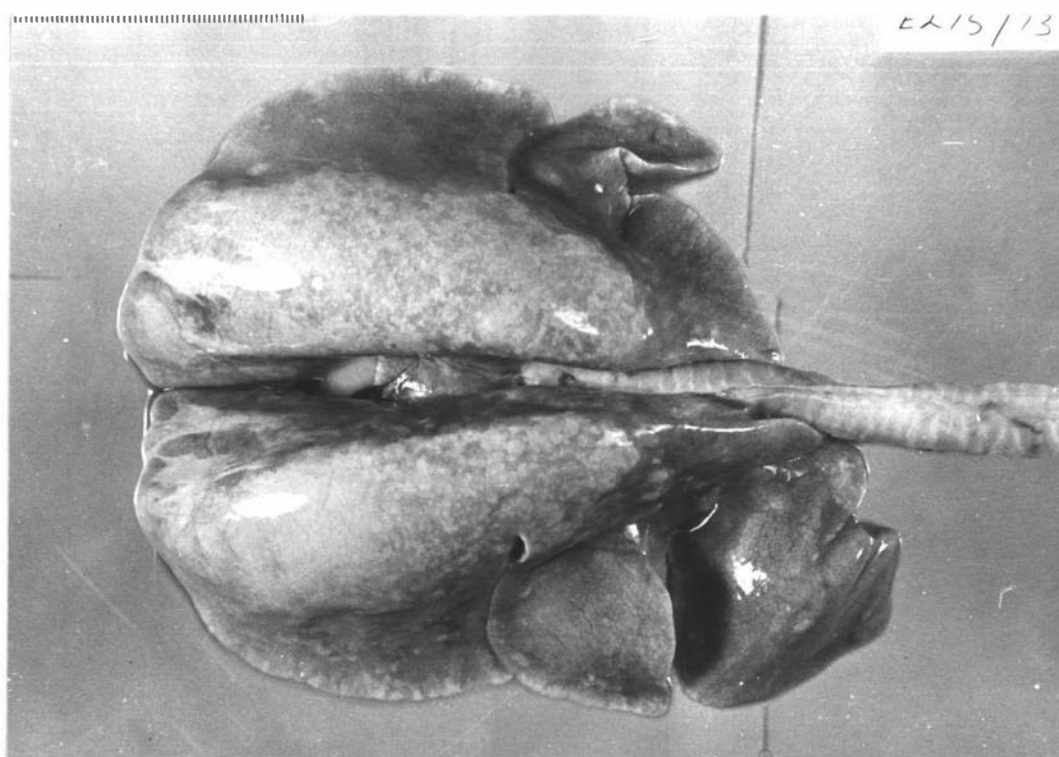
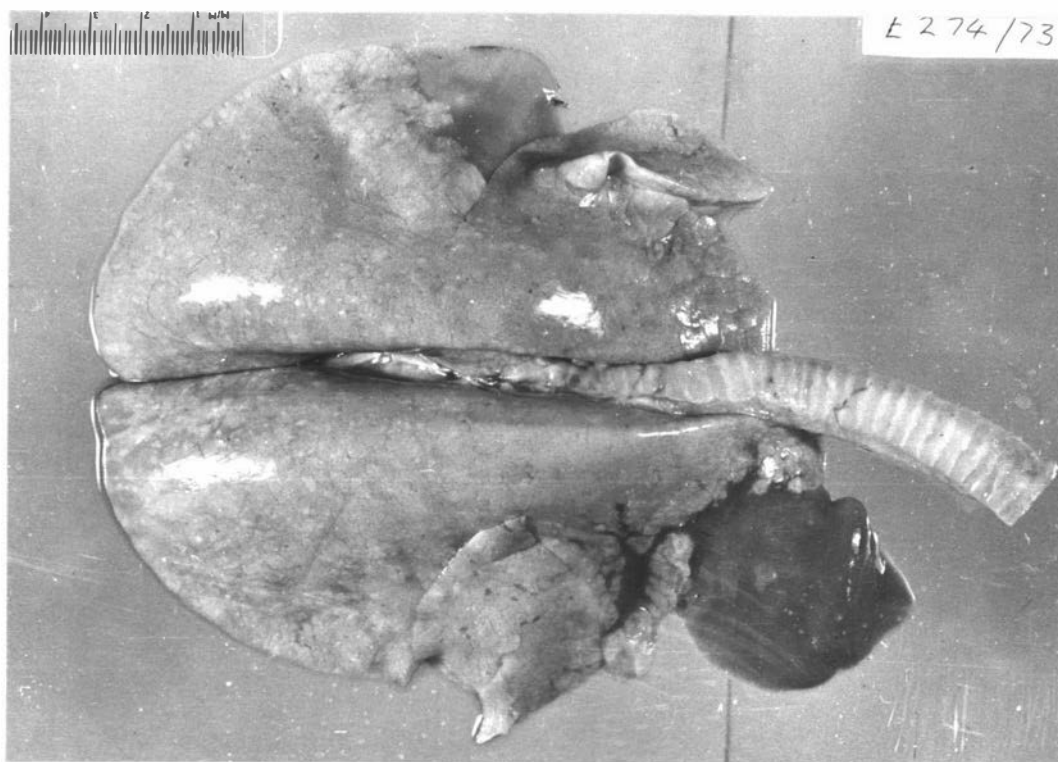
following page 178

Figure 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.

Figure 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.



and peribronchiolar thickening of alveolar septa were seen in the lambs sacrificed on days 2 and 4 while in the animals killed on days 6 and 10, focal lymphoid hyperplasia was found in some peribronchiolar areas.

In Group 2, one animal (E61) showed a temperature rise of 2°C on the 2nd day. During the next few days it coughed persistently and harsh, moist rales were audible on auscultation. On the 4th day it became depressed then recumbent and died on the following day. Extensive areas of dark red consolidation were present in the right lung and anterior parts of the left lung at necropsy. Within the ventral diaphragmatic lobe of the right lung was a large grey-red necrotic focus the surface of which was covered with thick strands of fibrin. The histological changes in the lungs embraced all the main features of acute enzootic pneumonia including severe congestion, intra-alveolar haemorrhage, fibrin exudation and necrotic cellular debris which often had an elongated "streaming" appearance. Many colonies of bacteria were present within alveolar spaces and P. haemolytica was isolated in large numbers from the affected tissue. In the animals sacrificed on days 4, 6 and 10 following inoculation no gross lesions were observed but a few macrophages were found in some alveolar ducts histologically and occasionally there was mild thickening of alveolar septa around bronchioles. Gross lung lesions typical of chronic enzootic pneumonia were seen in the animal killed on day 14 and the pathological features usually found in this disease were confirmed histologically. No lesions were seen in the lambs sacrificed at days 20 and 40 after inoculation but in the animal killed on day 30 several dull red linear lesions were found which consisted histologically of alveolar collapse, mild interstitial thickening and moderately severe peribronchiolar lymphoid hyperplasia.

DISCUSSION

The transmission experiments described here using pneumonic lung homogenate successfully produced lesions which were pathologically identical to naturally-occurring cases of either acute or subacute and chronic enzootic pneumonia in a significant proportion of the inoculated animals. In this study, the majority of work was concentrated on subacute and chronic pneumonia because little information is available on this form of the disease in New Zealand. In addition, its widespread distribution in the Manawatu district ensured that a ready source of fresh inoculum was available from local meatworks during the late summer and autumn months.

Use of the endobronchial inoculation technique proved effective in inducing acute pneumonia in the 10 to 12-month-old, worm-free, housed sheep that were used in this experiment. A technique of this type was necessary in order to deliver the large quantity of inoculum (10 ml) required to produce an acute pneumonic response. The resulting lesions were nevertheless, widely distributed in most cases and not typical of a focal, necrotizing aspiration pneumonia. The main differences between the microscopic lesions induced and naturally-occurring acute pneumonia lay in the variability of cellular response within the lung of a given animal. In the present cases, some areas adjacent to necrotic lobules contained exudate which was almost purely neutrophilic in character whereas in other areas macrophages, neutrophils, small mononuclear cells, alveolar epithelium and fibrin combined together to form a necrotic exudate in which many cells became elongated. While this variability of response fell within the range of microscopic lesions usually seen in acute pneumonia, variations of this degree in the natural disease were most often seen between individuals rather than within a given lung.

A large measure of the success in transmitting acute pneumonia can be ascribed to the susceptible nature of the experimental animals used. Their lack of contact with other sheep and exposure to internal parasites since birth was reflected in a low rate of nasal carriage of *P. haemolytica* (15% compared to 73% in lambs at slaughter) and the blemish-free appearance of the lungs of control animals at necropsy.

The unsuccessful transmission experiments by Salisbury (1957) and the dubious results obtained by Downey (1957) may be explained in part by the lack of fresh inoculum available for their transmission tests as both these authors worked in Upper Hutt with material derived from South Otago and Southland. The experimental animals used by Salisbury were described as "sheep of all ages" while Downey used flock ewes aged 2 years and older. It seems likely that adult flock sheep would be more resistant to transmission than younger animals, which may be a further factor contributing to the lack of success of these authors' experiments. In the present study these drawbacks were rectified, but nevertheless, the quantity of bacteria and foreign protein that it was necessary to administer in order to produce acute pneumonia, could not in any way be considered to represent a natural process of infection. It is clear therefore, that although the disease is experimentally transmissible by these means, under field conditions further important host and environmental factors will be necessary in order to depress pulmonary defence mechanisms to the point required for the natural disease to occur.

When the inoculated pneumonic lung was homogenised in a medium containing penicillin and streptomycin no significant lesions were produced in the experimental animals. This finding further serves to emphasise the essential role which bacteria undoubtedly have in the aetiology of acute enzootic pneumonia.

Although the pathological response to P. haemolytica cultures was erratic, focal areas of haemorrhagic necrosis were produced in 2 of the animals inoculated. These were closely similar in structure to those produced by Biberstein et al. (1967) who inoculated up to 12×10^9 P. haemolytica organisms into the lungs of adult sheep via the trachea.

No gross or microscopic lesions were observed following the endo-bronchial inoculation of M. arginini cultures. This lack of pulmonary response is in keeping with the generally accepted belief that the organism is of low pathogenicity in the respiratory tract of most species (Leach, 1970).

For the transmission of chronic pneumonia the endobronchial

technique was abandoned in favour of aerosol inoculation because of the ability of this method to deposit small quantities of inoculum in the ventral parts of the lung thus reducing the danger of evoking an aspiration-type pneumonia. By this method and with the careful choice of susceptible animals, lesions pathologically identical to naturally-occurring cases of subacute and chronic enzootic pneumonia were produced in a large proportion of the inoculated lambs. Inoculation of a 6-year-old ewe however, failed to produce either a clinical response or evidence of pathological reaction in the lung.

A greater range of pathological response from individual animals was seen in the studies on the development of chronic pneumonia by sequential slaughter following experimental transmission. This variability was a considerable handicap to these studies as it prevented a clear view of the sequence and timing of pathological events. In future studies this could best be overcome by the use of colostrum-deprived specific pathogen-free lambs and the sacrifice of greater numbers of animals at each time interval.

Despite this problem it was possible to observe a basic pattern of events which was similar to that described by Baskerville (1972) who performed transmission studies on enzootic pneumonia in young pigs. The main differences between the development of the lesions observed in the present study and those described in pigs by Baskerville lay in the degree and timing of the pathological changes rather than in the essential nature of the lesions. In the current investigation mild hyperplastic changes were seen in the bronchiolar epithelium as early as 2 days after inoculation whereas similar changes were not noted until 7 days in pigs. Peribronchial lymphoid hyperplasia on the other hand, developed more rapidly in pigs where it was seen 5 days after inoculation while corresponding aggregations were not seen until 6 to 8 days in lambs. Although only subjective comparisons can be made it appeared likely that alveolar collapse was more severe and extensive in the present study and that intra-alveolar cellular response was correspondingly less severe throughout the period studied than that described in pigs by Baskerville.

Several chemotherapeutic agents were effective in suppressing the

development of subacute and chronic pneumonia following experimental transmission. Ronidazole at 100 mg/Kg and oxytetracycline suppressed both the lesions and micro-organisms whereas tylosin and penicillin suppressed the development of the lesions but were not totally successful in inhibiting the growth of micro-organisms in the lung. Ronidazole was used in this trial because Smith (1973) recently demonstrated its efficacy in eliminating mycoplasmas (M. mycoides and M. capri) from the blood of experimentally-infected mice. Tylosin is similarly recognised for its strong activity against mycoplasmas and low activity against most gram-negative bacteria. Oxytetracycline on the other hand, has a broad spectrum of activity against both mycoplasmas and bacteria while the limited range of activity of penicillin against many bacteria is well known. The results of this trial therefore support the view that both mycoplasmas and bacteria have a role in the pathogenesis of chronic enzootic pneumonia of sheep.

The introduction of foreign proteins derived from pneumonic tissue into the lower respiratory tract, as was the case in the present experiments, raises the possibility that the pathological reaction observed in the lung represents either a simple response to the overloading of alveolar defence mechanisms or an auto-immune reaction to lung extract as has been reported previously in pigs (Roberts and Little, 1970). If either of these possibilities were the case, some lesions should also have been observed in the lungs of animals receiving pneumonic lung homogenate in which bacteria and mycoplasmas were suppressed either by the in vitro addition or in vivo administration of antibiotics. Similarly, these findings argue against the possibility that viruses have a primary role in the aetiology of the common forms of ovine enzootic pneumonia. If pathogenic viruses were being transmitted in the homogenised pneumonic lung, minimal lesions could be expected to occur in some parts of the respiratory tract of those animals receiving antibacterial and antimycoplasma chemotherapy.

From the results of the weight gain trial it can be concluded that the inoculation procedure used for the transmission of pneumonia produced a significant reduction in weight gain. This effect may have been due to a transitory bacteraemia or septicaemia during the 2 to 5 day period

following inoculation as was suggested by the mild rises in body temperature seen after inoculation in many sheep in previous transmission experiments. Although a high proportion of the lungs of inoculated sheep showed small focal areas of consolidation and linear collapse the production of severe pneumonic lesions was disappointingly low in this experiment. Several reasons can be advanced to explain these poor transmission results including; loss of infectivity of the inoculum, which was held for over 24 hours before use; poor inoculation technique, as the lambs were larger and more difficult to handle than those previously used; or the possibility that subacute pneumonia was already present in the flock, since its pneumonic status could not be unequivocally established before inoculation.

No correlation was detected between the pneumonic lesions that were present and the weight gain of individual animals. This result is in agreement with the findings of Kirton (1968) who found no relationship between the growth rate of lambs and the presence of enzootic pneumonia at slaughter. However, it is contrary to the experiences of many field veterinarians (Davis, 1969; Smith, 1970) and to convincing evidence from other species supporting a direct relationship between poor weight gain and the presence of chronic pneumonia (Betts and Beveridge, 1953).

Inoculation of lambs with M. ovipneumoniae cultures produced results which were inconclusive. While significant gross and microscopic lesions were found in 2 caesarian-derived, colostrum-deprived lambs little evidence of pathogenicity was observed in the lungs of 9 housed lambs that were subsequently inoculated. Part of the problem may have been due to the lower titre of the inoculum used in the second experiment (i.e. 1/35th of the first). A further possible explanation may lie in differences in the immune status of the 2 types of experimental animals, although the failure to isolate M. ovipneumoniae from the nasal cavities of the housed lambs suggests that their previous exposure to this organism was minimal. Similar studies have been carried out in Australia by St. George et al. (1971) with slightly more promising results. These authors produced pneumonic lesions in 3 out of 5 caesarian-derived lambs and 1 out of 4 normal lambs but they were unable to produce pneumonia in any lambs older than 4 days of age.

Because of the close association of the organism with ovine pneumonia further work on its pathogenicity is clearly necessary. The success of these experiments will be dependant to a large extent on the availability of suitable colostrum-deprived animals and housing facilities.

SUMMARY

Lesions pathologically identical to naturally-occurring cases of acute enzootic pneumonia were produced in 4 worm-free, housed lambs by means of endobronchial inoculation of lung homogenate derived from field cases of acute pneumonia. When the inoculum was homogenised with Eagle's medium containing penicillin and streptomycin no lesions were produced in a further 4 inoculated lambs. Endobronchial inoculation of 4 lambs with P. haemolytica cultures evoked an erratic response with the formation of focal areas of haemorrhagic necrosis in 2 of the inoculated animals. No lesions were seen following endobronchial inoculation of M. arginini cultures or in animals receiving nutrient media. These findings serve to emphasise the essential role bacteria have in the pathogenesis of acute pneumonia however, the excessive amount of inoculum and the method of inoculation required to produce the lesions suggests that host and environmental factors maybe equally important in the development of the natural disease.

The serial transmission of subacute and chronic pneumonia was achieved by intranasal aerosol inoculation of lung homogenate derived from a range of abattoir cases. The 5 to 7-month-old animals used were screened radiographically before inoculation. The clinical signs and pathological lesions exhibited were studied and found to be identical in most respects to the naturally-occurring disease. The development of the lesions was also studied by means of the sequential slaughter of 12, 7-month-old lambs from 2 to 12 days after inoculation. The earliest changes seen were alveolar collapse and the accumulation of small numbers of neutrophils in bronchioles and macrophages in alveolar ducts. A severe cellular response was seen at 6 days when epithelial hyperplasia also became prominent. Considerable variation in reaction was seen. The reasons for this are discussed and the results compared with similar studies in pigs.

The effect of various chemotherapeutic agents on the development of chronic pneumonia was investigated following transmission by intranasal aerosol inoculation of pneumonic lung homogenate. Both ronidazole at 100 mg/Kg and oxytetracycline were successful in suppressing the development of the disease while tylosin and penicillin suppressed the

development of lesions but did not completely inhibit the growth of bacteria and mycoplasmas in the lung. These findings therefore support the view that both mycoplasmas and bacteria have a role in the pathogenesis of chronic enzootic pneumonia in sheep.

A controlled experiment was carried out with grazing lambs to assess the effect of chronic pneumonia on weight gain. Although a significant difference was observed between the weight gain in the transmission group and the controls, no correlation could be found between the presence of pneumonic lesions at slaughter and the weight gain of individual animals. It was suggested that the weight difference between the 2 groups was caused by a transitory bacteraemia or septicaemia following inoculation.

Inoculation of M. ovipneumoniae cultures by intranasal aerosol produced equivocal results. Lesions were produced in 2, 3-week-old caesarian-derived lambs but no response was evoked in 9, 5-month-old housed lambs. It was concluded that further inoculation experiments with M. ovipneumoniae on colostrum-deprived lambs in suitable housing facilities are necessary before its pathogenicity can be accurately assessed.

CHAPTER 6

GENERAL DISCUSSION

The information presented in this thesis is compatible with the general concept that enzootic pneumonia in sheep results from the complex interaction of both host and environmental factors with infectious agents. The list of agents potentially capable of damaging the ovine respiratory tract is impressive, but no single agent has been shown to have the capacity to reproduce exactly the lesions of either acute or subacute and chronic pneumonia under conditions which resemble those likely to be encountered in pastoral grazing. Rather than examine the effect of any one of these agents in depth, the present study has sought to investigate the reaction of the pulmonary alveolus to injury in both natural and experimental situations, in order to define more clearly the nature and sequence of events which underlie the development of the disease. In the second part of this study the prevalence of micro-organisms commonly associated with pneumonia in sheep in this country was investigated with a view to ascertaining their relative importance in producing the characteristic lesions. In addition, transmission experiments were used to provide further information on the importance of infectious agents, the development of the disease and the effect of the disease on the host.

The morphological studies described in Chapter 2 confirm the established field observations that acute and chronic enzootic pneumonia are separate entities and suggest that these 2 forms of the disease have a different pathogenesis. The overwhelming numbers of bacteria found in acute pneumonia and their close association with the destructive changes observed, leaves little doubt as to the importance of bacteria in the production of the lesions in acute pneumonia. Further evidence supporting this view comes from the experimental results obtained in Chapter 3 in which lesions closely similar in character to acute enzootic pneumonia were produced in lambs by the endobronchial inoculation of large numbers of Staphylococcus aureus. Nevertheless, cultures of Pasteurella haemolytica consistently failed to produce lesions typical of acute pneumonia, an experience which has been shared by most other workers in this field (Montgomerie et al., 1938; Salisbury, 1957;

Downey, 1957; Hamdy and Pounden, 1959; Biberstein et al., 1967). Furthermore, the transmission of acute pneumonia could only be accomplished by the endobronchial inoculation of large doses of pneumonic lung homogenate into highly susceptible animals that were reared in isolation (Chapter 5), a procedure which is hardly analogous to the natural means of infection.

These findings therefore reinforce the commonly-held belief that systemic factors also play a vital role in the development of acute pneumonia. The most common environmental factors with which the disease is associated are, sudden changes or extremes of temperature; exposure to wet and windy conditions; and mustering or transportation. Whether these factors result in hypothermia, hypovolaemic shock, acidosis or merely elevated blood adrenaline levels, they all have the potential to induce pulmonary congestion and oedema by altering pulmonary haemodynamics and increasing capillary permeability (Chapter 1). The mechanisms whereby these factors act on the pulmonary capillaries is not completely understood. In some cases they act directly on endothelial cells and in others they may act via mediators such as histamine and plasmakinins (Henry, 1968) or by deposition of platelet-fibrin micro-emboli or neutrophils in the pulmonary vascular tree (Ratliff et al., 1970, 1971; Rapaport et al., 1973). However, the work of Kylstra (1965) suggests that filling of the alveoli with fluid, provided it is not universal may be a relatively benign occurrence and that failure of clearance by lymphatics may be a more important factor underlying the onset of pathological change. The presence of protein-rich fluid in the alveolar spaces for any length of time would in any case provide an excellent incubatory medium for bacteria inhaled from the upper respiratory tract and may reduce the phagocytic and bactericidal activity of alveolar macrophages (LaForce et al., 1973). The outcome of this sequence of events would be a lowered resistance to pulmonary infection and this has been best documented in human medicine where it is seen in patients recovering from hypothermia (Mant, 1969) severe burns (Rapaport et al., 1973) and traumatic or haemorrhagic shock (Eiseman, 1968).

In subacute and chronic pneumonia the earliest changes seen were peribronchiolar alveolar collapse, the accumulation of small numbers of

macrophages in alveolar spaces and mild thickening of alveolar septa with mononuclear cells. These changes were often accompanied by mild hyperplasia of the terminal bronchiolar epithelium and the infiltration of small numbers of neutrophils into the lumen of bronchioles and alveolar ducts. Initial changes very similar in nature to this were produced by the instillation of dilute nitric acid (Chapter 3) and following the intranasal aerosol inoculation of lung homogenate derived from cases of chronic pneumonia (Chapter 5). From these findings it can be postulated that these early changes represent a non-specific response of the ovine lung to low grade irritation of the terminal airways. The factors responsible for initiating the early lesions in subacute pneumonia remain undefined at the present time but from the current study it seems likely that they are of exogenous origin. Whether or not these changes progress to the overt lesions of chronic enzootic pneumonia may well depend on the continuing presence of irritants in the affected tissue. In this respect, colonization of the lesions by organisms from the nasal cavity such as Mycoplasma ovipneumoniae, P. haemolytica and Neisseria catarrhalis is likely to be essential in determining the development and severity of the lesions. Compelling evidence favouring this view comes from the high correlation obtained between the severity of histological lesions and the titres of bacteria and M. ovipneumoniae in the lung (Chapter 4) as well as from the suppressive effect of antimycoplasma and antibacterial drugs on the development of the disease following experimental transmission (Chapter 5).

The acceptance of this view of the pathogenesis of chronic enzootic pneumonia should not negate the possibility that systemic factors have a role in determining the initiation, progression and severity of the lesions. It is clear from observations on both natural and experimental cases that not all subacute lesions progress to the severe proliferative pneumonias seen in a proportion of affected animals. Some of the environmental factors associated with the occurrence of chronic pneumonia in lambs during the late summer and autumn are, hot weather and drought conditions; fatigue and dehydration during driving, yarding and handling; and debilitating diseases such as internal parasitism malnutrition and facial eczema (sporodesmin poisoning). Studies by

Holmes and Bremner (1971) on sheep infected with Ostertagia circumcincta have shown a significant reduction in food and water intake and a profound drop in the turnover of body water. Similarly, Siefert (1971) has shown that gastro-intestinal parasites significantly depressed packed-cell volume, haemoglobin concentration and plasma protein levels in cattle when pastures were poor. Future studies on the morphological effects of these factors on the ovine pulmonary alveolus and their physiological effects on pulmonary defence mechanisms may provide important clues linking the occurrence of debilitating diseases in sheep with the severity of chronic pneumonia.

The importance of the alveolar type I epithelial cell in maintaining the structural integrity of the alveolus was highlighted in the current investigations of both natural and experimental pulmonary injury. Type I cells were seen to endure a variety of degenerative changes before eventual sloughing and disintegration. The loss of the epithelium was a catastrophic event resulting in the accumulation of fibrin and the build up of leucocytes in the alveolar space. Studies on oxygen-poisoned mice by Adamson et al. (1970b) have also emphasized the function of the alveolar epithelium in preventing the flow of plasma into the alveolus following damage to the capillary endothelium and Bensch et al. (1967) have demonstrated that alveolar type I cells are the main obstacle to the passage of proteins across the pulmonary blood-air barrier.

Undoubtedly, one of the most outstanding ultrastructural features of both the natural and experimental alveolar injury observed in the present study was the degree to which the alveolar type II cells participated in the reparative process. Although both type I and II cells were vulnerable to injury, proliferation after injury was almost exclusively the prerogative of the type II cells. It therefore seems likely that some of these cells are metabolically inactive or undifferentiated in normal lungs and are thus able to escape damage and be available for repair. Further studies to confirm this hypothesis would require the use of autoradiographic and histochemical techniques at the ultrastructural level.

Although it was possible to induce hyperplasia of alveolar type II cells by the experimental instillation of dilute (1%) nitric acid into the lungs of lambs, the degree of epithelial proliferation produced was considerably less than that encountered in naturally-occurring cases of chronic enzootic pneumonia. The most likely explanation for these differences is, that in the case of the naturally-occurring disease, the agents responsible are micro-organisms which are continually present in the alveolar space and are thus able to produce a sustained irritation of the alveolar epithelium which it is difficult to reproduce by chemical means.

In assessing the role of P. haemolytica in ovine pneumonia, two important factors must be taken into account. Firstly, the variety of serotypes which can occur in the environment is considerable as has been demonstrated in Scotland by Biberstein et al. (1970) and secondly, the organism has an almost ubiquitous distribution in the nasal cavity of both normal and pneumonic sheep in New Zealand as indicated by the results obtained in Chapter 4. The possibility that certain serotypes of P. haemolytica in the normal flora of the nasal cavity may exert a protective effect against invasion by more virulent strains of the organism must therefore be considered. In this context, it is relevant to note that Crowe et al. (1973) have shown that the normal throat flora of children who do not become colonized with Group A streptococci contains bacteria with a significantly greater inhibitory activity against Group A streptococci than that from children who did become colonized. The bacteria responsible for most of this inhibitory activity were non-haemolytic streptococci, Streptococcus viridans and Neisseria sp.

Many important questions concerning the role of M. ovipneumoniae in the pathogenesis of subacute and chronic pneumonia remain to be answered. Although the present work has shown a widespread distribution of the organism, high titres in pneumonic lungs and a close association between these titres and the severity of histological lesions, conclusive evidence of its primary role in the aetiology of chronic pneumonia in New Zealand is lacking. The possibility that M. ovipneumoniae alone is capable of initiating the disease, as postulated by

Carmichael et al. (1972) and Sullivan et al. (1973a) for chronic pneumonia in sheep in Queensland, needs further investigation by more inoculation trials on colostrum-deprived lambs. It is possible that latent M. ovipneumonia infection may occur in a large proportion of New Zealand lambs from a few weeks of age as has been suggested occurs in sheep in Queensland by Sullivan et al. Production of an overt pneumonia may then occur through reactivation of the latent infection by some other factors. Such additional factors may be of endogenous origin such as the waning of maternally-derived antibody or alternatively, environmental stress, bacterial invasion or superinfection by a virus capable of inhibiting immune mechanisms. Experimentally, Singer et al. (1972) have demonstrated that it is possible to transform a smouldering, chronic Mycoplasma pulmonis infection of mice into a lethal one by the administration of the immunosuppressive agent, cyclophosphamide.

In the light of these possibilities, future studies might be profitably directed towards epidemiological investigations of the seasonal and age incidence of micro-organisms in the ovine respiratory tract and their relationship to environmental factors such as climate and husbandry procedures. Concomitant with these investigations should be the development of a reliable serological test for M. ovipneumoniae so that infection with this organism can be adequately monitored under both field and experimental conditions.

There is one aspect of pneumonia in sheep which requires high priority before any large scale expenditure on future research is undertaken. This is the need for precise information on the effect of subacute and chronic pneumonia on the growth rate of lambs. Although the serious economic effects of acute pneumonia cannot be disputed, no reliable data are available to support the commonly-held belief that chronic pneumonia in lambs has an adverse affect on weight gain. The difficulties inherent in designing suitable weight gain trials are considerable. Besides the problem of cross-infection when animals are run together there is the added difficulty in long-term trials, of ascertaining whether the lesions seen at necropsy represent recovery phases of previously severe lesions or early lesions whose effect on growth rate should be minimal. For this reason the careful correlation of

individual weight gains with histopathological findings in each case should be an integral part of any investigation of this type.

An area neglected in the present study has been the virological aspects of the ovine respiratory tract. While there is an obvious need for information on the nature and distribution of respiratory viruses in sheep in this country, current indications are that these agents do not have a major role in the aetiology of enzootic pneumonia. This view is based on the absence of pathological evidence of virus infection, such as intra-cellular inclusion bodies in the naturally-occurring disease as well as the strongly suppressive effect which antibiotics have on the transmission of the disease (Chapter 5). Although parainfluenza-3 virus has been isolated in New Zealand (Carter and Hunter, 1970), overseas experience indicates that this organism is not associated with naturally-occurring chronic pneumonia in lambs in Australia (St. George and Liefman, 1972) and produces only mild lesions in colostrum-deprived lambs when inoculated intranasally (Gilmour et al., 1968).

Future prospects for the control of pneumonia in sheep could involve the possibility of manipulating either the environmental factors or the micro-organisms with which the disease is associated. Unless conventional systems of sheep management can be radically altered, control of environmental factors does not seem feasible in the immediate future. Control of micro-organisms could be accomplished by the establishment of specific pathogen-free breeding stocks as has been successfully achieved in the pig and poultry industries. Further possibilities include the suppression of micro-organisms by the use of chemotherapeutic agents or the development of immunity by the use of multivalent vaccines.

REFERENCES

- Adamson, I.Y.R. & Bowden, D.H. (1974). The type 2 cell as progenitor of alveolar epithelial regeneration. A cytodynamic study in mice after exposure to oxygen. Lab. Invest. 30: 35-42.
- Adamson, I.Y.R., Bowden, D.H. & Wyatt, J.P. (1970a). A pathway to pulmonary fibrosis: An ultrastructural study of mouse and rat following radiation to the whole body and hemithorax. Am. J. Path. 58: 481-498
- Adamson, I.Y.R., Bowden, D.H. & Whatt, J.P. (1970b). Oxygen poisoning in mice ultrastructural and surfactant studies during exposure and recovery. Arch. Path. 90: 463
- Albert, R.E. & Arnett, L.C. (1955). Clearance of radioactive dust from the human lung. A.M.A. Arch. Industr. Health. 12: 99-106
- Alley, M.R. (1969). Seasonal variations in sheep pneumonia. N.Z. vet J. 17: 184
- Alley, M.R. & Marktelow, B.W. (1971). Alveolar epithelialization in ovine pneumonia. J. Path. 103: 219-224
- Alley, M.R., Marshall, R.B. & Pearson, R. (1970). The isolation of Neisseria sp. from pneumonic sheep lungs. N.Z. vet. J. 18: 18
- Allison, A.C. (1971). Effects of silica and asbestos on cells in culture. In Inhaled Particles III. Vol.1 Ed. by W.H. Walton, Gresham Press, England. p.437-445.
- Alpert, S.M., Gardner, D.E., Hurst, D.J., Lewis, T.R. & Coffin, D.C. (1971). Effects of exposure to ozone on defensive mechanisms of the lung. J. Appl. Physiol. 31: 247-252.
- Annual report of animal research stations. N.Z. Dept. of Agriculture (1962-63) p.51.
- Azzopardi, A. & Thurlbeck, W.M. (1969). The histochemistry of the nonciliated bronchiolar epithelial cell. Am. Rev. Resp. Dis. 99: 516-525.
- Baar, H.S. & Ferguson, E.F. (1963). Microlithiasis alveolaris pulmonum. Arch. Path. 76: 659-666
- Baldwin, D.E., Marshall, R.G. & Wessman, G.E. (1967). Experimental infection of calves with myxovirus parainfluenza-3 and Pasteurella haemolytica. Am. J. vet. Res. 28: 1773-1782
- Barber, T.L. & Fabricant, J. (1962). Primary isolation of Mycoplasma organisms (PPL0) from mammalian species. J. Bact. 83: 1268-1273

- Barter, R.A., Byrne, M.J. & Carter, R.F. (1966). Pulmonary hyaline membrane: late results of injury to the lung linings. Arch. Dis. Childh. 41: 489-495
- Baskerville, A. (1969). Ultrastructural changes in experimental lipid pneumonia. Res. vet. Sci. 10: 4-6.
- Baskerville, A. (1972). Development of the early lesions in experimental enzootic pneumonia of pigs: An ultrastructural and histological study. Res. vet. Sci. 13: 570-578
- Baskerville, A. & Wright, C.L. (1973) Ultrastructural changes in experimental enzootic pneumonia of pigs. Res. vet. Sci. 14: 155-160.
- Baskerville, A., Thomas, G., Wood, M. & Harris, W.J. (1974). Histology and ultrastructure of metaplasia of alveolar epithelium following infection of mice and hamsters with influenza virus. Br.J. exp. Path. 55: 130-137
- Beall, F.A. & Dalldorf, F.G. (1966) The pathogenesis of the lethal effect of anthrax toxin in the rat. J. Infect. Dis. 116: 377-389
- Bennell, D.G. (1966). A non-infectious respiratory disease of bovines N.Z. vet. J. 14: 73
- Bennet, G. (1836). Report on the epidemic catarrh or influenza prevailing among sheep in this colony. N.S.W. Calendar and General Post Office Directory. p224. Cited by St George and Sullivan (1973).
- Bensch, K.G., Schaefer, K. & Avery, M.E. (1964) Granular Pneumocytes: Electron microscopic evidence of their exocrine function. Science. 145: 1318-1319
- Bensch, K.G., Dominguez, E.A.M. & Liebow, A.A. (1967). Absorption of intact protein molecules across the pulmonary air-tissue barrier. Science. 157: 1204-1206
- Berdjis, C.C. (1971). Pathology of Irradiation. Baltimore, Williams and Wilkins.
- Bergofsky, E.H., Lehr, D.E. & Fishman, A.P. (1962). The effect of changes in hydrogen ion concentration on the pulmonary circulation. J. Clin. Invest. 41: 1492-1502
- Berry, W.B., McLaughlin, J.S., Clark, W.D. & Morrow, A.G. (1965) The effects of acute hypoxia on pressure, flow, and resistance in the pulmonary vascular bed. Surgery. 58: 404-411

- Berthiaume, L., Joncas, J., Boulay, G., & Pavilanis, V. (1973). Serological evidence of respiratory syncytial virus infection in sheep. Vet. Rec. 93: 337
- Betts, A.O. & Beveridge, W.I.B. (1953). Virus pneumonia of pigs. The effect of the disease upon growth and efficiency of food utilization. Vet. Rec. 65: 515-520
- Biberstein, E.L. & Thompson, D.A. (1966). Epidemiological studies on Pasteurella haemolytica in sheep. J. comp. Path. 76: 83-94
- Biberstein, E.L., Nisbet, D.I. & Thompson, D.A. (1967). Experimental pneumonia in sheep. J. comp. Path. 77: 181-192
- Biberstein, E.L., Shreeve, B.J. & Thompson, D.A. (1970). Variation in carrier rates of Pasteurella haemolytica in sheep flocks. I Normal flocks. J. comp. Path. 80: 499-507
- Bils, R.F. (1970). Ultrastructural alterations of alveolar tissue of mice. III. ozone. Arch. Environ. Health. 20: 468-480
- Blair, W.H., Henry, M.C. & Ehrlich, R. (1969). Chronic toxicity of nitrogen dioxide. II. Effect on histopathology of lung tissue. Arch. Environ. Health. 18: 186-192
- Bohm, G.M. (1966). Vascular permeability changes during experimentally produced pulmonary oedema in rats. J. Path. Bact. 92: 151-161.
- Boidin, A.G., Cordy, D.R. & Adler, H.E. (1958). A pleuropneumonia like organism and a virus in ovine pneumonia in California. Cornell. Vet. 48: 410.
- Boren, H.G. (1964). Carbon as a carrier mechanism for irritant gases. Arch. Environ. Health. 8: 119-124.
- Bosworth, T.J. & Lovell, R. (1944). The occurrence of haemolytic coccobacilli in the nose of normal sheep and cattle. J. comp. Path. 54: 168-171
- Bowden, D.H. & Adamson, I.Y.R. (1971) Reparative changes following pulmonary cell injury: Ultrastructural, cytodynamic, and surfactant studies in mice after oxygen exposure. Arch. Path. 92: 279-283
- Bowden, D.H., Adamson, I.Y.R. & Wyatt, J.P. (1968) Reaction of the lung cells to a high concentration of oxygen. Arch. Path. 86: 671-675
- Bowden, D.H., Adamson, I.Y.R., Grantham, W.G. & Wyatt, J.P. (1969). Origin of the lung macrophage: Evidence derived from radiation injury. Arch. Path. 88: 540-546

- Brewer, D.G., Heath, D. & Asquith, P. (1969). Electron microscopy of desquamative interstitial pneumonia. J. Path. 97: 317-323
- Brooks, R.E. (1971). Ultrastructure of lung lesions produced by ingested chemicals. I. Effect of the herbicide Paraquat on mouse lung. Lab. Invest. 25: 536-545
- Brown, J.H., Cook, K.M., Ney, F.G. & Hatch, T. (1950). Influence of particle size upon retention of particulate matter in the human lung. Amer. J. Public. Health. 40: 450-459.
- Bruce, A. (1868). J. Agric. Soc. N.S.W. 1. 6,22,35,49. Cited by St. George and Sullivan, (1973).
- Brunstetter, M., Hardie, J.A., Schiff, R., Lewis, J.P. & Cross, C.E. (1971) The origin of pulmonary alveolar macrophages: Studies of stem cells using the ES-2 marker of mice. Arch. Int. Med. 127: 1064-1068
- Buckley, R.D. & Loosli, G.G. (1969). Effects of nitrogen dioxide inhalation on germfree mouse lung. Arch. Environ. Health 18: 588-595
- Burroughs, A.L. (1967) Mxovirus parainfluenza-3 respiratory infection in dairy cattle. Am. J. vet. Res. 28: 359-364
- Butler, W.H. (1970) An ultrastructural study of the pulmonary lesions induced by pyrrole derivatives of pyrrolizidine alkaloids. J. Path. 102: 15-19
- Butler, C.I & Kleinerman, J. (1971) Paraquat in the rabbit. Brit. J. Industr. Med. 28: 67-71.
- Cameron, G.R. & Sheikh, A.H. (1951). The experimental production of pulmonary oedema with ammonium salts, together with a classification of lung oedemas. J. Path. Bact. 63: 609-617.
- Campbell, D., Campbell, R.S.F. & Dromey, L. (1949) Pneumonia in sheep associated with infection by a Pasteurella-like organism. Vet. Rec. 61: 64-65
- Carmichael, L.E., St. George, T.O., Sullivan, N.D. & Horsfall, N. (1972) Isolation, propagation and characterization studies of an ovine mycoplasma responsible for proliferative interstitial pneumonia. Cornell. Vet. 62: 654-679
- Carson, S., Goldhamer, R., & Carpenter, R. (1966) Mucus transport in the respiratory tract. Amer. Rev. Resp. Dis. 93: 86-92

- Carter, M.E. & Hunter, R. (1970). Isolation of parainfluenza type 3 virus from sheep in New Zealand. N.Z. vet. J. 18: 226-227
- Castleman, W.L., Dungworth, D.L. & Tyler, W.S. (1973). Cytochemically detected alterations of lung acid phosphatase reactivity following ozone exposure. Lab. Invest. 29: 310-319.
- Cheema, R.A., Kazimi, S.E. & Majeed, M.A. (1965) Studies on the etiology of lamb pneumonia. W. Pakist. J. agric. Res. 3: 97-105
- Clapper, W.E., & Meade, G.H. (1963). Normal flora of nose, throat and lower intestine of the dog. J. Bact. 85: 643-648
- Clark, D.G., McElliot, T.G. & Weston Hurst, E. (1966). The toxicity of Paraquat. Br. J. Industr. Med. 23: 126-132.
- Clarke, J.K., McFerran, J.B. & Gay, F.W. (1972). Use of allantoic cells for the detection of avian infectious bronchitis virus. Arch. Ges. Virusforsch. 36: 62-70
- Clarke, J.K., Brown, V.G. & Alley, M.R. (1974). Isolation and identification of mycoplasmas from the respiratory tract of sheep in New Zealand. N.Z. vet J. 22: 117-121
- Coalson, J. Beller, J.J. & Greenfield, L.J. (1971) Effects of 100 per cent oxygen ventilation on pulmonary ultrastructure and mechanics. J. Path. 104: 267-273
- Cole, A.M. (1970). The aetiology of calf pneumonia in Queensland. Aust. vet.J. 46: 576
- Conning, D.M., Fletcher, K. & Swan, A.A.B. (1969) Paraquat and related bipyridyls. Brit. Med. Bull. 25: 245-249.
- Cook, T.F. (1966) Sheep dipping mortalities. N.Z. vet J. 14: 55-61
- Corrin, B. & King, E. (1969) Experimental endogenous lipid pneumonia and silicosis. J. Path. 97: 325-330
- Cottew, G.S. Sutherland, A.K. and Meehan, J.F. (1952) Meliodosis in sheep in Queensland. Aust.vet J. 28: 113-123.
- Cottew, G.S. (1955) Meliodosis. Aust.vet. J. 31: 155-158
- Cottew, G.S. (1971) Characterisation of mycoplasmas isolated from sheep with pneumonia. Aust. vet. J. 47: 591-596
- Cottew, G.S., Watson, W.A., Arisoy, F., Erdag, O. & Buckley, L.S. (1968) J. comp. Path. 78: 275-282
- Cottrell, T.S., Levine, O.R. Senior, R.M., Wiener, J., Spiro, D. & Fishman, A.P. (1967). Electron microscopic alterations at the alveolar level in pulmonary oedema. Circul. Res. 21: 783-797

- Cowan, S.T. & Steel, K.F. (1965). Manual for the Identification of Medical Bacteria. London.
- Cowdry, E.V. & Marsh, H. (1927). Comparative pathology of South African Jaagsiekte and Montana progressive pneumonia of sheep. J. exp. Med. 45: 571-585
- Creasia, D.A. Nettesheim, P. & Hammons, A.S. (1973). Arch. Environ. Health. 26: 197-201
- Creech, G.T. & Gochenour, W.S. (1936). Chronic progressive pneumonia of sheep, with particular reference to its etiology and transmission. J. agric. Res. 52: 667-679
- Crowe, C.C., Sanders, W.E. & Longley, S. (1973). Bacterial interference II. Role of the normal throat flora in prevention of colonization by group A. streptococcus. J. Infec. Dis. 128: 527-532.
- Cuba-Caparo, A., de la Vega, E. & Copaira, M. (1961) Pulmonary adenomatosis of sheep: Metastasising bronchiolar tumours. Am.J. vet. Res. 22 673-682
- Culling, C.F.A. (1963) Handbook of Histopathological and Histochemical Techniques. 2nd Edition. London.
- Darbyshire, J.H. (1961) A fatal ulcerative mucosal condition of sheep associated with the virus of contagious pustular dermatitis. Br. vet.J. 117: 97-105
- Darbyshire, J.H. & Pereira, H.G. (1964) An adenovirus precipitating antibody present in some sera of different animal species and its association with bovine respiratory disease. Nature, London. 201: 895-897
- Darke, C.S. & Warrack, A.J.N. (1958). Bronchiolitis from nitrous fumes. Thorax 13: 327-333
- Davis, G.B. (1969) Pers. Comm.
- Davis, G.B. (1974): A sheep mortality survey in Hawke's Bay. N.Z. vet. J., 22: 39-42
- Degré, M. (1970). Synergistic effect in viral-bacterial infection. 2. Influence of viral infection on the phagocytic ability of alveolar macrophages. Acta. path. microbiol. scand. Sect.B. 78: 41-50.
- Degré, M. & Glasgow, L.A. (1968) Synergistic effect in viral-bacterial infection. I Combined infection of the respiratory tract in mice with parainfluenza virus and Hemophilus Influenza. J. infect. Dis. 118: 449-462

- de Kock, G. (1929). Are the lesions of jaagziekte in sheep of the nature of neoplasm? 15th Rep. Dir. Vet. Serv. Union of S. Afr. 611-641
- Dhanda, M.R., G.L. Sharma. & Bhalla, N.P. (1963). Possible occurrence of jaagsiekte in indigenous sheep. Indian J. vet. Sci. 33: 84-88
- Dieana, S. & Cereto, F. (1967). XXI Conv. Soc. Ital. Sci. Vet. Senigallia p.612. Cited by Krauss & Wandera (1970).
- Dillard, C.J., Urribarri, N. Reddy, K., Fletcher, B., Taylor, S., de Lumen, B., Langberg, S. & Tappel, A.L. (1972). Increased lysosomal enzymes in lungs of ozone-exposed rats. Arch. Environ. Health. 25: 426
- Diller, W., Bils, R.F., Kimmerle, G. & Huth, F. (1969) The early phases of phosgene poisoning, light microscope, electron microscope and roentgenological studies. Virchows. Arch. Abt. A. Path. 348: 230-248.
- Ditchfield, J. (1966). Isolation of parainfluenza-3 virus from Canadian sheep. Vet. Rec. 79: 773
- Doniach, I. (1947). Uremic edema of the lungs. Amer. J. Roentgenol. 58: 620-628
- Downey, N.E. (1957). A preliminary investigation into the aetiology of enzootic pneumonia of sheep ('Southland pneumonia') N.Z. vet. J. 5: 128-133
- Duke, H.N. (1954). The site of action of anoxia on the pulmonary blood vessels of the cat. J. Physiol. 125: 373-382
- Dungal, N. (1931) Contagious pneumonia in sheep. J. comp. Path. 44: 126-143
- Dungal, N. (1938). Epizootic adenomatosis of the lungs of sheep: its relation to verminous pneumonia and jaagseikte. Proc. royl Soc. Med. 31: 497-505
- Dungal, N. (1946). Experiments with jaagsiekte. Am. J. Path. 22: 737-759
- Dungworth, D.L. & Cordy, D.R. (1962a). The pathogenesis of ovine pneumonia. I Isolation of a virus of the PLV group. J. comp. Path. 72: 49-70

- Dungworth, D.L. & Cordy, D.R. (1962b). The pathogenesis of ovine pneumonia. II. Isolation of virus from faeces; comparison of pneumonia caused by faecal, enzootic abortion and pneumonitis viruses. J. comp. Path. 72: 71-79
- Duran-Reynals, F., Jungherr, E., Cuba-Caparo, A., Rafferty, K.A.Jr. & Helmbolt, C. (1958). The pulmonary adenomatosis complex in sheep. Ann. N.Y. Acad. Sci. 70: 726-742
- Durusan, R. & Dogyer, M. (1955) Turkiye de kuzularin akcigerlerinden izole ploropnomonia gurubuna dahil organizmler. Turk vet. Hekin. dern. Derg. 25: 2217-2230. Cited by Stevenson (1969).
- Eaton, R.M. (1947). Pulmonary oedema: Experimental observations on dogs following acute peripheral blood loss. J. Thorac. Cardiovasc. Surg. 16: 668-694
- Eber, A. (1899). Uber multiple adenombildung in den lungen der schafe. Zeitschrift fuer tiermedizin. 3: 161-172. Cited by Wandera (1971)
- Ehrlich, R. & Henry, M.C. (1968). Chronic toxicity of nitrogen dioxide I. Effect on resistance to bacterial pneumonia. Arch. Environ. Health. 17: 860-865
- Eiseman, B. (1968). Introduction to conference. J. Trauma. 8: 649-650
- Emery, J. (1969). The Anatomy of the Developing Lung. London
- Enson, Y., Guitini, C., Lewis, M.L., Morris, T.Q., Ferrer, I.M. & Harvey, R.M. (1964). The influence of hydrogen ion concentration and hypoxia on pulmonary circulation. J. clin. Invest. 43: 1146-1162
- Epling, B.P. (1964) Electron microscopy of the bovine lung: The normal blood-air barrier. Am. J. vet. Res. 25: 679-689
- Euler, U.S. von & Liljestrand, E. (1946). Observations on the pulmonary arterial blood pressure in the cat. Acta. physiol. scand. 12: 301-320
- Evans, M.J., Cabral, L.J., Stephens, R.J. & Freeman, G. (1973). Renewal of alveolar epithelium in the rat following exposure to NO₂. Am. J. Path. 70: 175-190
- Ewing, W.H. (1968). Differentiation of enterobacteriaceae by biochemical methods. National Communicable Disease Center, Atlanta, Ga.

- Farzaliev, M.M., Khalimbekov, M.M., Dandamaev, S.G. & Aliev, A.I. (1962). Pneumonia in lambs : The role of P.P.L.O. Veterinariya, Moscow, 2: 27-30. Cited by Stevenson, (1969).
- Faulkner, C.S. & Esterley, J.R. (1971). Ultrastructural changes in the alveolar epithelium in response to Freund's adjuvant. Amer. J. Path. 71: 559-564
- Faye, P., Charton, A. & Le Layec (1967). Presence, in sheep serum, of myxovirus parainfluenzae haemagglutination-inhibiting antibodies. Bull. Acad. vet. Fr., 40: 203-207
- Ferin, J. (1971). Emphysema in rats and clearance of dust particles. In Inhaled Particles III. Vol 1., by W.H. Walton. 283-292, England.
- Filmer, J.F. (1957). Pneumonia in sheep. N.Z. Dept. Agr. Anim. Res. Div., Ann. Rep. 156-57. 16-7
- Finegold, M.J. (1967). Interstitial pulmonary oedema. An electron microscopic study of the pathology of staphylococcal enterotoxemia in Rhesus monkeys. Lab. Invest. 16: 912-924
- Finlay-Jones, J.M., Robertson, T.A. & Walters, M.N.I. (1971). The effect of anaesthetics on pulmonary ultrastructure. Pathology. 3: 181-190
- Fischman, H.R., (1965). Presence of neutralizing antibody for myxovirus parainfluenza-3 in sheep sera. Proc. Expt. Biol. & Med. 118: 725-727
- Fischman, H.R. (1967). Epidemiology of parainfluenza-3 infection in sheep. Am. J. Epidem. 85: 272-281
- Fisher, H.K. & Clements, J.A. (1969). Effects of the herbicide Paraquat on mechanical properties of rat lungs. Fed. Proc. 28: 526
- Fishman, A.P., Fritts, H.W., & Cournand, A. (1960). Effects of breathing carbon dioxide upon the pulmonary circulation. Circulation. 22: 220-225
- Fishman, M. (1969). Induction of antibodies in vitro. Ann. Rev. Microbiol. 23: 199-222
- Fletcher, K. & Wyatt, I. (1970). The composition of lung lipids after poisoning with Paraquat. Br. J. Exp. Path. 51: 604
- Fletcher, K & Wyatt, I. (1972). The action of Paraquat on the incorporation of palmitic acid into dipalmitoyl lecithin in mouse lungs. Br. J. Exp. Path. 53: 225-230

- Florey, H.W. (1970). General Pathology. 4th Edition. London.
- Ford, R.J. & Kuhn, C. (1973). Immunologic competence of alveolar cells
I. The plaque-forming response to particulate and soluble antigens. Amer. Rev. Resp. Dis. 107: 763-770
- Francis, J. (1958). Tuberculosis in Man and Animals. London.
- Freeman, G., Crane, S.C., Stephens, R.J. & Furiosi, N.J. (1968). Pathogenesis of the nitrogen dioxide-induced lesion in the rat lung. Amer. Rev. Resp. Dis. 98: 429-443
- Freeman, G., Stephens, R.J., Coffin, D.L. & Stara, T.F. (1973). Changes in dogs lungs after long-term exposure to ozone - light and electron microscopy. Arch. Environ. Health. 26: 209-216.
- Frey, M.L., Hanson, R.P. & Anderson, D.P. (1968). A medium for isolation of avian mycoplasmas. Am. J. vet. Res. 29: 2163-2171
- Fuller, C.J. (1953). Farmer's lung: A review of present knowledge. Thorax. 8: 59-64
- Furlong, S.L. & Cottew, G.S. (1973). Identity of mycoplasmas from sheep pneumonia in Queensland and Victoria. Aust. vet. J. 49: 215.
- Gallagher, C.H. (1962). The effect of drenching technique on poisoning of sheep with carbon tetrachloride. Aust. vet. J. 38: 575-579
- Gallagher, C.H. (1964). Effects of carbon tetrachloride administered into the trachea or the rumen of sheep. Aust. vet. J. 40: 229-232
- Gardner, D.E., Holzman, R.S. & Coffin, D.L. (1968). Depression of phagocytosis by sulfamethazine. J. Bact. 96: 274-275
- Gell, P.G.H. & Coombs, R.R.A. (1968). Clinical Aspects of Immunology. 2nd Edition. Oxford.
- Georgsson, G. & Pålsson, P.A. (1971). The histopathology of maedi. A slow viral pneumonia of sheep. Vet. Path. 8: 63-80
- Gibb, F.R. & Morrow, P.E. (1962). Alveolar clearance in dogs after inhalation of an iron 59 oxide aerosol. J. Appl. Physiol. 17: 429-432
- Gil, J. & Thurnheer, U. (1971) Morphometric evaluation of ultrastructural changes in type II alveolar cells of rat lung produced by bromhexine. Respiration 28: 438-456
- Gilmour, N.J.L. & Brotherston, J.G. (1963). Clinical and pathological findings in an outbreak of pneumonia in sheep. J. comp. Path. 73: 324

- Gilmour, N.J.L., Drysdale, A., Stevenson, R.G., Hore, D.E. & Brotherston, J.G. (1968). Reaction of young adult sheep to vaccination and infection with myxovirus parainfluenza-3. J. comp. Path. 78: 463-458
- Gilruth, J.A., (1900) Eighth annual report of the New Zealand Department of Agriculture. p 197
- Goldstein, E. & Green, G.M. (1966). The effect of acute renal failure on the bacterial clearance mechanisms of the lung. J. Lab. Clin. Med. 68: 531-542
- Goldstein, E., Tyler, W.S., Hoeprich, P.D. & Eagle, C. (1971). Adverse influence of ozone on pulmonary bactericidal activity of murine lung. Nature. 229- 262-263
- Goldstein, E., Eagle, M.C. & Hoeprich, P.D. (1973). Effect of nitrogen dioxide on pulmonary bacterial defence mechanisms. Arch. Environ. Health. 26: 202-206
- Gourdon, R., Gourdon, J.M., Henry, M. & Quinchon, C. (1957). Recherches sur Pasteurella septica I. Etude bacteriologique de souches isolees d'animaux des especes porcine et ovine. Ann. Inst. Pasteur. 93: 251-256
- Gourlay, R.N., MacKenzie, A. & Cooper, J.E. (1970). Studies of the microbiology and pathology of pneumonic lungs of calves. J. comp. Path. 80: 575-584
- Green, G.M. (1968). Pulmonary clearance of infectious agents. Ann. Rev. Med. 19: 315-336
- Green, G.M. (1969). The response of the alveolar macrophage system to host and environmental changes. Arch. Environ. Health. Vol 18: 548-550
- Green, G.M. (1970a). In defence of the lung. Amer. Rev. Resp. Dis. 102: 691-703
- Green, G.M. (1970b) Integrated defence mechanisms in models of chronic pulmonary disease. Arch. Intern. Med. 126: 500-503
- Green, G.M. & Kass, E.H. (1964a). The role of the alveolar macrophage in the clearance of bacteria from the lung. J. Exp. Med. 119: 167-176
- Green, G.M. & Kass, E.H. (1964b). Factors influencing the clearance of bacteria by the lung. J. Clin. Invest. 43: 769-776
- Green, G.M. & Kass, E.H. (1965). The influence of bacterial species on pulmonary resistance to infection in mice subjected to hypoxia cold stress, and ethanolic intoxication. Brit. J. Exp. Path. 46: 360-366

- Green, G.M. & Carolin, D. (1967). The depressant effect of cigarette smoke on the in vitro anti-bacterial activity of alveolar macrophages. New Eng. J. Med. 276-421.
- Greenberg, S.O., Gyorkey, F., Jenkins, D.E. & Gyorkey, P. (1971). Alveolar epithelial cells following exposure to nitric acid. Arch. Environ. Health. 22: 655-662
- Grieg, A.S. (1955) The isolation of pleuropneumonia-like organisms from the respiratory tracts of sheep. Canad. J. comp. Med. and Vet. Sci. 19: 265-271
- Gross, P. & Westrick, M.L. (1954). The permeability of the lung parenchyma to particulate matter. Am. J. Pathol. 30: 195-207
- Gross, P. Braun, D.C. & de Treville, R.T.P. (1972) The pulmonary response to coal dust. Ann. N.Y. Acad. Sci. 200: 155-165
- Gudnadóttir, M. & Pálsson, P.A. (1965). Successful transmission of visna by intrapulmonary inoculation. J. infect. Dis. 115: 217-225
- Gudnadóttir, M. & Pálsson, P.A. (1967). Transmission of maedi by inoculation of a virus grown in tissue culture from maedi-affected lungs. J. infect. Dis. 117: 1-6
- Guillaume, J., Wattel, F., Macki-van-Mooleghem, C., Aerts, C. & Voisin, C. Etude de la phagocytose du virus grippal par les macrophages alveolaires de coybaye, II Effets de la phagocytose du virus grippal sur le metabolisme cellulaire. Ann. Inst. Pasteur. Lille. 16: 11-15
- Hahn, F.F., Goldstein, E. & Dungworth, D.L. (1971). Effect of whole body x-irradiation on pulmonary bactericidal function. Radiat. Res. 47: 461-471
- Halmagyi, D.F.J. (1961) Lung changes and incidence of respiratory arrest in rats after aspiration of sea and fresh water. J. Appl. Physiol. 16: 41-44.
- Hamdy, A.H. & Pouden, W.D. (1959). Experimental production of pneumonia in lambs. Am. J. vet. Res. 20: 78-83
- Hamdy, A.H., & Sanger, V.L. (1959). Characteristics of a virus associated with lamb pneumonia. Am. J. vet. Res. 20: 84-86
- Hamdy, A.H. Pouden, W.D. & Ferguson, L.C. (1959). Microbial agents associated with pneumonia in slaughtered lambs. Am. J. vet. Res. 20: 87-90
- Hanichen, T. (1964) Lesions in bronchial and alveolar epithelium in enzootic virus pneumonia of pigs. Zbl.f. Vet. Med. II 251-269

- Hargreave, T.B., Gresham, G.A. & Karayannopoulos, S. (1969).
Paraquat poisoning. Postgrad. Med. 45: 633-635
- Harris, D.L., Ross, R.F. & Switzer, W.P. (1969). Incidence of certain
micro-organisms in the nasal cavities of swine in Iowa.
Am. J. vet. Res. 30: 1621-1624
- Harrison, G. (1970). Lung ultrastructure effects of breathing
pure O₂ at 0.6 ATM for 90 days. Sept. Congres. de Internat.
de Microscopie Electronique. p851.
- Harrison, G.A. (1971). Ultrastructural changes in rat lung during
long-term exposure to oxygen. Exp. Med. Surg. 29: 96-107
- Hasleton, P.S., Heath, D. & Brewer, D.B. (1967). Hypertensive
pulmonary vascular disease in states of chronic hypoxia.
J. Path. Bact. 95: 431-440
- Hatch, T. (1961). Distribution and deposition of inhaled particles
in the respiratory tract, conference on air-borne infection.
Bacteriol. Rev. 25: 237-240
- Hatch, T.F. & P. Gross. (1964). Pulmonary Deposition and Retention
of Inhaled Aerosols. Published Academic Press.
- Haugaard, N. (1968). Cellular mechanisms of oxygen toxicity.
Physiol. Rev. 48: 311-373
- Hayes, J.A. & Shiga, Amy. (1970). Ultrastructural changes in
pulmonary oedema produced experimentally with ammonium sulphate.
J. Path. 100: 281-286
- Hayflick, L. (1965). Tissue cultures and mycoplasmas. Texas. Rep.
Biol. Med. 23: (Supplm 1). 285-303
- Heard, B.E. & Cooke, R.A. (1968). Busulphan lung. Thorax. 23:
187-193
- Heath, D. (1968). Hypertensive pulmonary vascular disease in
emphysema and states of chronic hypoxia. In Form and Function
in the Human Lung ed. by G. Cumming and L.B. Hunt. London
- Henning, M.W. & Brown, M.H.V. (1936) Pasteurellosis: An outbreak amongst
sheep. Onderstepoort. J. vet. Sci. No 7 113-131
- Henry, J.N. (1968). The effect of shock on pulmonary alveolar
surfactant. J. Trauma 8: 756-770
- Heppleston, A.G. (1954). Pathogenesis of simple pneumoconiosis in
coal workers. J. Path. Bact. 67: 51-63

- Hepplestone, A.G. (1963). The disposal of inhaled particulate matter: A unifying hypothesis. Am. J. Path. 42: 119-134
- Heppleston, A.G. & Styles, J.A. (1967). Activity of a macrophage factor in collagen formation by silica. Nature (London) 214: 521-522
- Heppleston, A.G. & Young, A.E. (1972). Alveolar lipo-proteinosis : An ultrastructural comparison of the experimental and human forms. J. Path. 107: 107-117
- Heppleston, A.G. & Young, A.E. (1973). Uptake of inert particulate matter by alveolar cells: An ultrastructural study. J. Path. 111: 159-164
- Hesse, F.E. & Loosli, C.G. (1949). The lining of the alveoli in mice, rats, dogs and frogs following acute pulmonary edema produced by ANTU poisoning. Anat. Rec. 105: 299-312
- Holmes, P.H. & Bremner, K.C. (1971). The pathophysiology of ovine ostertagiasis: Water balance and turnover studies. Res. vet. Sci. 12: 381-383
- Holt, P.G., & Keast, D. (1973). Cigarette smoke inhalation : Effects on cells of the immune series in murine lung. Life Sciences 12: 377-387
- Holt, S.J. & Withers, R.F.J. (1958). Studies in enzyme cytochemistry. V. An appraisal of endogenous reactions for esterase localization. Proc. Roy. Soc. B. 148: 520-532
- Holub, M. & Hauser, R.E. (1969). Lung alveolar histiocytes engaged in antibody production. Immunology 17: 207-226
- Hopkirk, C.S.M. (1936). Ann. Report of N.Z. Dept. of Agriculture p.30.
- Hore, D.E. (1966) Isolation of ovine strains of parainfluenza virus serologically related to type 3. Vet. Rec. 79: 466-467
- Hore, D.E. (1970). Pneumonia in sheep. Aust. vet. J. 46: 169-172
- Hore, D.E. & Stevenson, R.G. (1967). Experimental virus pneumonia in lambs. Vet. Rec. 80: 26-27
- Hore, D.E. & Stevenson, R.G. (1969). Respiratory infection of lambs with an ovine strain of parainfluenza virus type 3. Res. vet. Sci. 10: 342-350

- Hore, D.E., Stevenson, R.G., Gilmour, N.J.L., Vantsis, J.T. & Thompson, D.A. (1968). Isolation of parainfluenza virus from the lungs and nasal passages of sheep showing respiratory disease. J. comp. Path. 78: 259-265
- Howe, D.L., Woods, G.T. & Marquis, G. (1966). Infection of bighorn sheep (Cvis canadensis) with myxovirus parainfluenzae-3 and other respiratory viruses. Bull. Wildlife. Dis. A. 2: 34-37
- Huber, G.L. & La Force, F.M. (1970). Comparative effects of oxone and oxygen on pulmonary antibacterial defence mechanisms. Antimicrob. Agents and Chemother. 129-136
- Huber, G.L., LaForce, F.M., Mason, R.J. & Monaco, A.P. (1970). Acute and chronic effects of immunosuppressive agents on intrapulmonary defence mechanisms. Clin. Res. 18: 485
- Humberstone, F.D. (1963) A gram-type stain for the simultaneous demonstration of gram-positive and gram-negative micro-organisms in paraffin sections. J. med. Lab. Technol. 20: 153-156
- Hurst, D.J., Gardiner, D.E. & Coffin, D.L. (1970). Effect of ozone on acid hydrolases of the pulmonary alveolar macrophage. J. Reticuloendothel. Soc. 8: 288-300
- Hyadon, G.B., Freeman, G. & Furiosi, N.A. (1965) Covert pathogenesis of NO₂ induced emphysema in the rat. Arch. Environ. Health. 11: 776-783
- Jack, E.J. (1968). *Salmonella abortus-ovis*: An atypical salmonella. Vet. Rec. 82: 558-561
- Jenkins, M.T., Jones, R.F., Wilson, B. & Moyer, C.A. (1950). Congestive atelectasis - a complication of the intravenous infusion of fluids. Ann. Surg. 132: 327-347
- Jericho, K.W. (1966). Intra-pulmonary lymphoid tissue in pigs. Vet. Bull. 36: 687-707
- Jolly, R.D. & Ditchfield, J. (1965). Bronchopneumonia of calves caused by parainfluenza virus type 3. Can. vet. Jour. 6: 295-297
- Jowett, W. (1930). Pyobacillosis in sheep. J. comp. Path. 43: 109-117.

- Jubb, K.V.F. & Kennedy, P.C. (1970) Pathology of Domestic Animals, 2nd Edition. New York and London.
- Karrer, H.E. (1956) The ultrastructure of mouse lung. J. Biophys. Biochem. Cytol. 2: 241-251
- Kass, E.H., Green, G.M. & Goldstein, E. (1966). Mechanisms of antibacterial action in the respiratory system. Bact. Rev. 30: 488-496
- Kennedy, R.C., Eklund, C.M. Lopez Carlos, Hadlow, W.E. (1968) Isolation of a virus from the lungs of Montana sheep affected with progressive pneumonia. Virology. 35: 483-484
- Kikkawa, Y., Motoyama, E.K. & Cook, C.D. (1965). The ultrastructure of the lungs of lambs. Am. J. Path. 47: 877-903
- Kilburn, K.H. (1969). Alveolar clearance of particles. A bullfrog lung model. Arch. Environ. Health. 18: 556-563
- Kilburn, K.H. (1970). Alveolar microenvironment. Arch. Intern. Med. 126: 435-449
- Kimbrough, R.D. & Gaines, T.B. (1970). Toxicity of Paraquat to rats and its effect on rat lungs. Tox. Appl. Pharmacol. 17: 679-690
- Kimbrough, R.D. & Linder, R.E. (1973). The ultrastructure of the Paraquat lung lesion in the rat. Environ. Res. 6: 265-273
- Kirton, A.H. (1968). The seasonal incidence of pneumonia in lambs and its effect on growth. Ann. Rep. Res. Div. (1967-68) N.Z. Dept. of Agric. p 38
- Kistler, G.S., Caldwell, P.R.B. & Weibel, E.R. (1967). Development of fine structural damage to alveolar and capillary lining cells in oxygen poisoned rat lungs. J. Cell. Biol. 32: 605-628
- Kitamura, H. (1972). Studies of experimental pneumonitis in rat induced by anti-rat-lung serum. Acta Path. Jap. 22: 823-830
- Krauss, A. & Wandera, J.G. (1970). Isolation and properties of mycoplasma from the respiratory tract of sheep with jaagsiekte. J. comp. Path. 80: 389-396
- Kuida, H., Hinshaw, L.B., Gilbert, R.P. & Visscher, M.B. (1958). Effect of gram-negative endotoxin on pulmonary circulation. Amer. J. Physiol. 192: 335-344
- Kylstra, J.A. (1965) Survival of submerged mammals. New Eng. J. Med. 272: 198-200

- LaBelle, C.W. & Brieger, H. (1961). Patterns and mechanisms in the elimination of dust from the lung. In Inhaled Particles and Vapours p356-365. Ed. C.N. Davies. New York and London
- LaForce, F.M. & Huber, G.L. (1970). Effect of a subacute extrapulmonary infection on antibacterial defense mechanisms of the lung. Antimicrob. Agents and Chemother. 332-334.
- LaForce, F.M., Mullane, J.F., Boehme, R.F., Kelly, W.J. & Huber, G.L. (1973). The effects of pulmonary edema on antibacterial defenses of the lung. J. Lab. Clin. Med. 82: 634-648
- Langham, R.F., Thorp, F. & Ingle, R.T. (1941). Some observations on the pathology of pneumonia of the food-producing animals. J. Am. Vet. Med. Assn. 99: 207
- Laurenzi, G.A. & Guarneri, J.J. (1966). The study of mechanisms of pulmonary resistance to infection: The relationship of bacterial clearance to ciliary and alveolar macrophage function. Am. Rev. Resp. Dis. 93: 134-141
- Laurenzi, G.A., Berman, L., First, M. & Kass, E.H. (1964). A quantitative study of the deposition and clearance of bacteria in the murine lung. J. clin. Invest. 43: 759-768
- Lauweryns, J.M. & Boussauw, L. (1969). The ultrastructure of pulmonary lymphatic capillaries of newborn rabbits and babies. Lymphology 2: 108-129
- Laws, L. & Hall, W.T.K. (1964). Meliodosis in animals in North Queensland IV. Epidemiology. Aust. vet. J. 40: 309-314
- Leach, R.H. (1970). The occurrence of Mycoplasma Arginini in several animal hosts. Vet. Rec. 87: 319-320
- LeRoy, E.P. (1969). The blood-air barrier in desquamative interstitial pneumonia. Virchows. Arch. Abt. A. Path. 348: 117-130
- LeRoy, E.P., Liebner, E.J. & Jensik, R.J. (1965). The ultrastructure of alveoli after supervoltage radiation of the lung. Am. J. Path. 46: 26a
- Leyshon, W.J. (1932). A note on an ovine affection. Vet. J. 88: 256-259.
- Liebow, A.A. (1962). Recent advances in pulmonary anatomy. In Ciba Foundation Symposium on Pulmonary Structure and Function. Ed. A.V.S. de Reuck and M.O'Connor. London.

- Liljestrand, G. (1958). Chemical control of the distribution of pulmonary blood flow. Acat. physiol. scand. 14: 120
- Littler, W.A., Kay, J.M., Hasleton, P.S. & Heath, D. (1969) Busulphan lung. Thorax 24: 639-655
- Loosli, C.G., Hertweck, M.S. & Hockwald, R.S. (1970). Airborne influenza PR8-A virus infections in actively immunized mice. Arch. Environ. Health. 21: 332-346.
- Low, F.N. (1952). Electronmicroscopy of the rat lung. Anat. Rec. 113: 437-443
- Lucam, F. (1942). La "Bouhite" ou "Lymphomatose pulmonaire maligne du mouton". Rec. Med. vet. 118: 273-284
- Luke, D. (1958). Tuberculosis in the horse, pig, sheep and goat. Vet. Rec. 70: 529-536
- Lullmann-Rauch, R., Reil, G.H., Rossen, E. & Seiler, K.U. (1972). The ultrastructure of rat lung changes induced by an anorectic drug (chlorphentermine). Virch. Arch. B. Zellpath. 11: 167-181
- Mackay, J.M.K. (1966). Lung tumours in animals. Proc. III. Int. Conf. Cancer. Perugia. 1965. pp.403-412
- Mackay, J.M.K. (1969). Tissue culture studies of sheep pulmonary adenomatosis (Jaagsiekte) I and II. J. Comp. Path. 79: 141-150
- Mackay, J.M.K. & Nisbet, D.I. (1966). Jaagsiekte - a hazard of intensified sheep husbandry. Vet. Rec. 78: 18-20
- Mackay, J.M.K., Nisbet, D.I. & Foggie, A. (1963). Isolation of PPD's (genus Mycoplasma) from cases of sheep pulmonary adenomatosis. S.P.A. Vet. Rec. 75: 550-551
- McGowan, B. Mculton, J.E. & Shultz, G. (1957). Pneumonia in California lambs. J. Am. vet. Med. Ass. 131: 318-323
- McKercher, D.G., (1952). A virus possibly related to the psittacosis-lymphogranuloma-pneumonitis group causing a pneumonia in sheep. Science 115: 543-545
- McNab, J. (1974). Pers. Comm.
- McQueen, J.L. & Davenport, F.M. (1963). Experimental influenza in sheep. Proc. Soc. exp. Biol. Med. 112: 1004-1006
- Magwood, S.E., Barnum, D.A. & Thomson, R.G. (1969). Nasal bacterial flora of calves in healthy and in pneumonia-prone herds. Canad. J. comp. Med. 33: 237-243

- Malmquist, W.A., Krauss, H.H., Moulton, J.E. & Wandera, J.G. (1972). Morphologic study of virus-infected lung cell cultures from sheep pulmonary adenomatosis (Jaagsiekte). Lab. Invest. 26: 528-533
- Manktelow, B.W. (1967a). The loss of pulmonary surfactant in Paraquat poisoning. Br. J. exp. Path. 48: 366-369.
- Manktelow, B.W. (1967b) Pulmonary surfactant in pathology. Ph.D. Thesis, Bristol University.
- Mant, A.K. (1969). Autopsy diagnosis of accidental hypothermia. J. Forensic. Med. 16: 126-129
- Marino, A.A. & Mitchell, J.T. (1972). Lung damage in mice following intraperitoneal injection of butylated hydroxytoluene (36407). Proc. Soc. exp. Biol. & Med. 140: 122-125
- Markson, L.M. & Terlecki, S. (1964) The experimental transmission of ovine pulmonary adenomatosis. Path. vet. 1, 269-288
- Marsh, H. (1923). Progressive pneumonia in sheep. J. Am. vet. med. Ass. 62(13) 458-473
- Marsh, H. (1953). The role of pasteurilla in sheep diseases. J. Am. vet. med. Assn. 123: 205-208
- Mettam, R.W.M. (1927). Investigations into lung diseases of lambs and sheep. Ann. Report. Dept. of Agric. Kenya. 163-179
- Mettam, R.W.M. (1929). Laikipia lung disease. Ann. Report. Dept. of Agric. Kenya. 333-362
- Meyrick, B. & Reid, L. (1970). The alveolar wall. Brit. J. Dis. Chest. 64: 121-140
- Meyrick, B. Miller, J. & Reid, L. (1972). Pulmonary oedema induced by ANTU, or by high or low oxygen concentrations in rat - an electron microscopic study. Br. J. Expt. Path. 53: 348-358.
- Milic-Emili, J., Henderson, J.A.M. & Kaneko, K. (1968). Regional Distribution of pulmonary ventilation. In Form and Function in the Human Lung. Ed. by Cumming, G. & Hunt, L.G. Edinburgh and London.
- Millar, R.A., Warden, J.C., Cooperman, L.H. & Price, H.L. (1970). Further studies of sympathetic actions of anaesthetics in intact and spinal animals. Brit. J. Anaesth. 42: 366-378

- Miller, K. & Harington, J.S. (1972). Some biochemical effects of asbestos on macrophages. Brit. J. Exp. Path. 53: 397-405
- Miller, N.J. (1940) Diseases incident to the fattening of lambs. J. Am. vet. med. Assn. 96: 24-29
- Ministry of Agriculture, Fisheries and Food. Animal Disease Surveys Rep. No 3. (1964). London : H.M. Stat. Off. pp17-27.
- Montgomerie, R.F., Bosworth, T.J. & Glover, R.E. (1938). Enzootic pneumonia in sheep. J. comp. Path. 51: 87-107
- Moran, T.J. (1955). Experimental aspiration pneumonia. IV. Inflammatory and reparative changes produced by intratracheal injections of autologous gastric juice and hydrochloric acid. Arch. Path. 60: 122-129
- Morcos, Z., Zaki, O.A. & Zaki, R. (1946). Contagious ovine pneumonia caused by a filterable virus. Vet. Med. 41: 202-204
- Morrow, P.E. (1972). Lymphatic drainage of the lung in dust clearance. Ann. N.Y. Acad. Sci. 200: 46-65
- Newsom, I.E. & Cross, F. (1932). An outbreak of hemorrhagic septicaemia in sheep. J. Am. vet. med. Ass. 80: 711-719
- Nieberle, K. & Cohrs, P. (1967). Textbook of the Special Pathological Anatomy of Domestic Animals. First English Edition. Oxford. p 270
- Niemoeller, H. & Schaefer, K.E. (1962). Development of hyaline membranes and atelectasis in experimental chronic respiratory acidosis. Proc. Soc. exp. Biol. 110: 804-808
- Nienhaus, H. & Ehrewfeld, M. (1971). Pathogenesis of lung disease in Paraquat poisoning. Beitrag. Pathol. 142: 244-267
- Nisbet, D.I., Mackay, J.M.K., Smith, W. & Gray, E.W. (1971). Ultrastructure of sheep pulmonary adenomatosis (jaagsiekte). J. Path. 103: 157-162
- Nobel, T.A. (1958). Pulmonary adenomatosis (jaagsiekte) in sheep with special reference to its occurrence in Israel. Refuah Vet. 15: 98-101
- Oikawa, M. (1970). Pulmonary alveolar surfactant in irradiated lung of rat. Tohoku. J. exp. Med. 102: 265-272
- Okada, Y. & Genka, K. (1966). Histological and electron microscopic observation of adenomatous hyperplasia of the alveolar epithelial cells in the human lung. Acta. tuberc. jap. 16:

- Oliner, H., Schwartz, R., Rubio, F. & Dameshek, W., (1961).
Interstitial pulmonary fibrosis following bulsulphan therapy.
Amer. J. Med. 64: 154
- Omar, A.R. (1964). The characteristic cells of the lung and their
reaction to injury. Part II. Vet. Bull. 34: 431-443
- Omar, A.R. (1966). The aetiology and pathology of pneumonia in calves.
Vet. Bull. 36: 259-273
- Page, A.R. (1972). Inflammatory mechanisms and fever. In Pathophysiology.
Altered Regulatory Mechanisms in Disease. Ed. by E.D. Frohlich
Philadelphia, Toronto.
- Palsson, P.A. (1973). Maedi-visna. J. clin. Path. 25: Suppl 6:
115-120
- Parish, W.E. & Pepys, J. (1968). The lung in allergic disease.
In Clinical Aspects of Immunology. Ed. by P.G.H. Gell and
R.R.A. Coombs. Oxford and Edinburgh.
- Parkinson, D.R. & Stephens, R.J. (1973). Morphological surface changes
in the terminal bronchiolar region of NO₂-exposed rat lung.
Environ. Res. 6: 37-51
- Parks, J.B., Post, G., Thorne, T. & Nash, P. Parainfluenza-3 virus
infection in rocky moutain bighorn sheep. J. Am. vet. med. Ass.
161 : 669-672
- Parrott, R.H. (1963). Viral respiratory tract illnesses in children.
Bull. New York Acad. Med. 39: 629-648
- Pascoe, R.R. & McGavin, M.D. (1969). Atypical interstitial pneumoria.
Vet. Rec. 85: 376-377
- Pattle, R.E. (1961). The retention of gases and particles in the
human nose. In Inhaled Particles and Vapours. Ed. by C.N. Davies
Pergamon Press. Oxford. p302-309
- Pearse, A.G.E. (1960). Histochemistry, Theoretical and Applied,
2nd Ed. London.
- Pepys, J. (1966). Pulmonary hypersensitivity disease due to
inhaled organic antigens. Postgrad. med. J. 42: 698
- Pine, J.H., Richter, W.R. & Esterly, J.R. (1973). Experimental
lung injury. I Bacterial pneumonia : Ultrastructural,
autoradiographic and histochemical observations.
Am. J. Pathol. 73: 115-130

- Pinkett, M.O., Cowdrey, C.R. & Nowell, P.C. (1966) Mixed hematopoietic and pulmonary origin of alveolar macrophages as demonstrated by chromosome markers in mice. Amer. J. Pathol. 48: 859-867
- Pirie, H.M., Dawson, C.O., Breeze, R.G. & Wiseman, A. (1971). A bovine disease similar to farmer's lung : Extrinsic allergic alveolites. Vet. Rec. 88: 346-351
- Plummer, M.J. & Stone, R.S. (1964). The pathogenesis of viral influenzal pneumonia in mice. Amer. J. Path. 45: 95-113
- Policard, A., Collet, A. & Pregermain, S. (1959). Recherches au microscope electronique sur les cellules parietales alveolaires du poumon des mammiferes. Z. Zellforsch. 50: 651
- Pyke, B.N. (1974). Sheep mortality in the King country. N.Z. vet. J., 22: 196-197
- Rajya, B.S. & Singh, C.M. (1964). The pathology of pneumonia and associated respiratory disease in sheep and goats. I Occurrence of jaagziekte and maedi in sheep and goats in India. Am. J. vet. Res. 25: 61-67
- Rapaport, F.T., Nemirovsky, M.S., Bachvaroff, R. & Ball, S.K. (1973) Mechanisms of pulmonary damage in severe burns. Ann. Surg. 117: 472-477
- Ratliff, N.B., Wilson, J.W., Hackel, D.B. & Martin, A.M. (1970). The lung in haemorrhagic shock. II. Observations on alveolar and vascular ultrastructure. Am. J. Path. 58: 353-373
- Ratliff, N.B. Wilson, J.W., Mikat, E., Hackel, D.B. & Graham, T.C. (1971). The lung in haemorrhagic shock. IV. The role of neutrophilic polymorphonuclear leucocytes. Am. J. Path. 65: 325-331
- Read, J. (1958). The pathological changes produced by anti-lung serum. J. Path. Bact. 76: 403-417
- Reidbord, H.E., (1967). An electron microscopic study of the alveolar capillary wall following intratracheal administration of saline and water. Am. J. Path. 50: 275
- Reidbord, H.E. & Spitz, W.U. (1966) Ultrastructural alterations in rat lungs: Changes after intratracheal perfusion with freshwater and seawater. Arch. Path. 81: 103-111

- Ressang, A.A., DeBoer, G.F. & De Wijn, G.C. (1966). The lung in zweegerziekte. Path. Vet. 5: 353-369
- Reynolds, E.S. (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell. Biol. 17: 203-213
- Richter, C.P. (1952). The physiology and cytology of pulmonary edema and pleural effusion produced in rats by alpha-naphthyl thiorea (ANTU). J. Thoracic Surg. 23: 66-91
- Robbins, S.L. (1964). Textbook of Pathology with Clinical Application Philadelphia and London. p580.
- Roberts, D.H. & Little, T.W.A. (1970). An auto-immune response associated with porcine enzootic pneumonia. Vet. Rec. 86: 328
- Roberts, E.D., Switzer, W.P. & L'Ecuyer, C. (1962). Influence of Pasteurella multocida and Mycoplasma hyorhinis (PPLO) on the histopathology of field cases of swine pneumonia. Cornell Vet. 52: 306-327
- Roberts, J.A. (1962). Histopathogenesis of mousepox. I. Respiratory infection. Brit. J. Exp. Pathol. 43: 451-461
- Robertson, B., Enhorning, G. Ivemark, B. Malmquist, E. & Modée, J. (1971). Experimental respiratory distress induced by paraquat. J. Path. 103: 239-244
- Robertson, W. (1904). Jaagziete or chronic catarrhal pneumonia (sheep) J. comp. Path. Ther. 17: 221-224
- Roehm, J.N. Hadley, J.G. & Menzel, D.B. (1971). Antioxidants vs lung disease. Arch. intern. med. 128: 88-93
- Rogers, P.A.M., Spillane, T.A., Fenlon, M. & Henaghan, T. (1973). Suspected Paraquat poisoning in pigs and dogs. Vet. Rec. 92: 44-45
- Romváry, J. (1962) Pneumonitis of sheep caused by a virus of the psittacosis-lymphogranuloma group. Abstracted in Vet. Bull. Weybridge 1963. 33. Mag. állator. Lapja. 17: 323-327. Cited by Stevenson. (1969).
- Romváry, J., Takatsy, G., Barb, K. & Farkas, E. (1962). Isolation of influenza virus from strains of animals. Nature. Lond. 193: 907-908

- Rosenbusch, C.T. & Merchant, I.A. (1939). A study of the hemorrhagic septicemia pasteurellae. J. Bact. 37: 69-89
- Ross, I.S. (1939). Pulmonary epithelium and proliferative reactions in the lungs. Arch. Path. 27: 478-496
- Roth, F.J. & Goldstein, M.I. (1961). Inhibition of growth of pathogenic yeasts by human serum. J. invest. Dermat. 36: 383
- Roth, J., Winkelmann, H. & Meyer, H.W. (1973). Electron microscopic studies in mammalian lungs by freeze-etching. IV Formation of the superficial layer of the surfactant system by lamellar bodies. Exp. Path. 8: 354-362
- Russo, A.G. (1972) Isolement D'un virus dans une infection respiratoire de l'agneau. Bull. Acad. Vet. 45: 207-213.
- Rylander, R. (1968) Pulmonary defence mechanisms to airborne bacteria. Acat. Physiol. Scand. Supplm. 306.
- Rylander, R. (1969). Alterations of lung defense mechanisms against airborne bacteria. Arch. Environ. Health. 18: 551-555
- Rylander, R. (1970). Studies of lung defense to infections in inhalation toxicology. Arch. Intern. Med. 126: 496-499
- Sackner, M.A., Will, D.H. & Dubois, A.B. (1966). The site of pulmonary vasomotor activity during hypoxia or serotonin administration. J. clin. Invest. 45: 112-121
- Salisbury, R.M. (1957). Enzootic pneumonia of sheep in N.Z. N.Z. vet. J. 5: 124-127
- Sanders, C.L., Jackson, T.A., Adey, R.R. Powers, G.J. & Wehner, A.P. (1971). Distribution of inhaled metal oxide particles in pulmonary alveoli. Arch. Intern. Med. 127: 1085-1089
- Sărăteanu, D., Popescu, G., Gheorghiu, V., Demetrescu, R. & Petrescu, A. (1965). Revue. roum. Intra.microbiol. 2: 63-69. Cited by Stevenson (1969)
- Scadding, J.G. (1937) Brit. med. J. ii 956. Cited by Spencer, H. (1968)
- Scarpelli, E.M. (1968). The Surfactant System of the Lung. Philadelphia.
- Schaefer, K.E., Avery, M.E. & Bensch, K. (1964). Time course of changes in surface tension and morphology of alveolar epithelial cells in CO₂- induced hyaline membrane disease. J. clin. Invest. 43: 2080-2093

- Schaffner, F., Felig, P., Wright-Patterson, A.F.B. & Trachtenberg, E. (1967). Structure of rat lung after protracted oxygen breathing. Arch. Path. 83: 99-107
- Scheel, L.D., Dobrogorski, O.J., Mountain, J.T., Svirbely, J.L. (1959) Physiologic, biochemical, immunologic and pathologic changes following ozone exposure. J. Appl. Physiol. 14: 67-80
- Schiller, E. (1971). Experimental pathology and pharmacology of dust exposure : An electron microscopic study. In Inhaled Particles III. Vol 1. Ed. W.H. Walton, England. 403-412.
- Schulz, H. (1959) Die Submikroskopische Anatomie und Pathologie der Lunge. Heidelberg.
- Schulz, H. (1962). Some remarks on the sub-microscopic anatomy and pathology of the blood-air pathway in the lung. In Ciba Foundation Symposium on Pulmonary Structure and Function. Ed. by A.V.S. de Reuck and M.O'Connor. London
- Schulz, L.C.I., Somoza, A., & Weiland, F. (1965). Zum auftreten und zum tumour charakter der enzootischen lungenadenomatose des schafes vergleichende untersuchungen in Chile und in der Bundesrepublik Deutschland. Deutsche tierärztliche Wochenschrift. 72: 458-461
- Sealy, W.C. (1968). The lung in haemorrhagic shock. J. Trauma 8: 774-781
- Seddon, H.R. (1967). Diseases in Domestic Animals in Australia. 2nd Ed., revised by H.E. Albiston. Part I p171, Dept. of Health Canberra.
- Seller, M.J. & Spector, R.G. (1964) The effects of anoxia on the newborn and adult rat lungs. J. Path. Bact. 88: 309-311
- Shires, T.K., Johnson, M. & Richter, K.M. (1969). Haematoxylin staining of tissues embedded in epoxy resin. Stain technol. 44: 21-25
- Shirlaw, J.F. (1936). A preliminary note on successful vaccination of sheep in Kenya against progressive enzootic pneumonia (Laikipia lung disease). Bull. Epizoot. Dis. Afr. 4: 57-59
- Shirlaw, J.F. (1959). The pneumonias of sheep with special reference to enzootic pneumonia and jaagsiekte. Vet. Rev. Annot. 5: 37-57

- Shorter, R.G., Titus, J.L. & Divertie, M.B. (1964). Cell turnover in the respiratory tract. Dis. chest. 46: 138-142
- Shortland, J.R., Darke, C.S. & Crane, W.A.J. (1969). Electron microscopy of desquamative interstitial pneumonia. Thorax 24: 192-208.
- Siefert, G.W. (1971). Ecto- and endoparasitic effects on the growth rates of zebu crossbred and british cattle in the field. Aust. J. agric. Res. 22: 839-850
- Sigurdsson, B. (1958). Adenomatosis of sheeps lungs. Experimental transmission. Arch. ges. Virusforsch. 8: 51-58
- Sigurdsson, B., Grimsson, H. & Pálsson, P.A. (1952). Maedi, a chronic, progressive infection of sheep's lungs. J. infect. Dis. 90: 233-241
- Sigurdsson, B., Pálsson, P.A. & Tryggvadóttir, A. (1953). Transmission experiments with maedi. J. infect. Dis. 93: 166-175
- Sigurdardóttir, B., & Thormar, H. (1964). Isolation of a viral agent from the lungs of sheep affected with maedi. J. infect. Dis. 114: 55-60.
- Singer, S.H., Noguchi, P. & Kirschstein, R.L. (1972). Respiratory disease in cyclophosphamide treated mice. II Decreased virulence of PR8 influenza virus. Infect. Immunity 5: 957-960
- Singh, K.V., & Ata, F.A. (1967). Presence of antibodies against parainfluenza 3 virus in camel and sheep sera. Vet. Rec. 81: 84
- Sjogren, N. (1933). Pneumonia in animals and its treatment. Tasm. J. agric. 4: 61-69
- Slama, H., Schoedel, W. & Hansen, E. (1973). Lung surfactant : Film kinetics at the surface of an air bubble during prolonged oscillation of its volume. Resp. Physiol. 19: 233-243
- Smith, B. (1970) Pers. comm.
- Smith, B.L. (1969) Pers. comm.
- Smith, B.L., Alley, M.R. & McPherson, W.B. (1969). Lipid pneumonia in a cow. N.Z. vet. J. 17: 65-67

- Smith, G.R. (1957) In discussion following a paper by A.J. Stevens. Vet. Rec. 69: 1254-1257
- Smith, G.R. (1959). Isolation of two types of Pasteurella haemolytica from sheep. Nature, Lond. 183: 1132-1133
- Smith, G.R. (1960a). The pathogenicity of Pasteurella haemolytica for young lambs. J. comp. Path. 70: 326-338.
- Smith, G.R. (1960b). Virulence and toxicity of Pasteurella haemolytica in the experimental production of ovine septicaemia. J. comp. Path. 70: 429-436.
- Smith, G.R. (1961a). Further observations on the pathogenicity of Pasteurella haemolytica for young lambs. J. comp. Path. 71: 94-99
- Smith, G.R. (1961b). The characteristics of two types of Pasteurella haemolytica associated with different pathological conditions in sheep. J. Path. Bact. 81: 431-440
- Smith, G.R. (1964). Production of pneumonia in adult sheep with cultures of Pasteurella haemolytica type A. J. comp. Path. 74: 241-249
- Smith, G.R. (1973). Tests in mice on the activity of ronidazole against the causal mycoplasmata of contagious bovine and caprine pleuropneumonia. Vet. Rec. 93: 440-444
- Smith, J.C. (1963). Radiation pneumonitis : a review. Am. Rev. Resp. Dis. 87: 647-655
- Smith, P. & Heath, D. (1974). The ultrastructure and time sequence of the early stages of Faraquat lung in rats. J. Path. 114: 177-184
- Smith, P., Heath, D & Hasleton, P.S. (1973) Electron microscopy of chlorphentermine lung. Thorax. 28: 559-566
- Sorokin, S.P. (1970). Properties of alveolar cells and tissues that strengthen alveolar defenses. Arch. Int. Med. 126: 450-462
- South, M.A., Cooper, M.D., Wollheim, F.A. & Good, .R.A. (1968) The IgA system. II The clinical significance of IgA deficiency : Studies in patients with agammaglobulinemia and ataxia-telangiectasia. Am. J. Med. 44: 168-178
- Spencer, H. (1968). Pathology of the Lung (Excluding pulmonary Tuberculosis). 2nd Ed. London.

- Spencer, H. & Shorter, R.G. (1962) Cell turnover in pulmonary tissues. Nature (London) 194: 880
- Spitz, W. & Blanke, R.V. (1961). Mechanism of death in freshwater drowning. Arch. Path. 71: 661-668
- Spray, R.S. (1923). Bacteriologic study of pneumonia in sheep. J. infect. Dis. 33: 97-112
- Stamp, J.T., Watt, J.A.A. & Thomlinson, J.R. (1955) Pasteurella haemolytica septicaemia of lambs. J. comp. Path. 65: 183-196
- Stamp, J.T. & Nisbet, D.I. (1963) Pneumonia of sheep. J. comp. Path. 73: 319-328
- Stanley, N.F. (1967) Reoviruses. Br. med. Bull. 23: 150-155
- Stephens, R.J., Freeman, G., Evans, M.J. (1972). The early response of lungs to low levels of NO₂ : Light and electron microscopy. Arch. Environ. Health. 24: 168-179
- Stephens, R.J., Freeman, G., Stara, J.F. & Coffin, D.L. (1973). Cytologic changes in dog lungs induced by chronic exposure to ozone. Am. J. Path. 73 : 711-726
- Stevens, A.J. (1957) Respiratory diseases of sheep. Vet. Rec. 69: 1249-1254
- Stevenson, R.G. (1969). Respiratory diseases of sheep. Vet. Bull. 39: 747-759.
- Stevenson, R.G. & Hore, D.E. (1970). Comparative pathology of lambs and calves infected with parainfluenza virus type 3. J. comp. Path. 80: 613-618
- Stevenson, R.G. & Robinson, G. (1970). The pathology of pneumonia in young lambs inoculated with bedsonia. Res. vet. Sci. 11: 469-474
- St. George, T.D. (1969) The isolation of myxo virus parainfluenza type 3 from sheep in Australia. Aust. vet. J. 45: 321-325
- St. George, T.D. (1971). Actively and passively acquired antibody to mucosal disease and parainfluenza type 3 viruses in sheep. Aust. vet. J. 47: 428-433.
- St. George, T.D. (1972) Investigations of respiratory disease of sheep in Australia. Aust. vet J. 48: 318-322

- St. George, T.D. & French, E.L. (1966). Isolation of a bovine strain of myxovirus parainfluenza type 3 in Australia. Aust. vet. J. 42: 438-439
- St. George, T.D. & Liefman, C.E. (1972). A field experiment to assess the role of parainfluenza type 3 virus in pneumonia in a flock of sheep in Victoria. Aust. J. biol. Sci. 25: 319-325
- St. George, T.D. & Sullivan, N.D. (1973). Pneumonias of sheep in Australia. Veterinary Review No 13. The Post-Graduate foundation in Veterinary Science, University of Sydney.
- St. George, T.D. Sullivan, N.D., Love, J.A. & Horsfall, N. (1971) Experimental transmission of pneumonia in sheep with a mycoplasma isolated from pneumonic sheep lung. Aust. vet. J. 47: 282-283
- Stone, L.A. & Loew, E.R. (1949). Effect of various drugs on epinephrine-induced pulmonary edema in rabbits. Proc. Soc. Exp. Biol. Med. 78: 122-126
- Stuart-Harris, C.H. (1950). Atypical pneumonia. Brit. Med. J. ii. 282-283
- Sukhandan, R. & Thal, A.D. (1965). The effect of endotoxin and vasoactive agents on dibenzyline-pretreated lungs. Surgery. 58: 185-196
- Sullivan, N.D., St. George, T.D. & Horsfall, N. (1973a) A proliferative interstitial pneumonia of sheep associated with mycoplasma infection : 1. Natural history of the disease in a flock. Aust. vet. J. 49: 57-62
- Sullivan, N.D., St. George, T.D. & Horsfall, N. (1973b). A proliferative interstitial pneumonia of sheep associated with mycoplasma infection : 2. The experimental exposure of young lambs to infection. Aust. vet. J. 49: 63-68
- Sun, C.N. & Saueressig, S (1965). Subcellular alterations in cortisone treated rat lungs. Zeitsch. Zellforsch. 67: 718-722
- Teabeaut, J.R. (1952). Aspiration of gastric contents : An experimental study. Am. J. Path. 28: 51

- Teplitz, C. (1968). The ultrastructural basis for pulmonary pathophysiology following trauma : Pathogenesis of pulmonary oedema. J. Trauma. 8: 700-714
- Thomas, P. & Amor, O.F. (1968). A case of diquat poisoning in cattle. Vet. Rec. 83: 674-676
- Thompson, S.W. (1966) Selected Histochemical and Histopathology Methods. Illinois.
- Thomson, R.G., Benson, M.L. & Savan, M. (1969). Pneumonic pasteurellosis in cattle : Microbiology and immunology. Can. J. Comp. Med. 33: 194-206
- Thormar, H. (1965). A comparison of visna and maedi viruses. I. Physical, chemical and biological properties. Res. vet. Sci. 6: 117-129
- Thormar, H. & Helgadóttir, H. (1965) A comparison of visna and maedi viruses. II. Serological relationship. Res. vet. Sci. 6: 456-465
- Timbrell, V. & Skidmore, J.W. (1971). The effect of shape on particle penetration and retention in animal lungs. In Inhaled Particles III. Ed. by W.H. Walton. Gresham Press. England.
- Toigo, A., Imarisio, J.J., Murmall, H. & Lepper, M.N. (1963). Clearance of large carbon particles from the human tracheo-bronchial tree. Am. Rev. Resp. Dis. 87: 487-492
- Tomasi, T.B. (1970). Structure and function of mucosal antibodies. Ann. Rev. Med. 21: 281-295
- Totten, R.S., Pierce, G.B. (1964). Cortisone and atypical pulmonary epithelial hyperplasia. Amer. J. Path. 45: 977-990
- Tourville, D.R., Adler, R.H., Bienenstock, J. & Tomasi, T.B. (1969) The human secretory immunoglobulin system. Immunohistochemical localization of gamma A, secretory "piece" and lactoferrin in normal human tissues. J. Exp. Med. 129: 411-423
- Trump, B.F. & Ginn, F.L. (1969) The pathogenesis of subcellular reaction to lethal injury. In. Meth. Achievm. exp. Path. 4: 1-29. Ed. by E. Bajusz, New York
- Tustin, R.C. (1969). Ovine Jaagsiekte. S. Afr. vet. med. Ass. 40: 3.-23

- Tustin, R.C. & Geyer, S.M. (1971) Transmission of ovine jaagsiekte using neoplastic cells grown in tissue culture. Jl. S. Afr. vet. med. Ass. 42: 181-182.
- Untersee, P. Gil, J. & Weibil, E.R. (1971). Visualization of extracellular lining layer of lung alveoli by freeze-etching. Respir. Physiol. 13: 171-185
- Valdivia, E. & Sonnad, J. (1966). Fatty change of the granular pneumocyte after CCl_4 intoxication. Arch. Path. 81: 514.
- Valdivia, E., Lalich, J.J., Hayashi, Y. & Sonnad, J (1967). Alterations in pulmonary alveoli after a single injection of monocrotaline. Arch. Path. 84: 64-75
- Van der Veen, R.R. & Zumpt. I.F. (1967). Enzootic pneumonia of sheep in the Mafeking district. Jl. S.Afr. Vet. Med. Ass. 38: 415-419
- Velo, G.P. & Spector, W.G. (1973). The origin and turnover of alveolar macrophages in experimental pneumonia. J. Path. 109: 8-19
- Vijeyaratnam, G.S. & Corrin, B. (1971). Experimental Paraquat poisoning : A histological and electron-optical study of the changes in the lung. J. Path. 103: 123-129
- Voisin, C., Vivier, E., Aerts, C., Petitprez, A. & Wattel, F. (1965) Etude de la phagocytose du virus grippal par les macrophages alveolaires de coybaye. I Aspects morphologiques et cinetiques en microscopie electronique et en microcinematographie en contraste de phase. Ann. Inst. Pasteur, Lille. 16: 1-10
- Voisin, C., Tonnel, A.B., Aerts, C. & Wattel, F. (1971). Le macrophage alveolaire son role dans la defense du tissu pulmonaire contre l'infection. Bull. de Physiol Pathologie Respiratoire. 7: 776-808
- Wachstein, M. & Meisel, E. (1956). Histochemistry of hepatic phosphatases at physiologic pH. Am. J. Clin. Path. 27: 13-23
- Wagner, J.C. (1965). The sequelae of exposure to asbestos dust. Ann. N.Y. Acad. Sci. 132: 691-695
- Wandera, J.G. (1967). Pneumonia in sheep in Kenya. I. Bacterial and parasitic. Epizoot. Dis. Afr. 15: 245-258

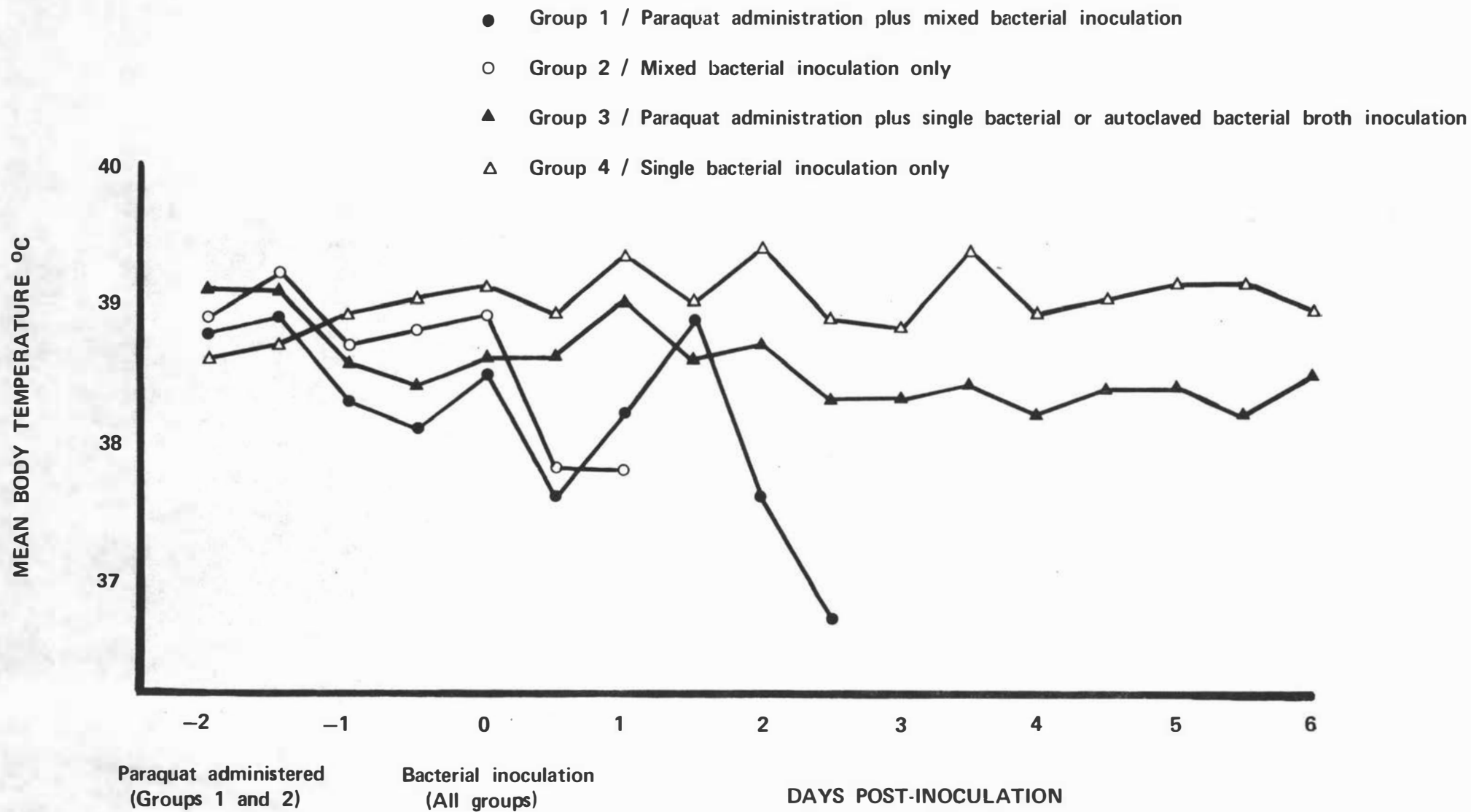
- Wandera, J.G. (1970). Clinical pulmonary adenomatosis of sheep produced experimentally. Br. vet. J. 126: 185
- Wandera, J.G. (1971) Sheep pulmonary adenomatosis (Jaagsiekte) Adv. vet. Sci. 15: 251-283
- Wandera, J.G. & Krauss, H. (1971). The ultrastructure of sheep pulmonary adenomatosis. Zbl. Vet. Med. A. 18: 325-334
- Wang, N.S. Huange, S.N., Sheldon, H. & Thurlbeck, W.M. (1971) Ultrastructural changes of Clara and type II alveolar cells in adrenalin-induced pulmonary oedema in mice. Am. J. Pathol. 62: 237-245
- Warren, S. & Gates, O. (1940). Radiation pneumonitis experimental and pathologic observations. Arch. Path. 30: 440-460
- Warren, S. & Spencer, J. (1940). Am. J. Roetgenol. 43: 682
Cited by Spencer, H. (1968).
- Weibel, E.R. & Gil, J. (1968). Electron microscopic demonstration of an extra-cellular duplex lining layer of alveoli. Resp. Physiol. 4: 42-57
- Wells, W.F. (1955) Airborne Contagion and Air Hygiene. Cambridge. Mass.
- Werthamer, S., Schwarz, L.H., Carr, J.J. & Soskind, L. (1970) Ozone-induced pulmonary lesions : Severe epithelial changes following sublethal doses. Arch. Environ. Health. 20: 16-21
- Whitworth, S.H. (1926). Observations on a lung affection of lambs and sheep. Ann. Report. Dept. of Agric. Kenya. 102-110
- Willwerth, B.M., Crawford, F.A., Young, W.G. & Sealy, W.D. (1967) The role of functional demand on the development of pulmonary lesions during haemorrhagic shock. J. Thorax. Cardiovasc. Surg. 54: 658-665
- Wilson, G.S. & Miles, A.A. (1964). Topley and Wilson's Principles of Bacteriology and Immunology. 5th Edition. Vol. 1. London
- Wiseman, A., Selman, I.E., Dawson, C.O., Breeze, R.G. & Pirie, H.M. (1973). Bovine farmers lung : A clinical syndrome in a herd of cattle. Vet. Rec. 93: 410-417

- Woods, G.T., Sibinovic, K., Starkey, A.L. (1965) Exposure of colostrum-deprived calves to bovine myxovirus parainfluenza-3
Am. J. Vet. Res. 26: 262
- Young, R.L., Nagland, H., Vaughn, T.R. & Staub, N.C. (1963). Pulmonary capillary blood volume in the dog: Effects of 5-hydroxytryptamine. J. App. Physiol. 18: 264-268
- Zaidi, S.H., Dograr, K.S., Shanker, Ravi, & Chandra, Satya. V. (1971) Experimental farmers lung in guinea-pigs. J. Path. 105: 41-48
- Zenker, F.A. (1867). U Ber Staubinhalationscrankheiten der lungen. Arch. Clin. Med. 2: 116-171. cited by: Pendergrass, E.P., Lainhart, W.S., Bristol, W.S., Felson, B. & Jacobson, G. (1972). Historical perspectives of coal workers' pneumoconiosis in the United States. Ann. N. Y. Acad. Sci. 200: 835-854
- Zollar, L.M., Krause, H.E. & Mufson, M.A. (1973). Microbiologic studies on young infants with lower respiratory tract disease. Am. J. Dis. Child. 126: 56-60
- Zollinger, H.U. (1948). Cytological studies with the phase microscope: The formation of "blisters" on cells in suspension (potocytosis), with observations on the nature of the cellular membrane. Amer. J. Path. 24: 545-567

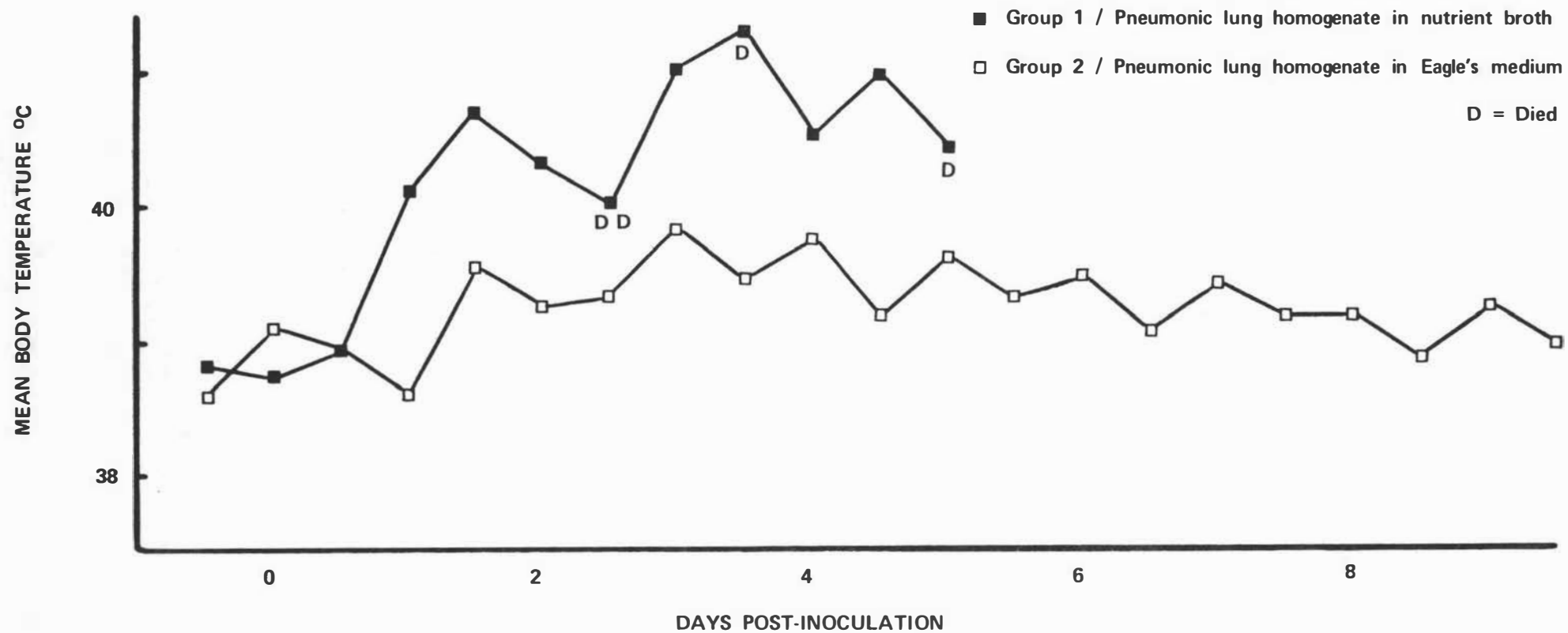
APPENDIX 1

METHOD FOR STAINING 'THICK' EPOXY EMBEDDED HISTOLOGICAL SECTIONS.

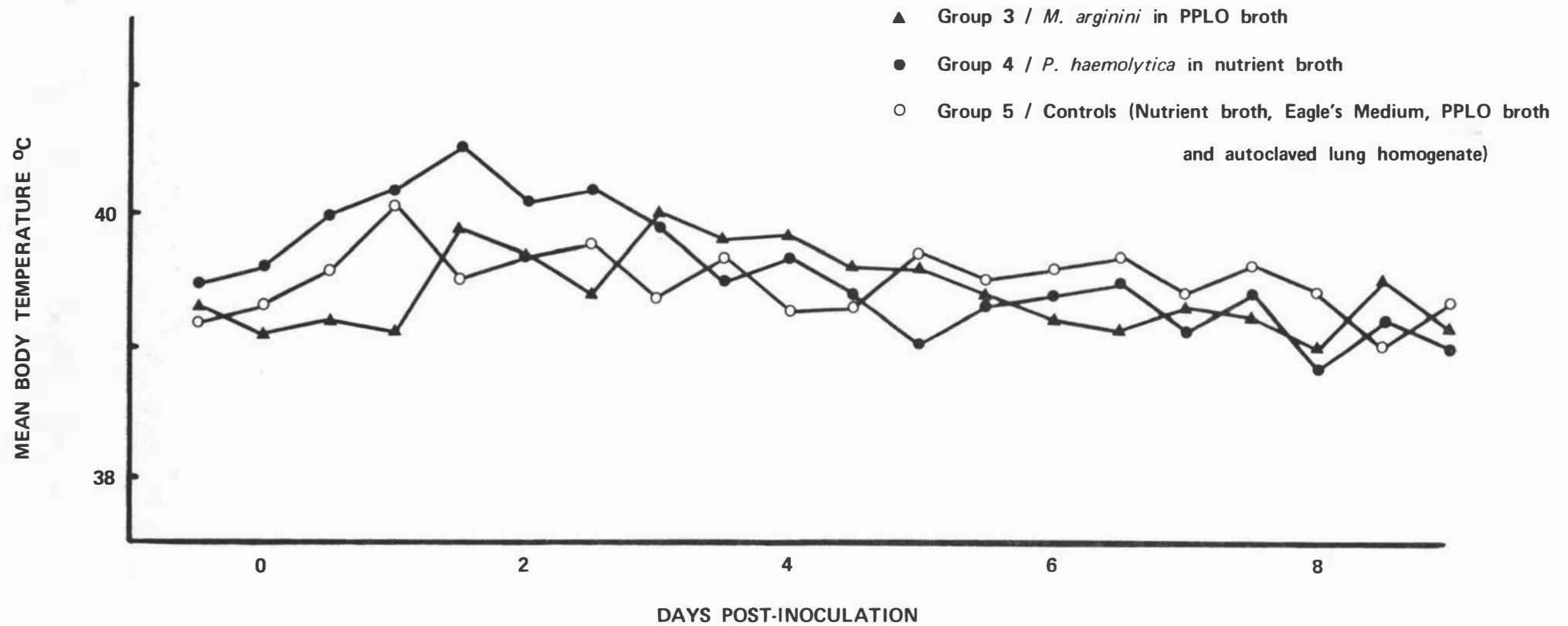
1. Cut sections at 0.5 to 1.0 μm and pick up with an eyelash or float off in a water bath and pick up with a coverslip.
2. Transfer sections to a drop of 10% acetone on a glass slide then heat-dry on a hot plate at 80°C for about a minute.
3. While at this temperature, cover sections with a few drops of 1% toluidine blue and leave for 1 to 2 minutes or until the stain begins to evaporate at the edges.
4. Rinse under running water.
5. Return the slide to the hot plate and counterstain with 4% basic fuchsin for 1 to 2 minutes.
6. Rinse in running water, air-dry at room temperature and mount in D.P.X.



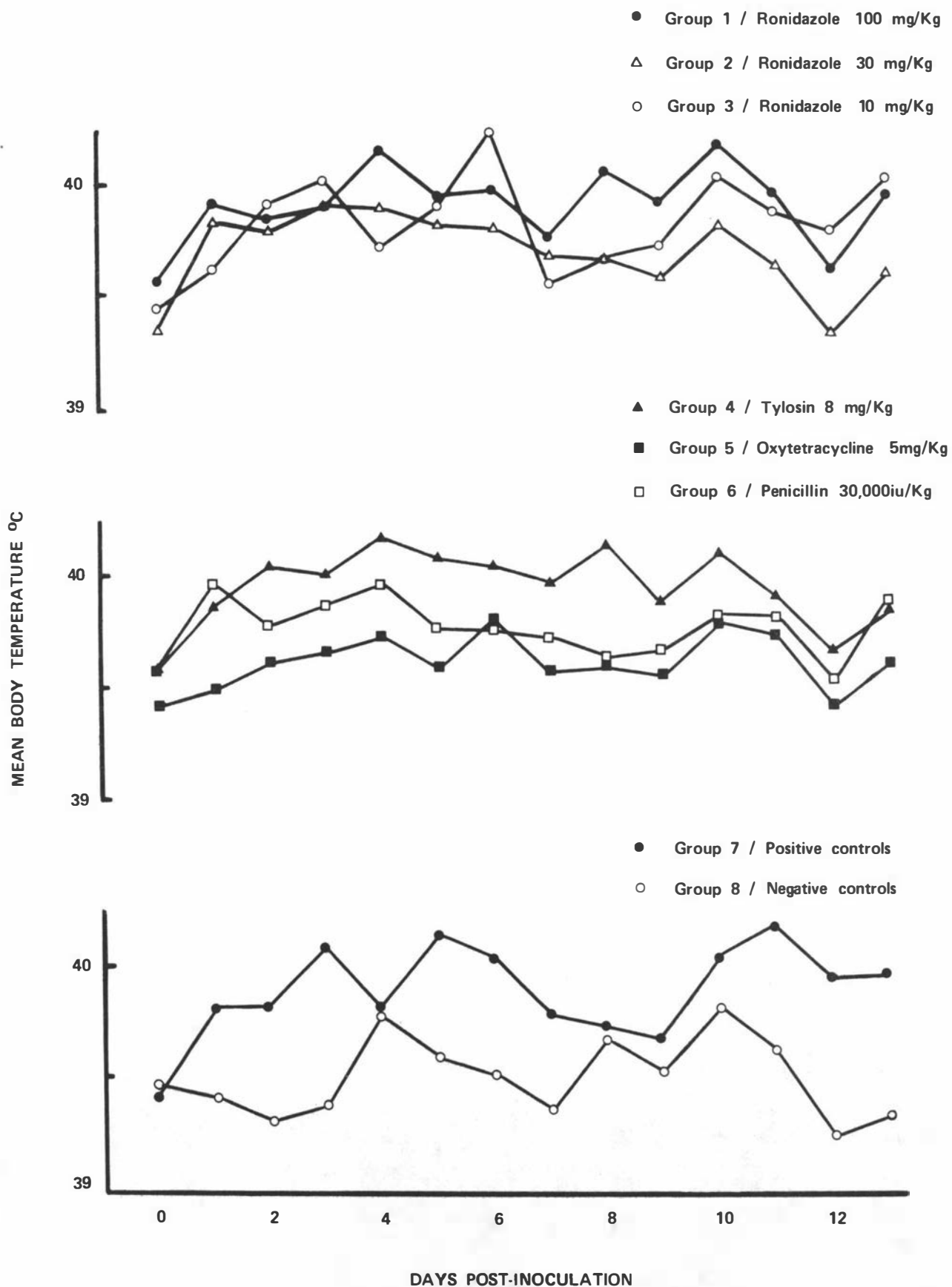
Appendix 2. Mean body temperatures following paraquat administration and endobronchial bacterial inoculation of lambs



Appendix 3. Mean body temperatures following endobronchial inoculation of worm-free sheep with pneumonic lung homogenate.



Appendix 4 . Mean body temperature following endobronchial inoculation of worm-free sheep with *M. arginini*, *P. haemolytica* and nutrient media or autoclaved lung homogenate.



Appendix 5.

Mean body temperature of sheep following intranasal transmission of chronic pneumonia and drug administration.