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THE TRACHEOBRONCHIAL AIRWAYS
OF NORMAL AND PNEUMONIC SHEEP:
CYTOLOGY AND CYTOPATHOLOGY

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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ABSTRACT
ACKNOWLEDGEMENTS
TABLE OF CONTENTS
LIST OF FIGURES
LIST OF TABLES
INTRODUCTION

CHAPTER 1 GENERAL REVIEW OF LITERATURE
   SECTION 1 THE TRACHEOBRONCHIAL AIRWAY OF MAMMALS
   SECTION 2 MUCUS SECRETION IN THE TRACHEOBRONCHIAL AIRWAYS
   SECTION 3 CHRONIC NON-PROGRESSIVE PNEUMONIA OF SHEEP IN NEW ZEALAND
   SECTION 4 TRACHEAL ORGAN CULTURE

CHAPTER 2 THE MORPHOLOGY OF THE TRACHEOBRONCHIAL EPITHELIUM IN NORMAL SHEEP

CHAPTER 3 THE MORPHOLOGY OF THE TRACHEOBRONCHIAL EPITHELIUM IN OVINE CHRONIC NON-PROGRESSIVE PNEUMONIA

CHAPTER 4 MORPHOMETRIC STUDIES OF TRACHEOBRONCHIAL AIRWAYS

CHAPTER 5 TYPES AND DISTRIBUTION OF GLYCOPROTEINS IN THE TRACHEOBRONCHIAL SUBMUCOSAL GLANDS

CHAPTER 6 OVINE TRACHEAL ORGAN CULTURE STUDIES

CHAPTER 7 GENERAL DISCUSSION

BIBLIOGRAPHY

APPENDICES
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ABSTRACT

As a basis for subsequent pathological studies the histology, topographical morphology and ultrastructure of the normal ovine tracheobronchial epithelium at five different levels were investigated. In addition, the topographical studies were extended to involve the normal alveolus. The lining epithelium of the trachea and bronchi consisted of pseudostratified ciliated columnar and goblet cells, while from the small bronchi distally, the airways were lined by low columnar or cuboidal ciliated and non-ciliated cells. A slightly lower proportion of mucous cells were present in the upper trachea compared to the lower trachea which also contained Clara cells with PAS-positive granules. Topographically, there was a marked change from a predominance of ciliated cells in the trachea and bronchi to non-ciliated cells in the distal bronchioli. Ten different types of epithelial cell were identified ultrastructurally. These were; two types each of ciliated, goblet and unknown secretory cells together with Clara, brush, basal and intermediate cells. Several cell types of unknown function which have not been previously described were observed. It was concluded that the ovine lung was similar to that of cattle but different from other mammals in two important features. Firstly, interalveolar pores of Kohn were uncommon in young sheep and secondly, there was a relative paucity of alveolar macrophages in alveolar spaces. It is thought that these features may have an influence on the pathophysiology and pathogenesis of pneumonia in sheep and the resistance of the ovine lung to infection.

The pathological changes which occurred in the tracheobronchial epithelium at five different levels were studied in both early and advanced lesions of chronic non-progressive pneumonia (CNP) in lambs 3 to 10 months old. In addition, the alveolar topographical changes were investigated. The most common topographical finding was loss of cilia from the epithelial surface which was more severe in early lesions. The tracheobronchial epithelium in advanced pneumonic lesions showed large areas of squamous metaplasia, while focal areas were observed in early lesions. Extensive inflammatory cell infiltration of the tracheobronchial epithelium was one of the main histological features seen in both early and advanced pneumonic
lesions indicating that active inflammatory changes were occurring at all stages of the disease. Aggregations of lymphoid cells together with submucosal gland hyperplasia and metaplasia were more extensive in advanced cases. Striking changes to Clara cells were observed by scanning electron microscopy in bronchioli in both stages of the disease. Mycoplasmas were commonly found attached by means of pili-like structures to the cilia of epithelial cells of the trachea and bronchi in early lesions and to tracheal and bronchiolar epithelial cilia in advanced lesions. Their presence in early pneumatic lesions suggested that they may compromise the effectiveness of the mucociliary system, allowing other destructive bacteria normally resident in the upper respiratory tract to penetrate into the pulmonary parenchyma and produce more severe lesions.

To quantitate the proliferative changes observed the epithelial and submucosal thicknesses of the tracheobronchial airways of sheep affected with CNP were measured at 6 levels and submucosal gland size and number were measured at 4 levels. The mean thickness of the tracheobronchial mucosal layers of normal sheep decreased regularly from the upper trachea to the distal bronchioli, while in pneumatic lesions the decrease in mucosal thickness was more irregular. Small bronchi and bronchioli were the most severely affected and the percentage increase above normal was 146.5% and 268.2% respectively. Comparative statistical analysis of the results showed that in early lesions the epithelium of the trachea and bronchi were worst affected, whereas in advanced lesions the epithelium of the peripheral airways showed the most severe change. It is thought that the increase in the thickness of the wall of peripheral airways together with the accumulation of inflammatory cells and mucus may result in partial or complete obstruction of the lumen of small airways in affected areas. Statistical analysis of sectional areas of submucosal gland of normal sheep showed that they decreased regularly from the upper trachea to the small bronchi, but this pattern became irregular in the pneumatic lesions. The most significant changes in submucosal gland parameters of early pneumatic sheep occurred in the intrapulmonary bronchi. In sheep with advanced pneumatic lesions changes were most severe in both intrapulmonary and extrapulmonary bronchi. Enlargement of the submucosal glands in pneumatic lesions was found to be due to both hyperplasia and hypertrophy and these changes were more severe in advanced than early lesions.
The histochemistry of the submucosal gland glycoproteins in normal and pneumonic sheep was also studied and statistical analysis of the results showed a change in the types present. It was found that most mucous cells of submucosal gland at all levels of the normal ovine tracheobronchial tree contained either neutral or mixed types of glycoprotein and very few contained the acid type. The submucosal glands of normal bronchi contained significantly more neutral glycoprotein and less mixed and acid glycoproteins than those of the trachea. In pneumonic lungs there were no significant differences in the amount and types of glycoprotein between levels. Comparative statistical analysis showed that in the intrapulmonary bronchi, acid glycoprotein increased and neutral glycoprotein decreased in advanced pneumonic lesions when compared to normal and early pneumonic sheep. It was concluded that the ovine tracheobronchial airways respond to unspecified noxious agents by changing the chemical and physical nature of their mucous secretions.

Ovine tracheal organ cultures were used to investigate the pathogenicity of Mycoplasma ovipneumoniae, Bordetella parapertussis, Pasteurella haemolytica and Neisseria catarrhalis. The ciliary activity, histology, topographical morphology, ultrastructure and microbiology of these experiments are described in detail. Four different titres of each microorganism were used. It was found that all the microorganisms caused cytopathological changes and the ciliostasis produced was dose dependent. Mycoplasma ovipneumoniae and B. parapertussis attached to cilia at 30 min and 1 hr respectively and produced ciliostasis as early as 13 hrs and 1 hr respectively. The means of attachment of both organisms was investigated with both scanning and transmission electron microscopes. A fimbria or pili-like structure was found in close proximity to cilia with both microorganisms. Pasteurella haemolytica and N. catarrhalis failed to attach to cilia but they produced cytopathological changes and the ciliostatic effect was achieved as early as 3 hrs and 4 hrs respectively. Although both of these organisms behaved in a similar manner in organ culture and produced similar cytopathological changes, P. haemolytica was more destructive and produced ciliostatic effects faster than N. catarrhalis. Of the four microorganisms used it was found that only M. ovipneumoniae and B. parapertussis had both an affinity for tracheal epithelial cells and the ability to produce destructive changes in organ culture. On the basis of this work both
M. ovipneumoniae and B. parapertussis could be considered as likely candidates for organisms which in vivo could initiate bronchiolar disease and thus allow the development of CNP. This hypothesis in regard to M. ovipneumoniae is strongly supported by several other workers. The role of B. parapertussis remains to be more fully investigated.
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Diagrammatic representation of the tracheobronchial airways of sheep showing the sites selected for study.</td>
</tr>
<tr>
<td>2.2</td>
<td>The epithelial surface of normal trachea stained with toluidine blue. Two types of ciliated cell are present. The first type (CF) has a narrow luminal border and the cytoplasm stains light blue. The second type (CS) has a wide luminal border and the cytoplasm stains more darkly. Goblet cells (G) stain dark blue and contain a large number of faint blue granules. Basal cells (B) and intermediate cells (I) are attached to the basal lamina but do not extend to the luminal surface. Toluidine blue. X 312.</td>
</tr>
<tr>
<td>2.3</td>
<td>Bronchial epithelium consisting of ciliated cells (C), goblet cells (G), intermediate cells (I) and basal cells (B). It is histologically similar to the lower trachea although the height of the epithelium is reduced. Toluidine blue. X 312.</td>
</tr>
<tr>
<td>2.4</td>
<td>Bronchiolar epithelium of normal lung composed of three cell types; ciliated (C), mucous (M) and Clara (Cl). Toluidine blue. X 1250.</td>
</tr>
<tr>
<td>2.5</td>
<td>The epithelial surface of normal trachea. Dense cilia cover most of the surface, but among them are interspersed single non-ciliated cells (N) and secretory openings (S) of submucosal glands. SEM. X 670. The inset figure shows the mucosal surface of a non-ciliated cell covered by short microvilli (arrow). SEM. X 8000.</td>
</tr>
<tr>
<td>2.6</td>
<td>The epithelial surface of a normal bronchus. Ciliated cells predominate over the majority of the surface but a large number of non-ciliated cells (arrows) are also present. SEM. X 1335.</td>
</tr>
<tr>
<td>2.7</td>
<td>The surface of non-ciliated cells. Very few microvilli are present (MV) and large pits or pores (P) open onto the surface. SEM. X 5700.</td>
</tr>
<tr>
<td>2.8</td>
<td>The epithelial surface of a normal bronchiole.</td>
</tr>
</tbody>
</table>
mixture of ciliated cells (C), Clara cells (Cl), cells with a granular surface (G) and cells with microvilli on their luminal surface (black arrow in inset figure) can be seen. The empty bladder-like structure may be an evacuated Clara cell (arrows). SEM. X 6000 (Inset X 4000).

Figure 2.9 Parenchyma of normal lung observed by SEM. Three cell types can be seen within alveoli. These are smooth surfaced type I epithelial cells (T1), granular or type II pneumocytes (T2) and alveolar macrophages (AM). X 340. The inset figure shows a higher magnification of the type II pneumocytes (arrows). Note the low number of interalveolar pores of Kohn (K). SEM. X 1000.

Figure 2.10 Ciliated cells from a tracheobronchial airway in normal lung. Two types can be recognised. The first type (CF) has numerous smooth vesicles (V) in the upper part of the cell. In the second type (CS) the cytoplasm is more electron-lucent and the luminal surface has less cilia (C) but more cytoplasmic processes (PR) and microvilli (M) than the first. TEM. X 5440.

Figure 2.11 High magnification of the apical part of a ciliated epithelial cell. Most of the mitochondria (MC) have accumulated near the apex. A large number of cilia (C) are found on the luminal surface and interspersed between them are microvilli (MV) and fine cytoplasmic processes (PR). An irregular intercellular space is present between these cells except at the apical part where tight junctions (T) are observed. Large numbers of vesicles (V) containing an amorphous material are present near the surface. TEM. X 14,360.

Figure 2.12 High magnification of the lower part of a group of ciliated cells. There are scattered profiles of rough (R) and smooth (S) endoplasmic reticulum, free ribosomes, smooth surfaced vesicles (V), lysosomes and glycogen particles (arrows). The Golgi apparatus (GA) is situated below the nucleus (NU) which contains a prominent nucleolus. TEM. X
Figure 2.13 High magnification of the upper part of both the first (CF) and second (CS) types of ciliated cell. A large number of cilia (C), and microvilli (MV) are found on the luminal surface of both cell types. Atypical or compound cilia (AC) and cytoplasmic processes (PR) are observed mostly on the luminal surface of the second type of ciliated cell. TEM. X 11,010.

Figure 2.14 The first cell type of goblet cell. It contains homogenised small electron-dense granules (G) and a small number of ribosomes (RS). TEM. X 8,540.

Figure 2.15 The second cell type of goblet cell. This contains mucous granules (G) which are larger and less electron-dense than the first type and more numerous ribosomes. Inset: The Golgi apparatus (GA) is well developed and usually situated above the nucleus. TEM. X 3,400.

Figure 2.16 High magnification of the apical part of the first type of unknown secretory cell. The cytoplasm of the cell is electron-dense and contains a few mucus-like granules ((G). The luminal surface shows relatively few long microvilli (MV). TEM. X 15,300.

Figure 2.17 The second type of unknown secretory cell (U). These tall and irregular cells have variable numbers of small spherical vacuoles (V) near their surface. TEM. X 8,900. The inset figure shows some of the vacuoles which appear to be emptying onto the surface. TEM. X 28,000.

Figure 2.18 Clara cell showing apical cytoplasm containing large numbers of free ribosomes (R) and abundant SER (S). The mitochondria are virtually devoid of cristae. Electron-lucent, heterogeneous, membrane-bound inclusions (I) are stained incompletely. TEM. X 12,150.

Figure 2.19 A tracheal brush cell (T) showing its luminal brush border of microvilli (arrows). The cytoplasm
is electron-dense and contains a moderate number of mitochondria (MC), a large amount of RER (R), SER (S) and a few empty vacuoles (V). TEM. X 7,900.

Figure 2.20 A bronchial brush cell showing the typical luminal brush border. Its electron-dense cytoplasm contains abundant RER (R), numerous mitochondria and a large number of ribosomes (R). The filament bundles (F) can be seen in the apical part of the cell. TEM. X 1,340.

Figure 2.21 Basal and intermediate cells. There is a wide, irregular intercellular space (arrows) around both cells and they have a large number of cytoplasmic processes (PR) on their surface. The intermediate cells have a large nucleus (NU) and an electron-lucent cytoplasm. TEM. X 8,400.

Figure 3.1 The epithelium of early pneumonic trachea showing moderate hyperplasia. The luminal border has lost most of its cilia. Moderate numbers of neutrophils and macrophages are present in the lamina propria (L) and large numbers of neutrophils (arrows) are found between epithelial cells. H&E. X 156.

Figure 3. Bronchial epithelium of an early pneumonic lesion. There are moderate numbers of neutrophils (arrows) between the epithelial cells with early lymphoid aggregations immediately beneath the epithelial layer. H&E. X 312.

Figure 3.3 Bronchiole from an early pneumonic lesion showing moderate epithelial hyperplasia and mild peribronchial lymphoid aggregations. The lumen contains necrotic material and inflammatory cells. H&E. X 156.

Figure 3.4 The tracheal epithelial surface in early pneumonia. The mucosal surface contains approximately equal numbers of ciliated and non-ciliated cells. Observe the goblet cell openings (G) and submucosal gland opening (S). SEM. X 800.

Figure 3.5 The luminal surface of the lower trachea in early pneumonia showing a thick layer of mucus secretion (arrows) which covers most of the ciliated but not the non-ciliated surface. SEM. X 10,000. The
inset micrograph shows some of the large number of mycoplasma-like organisms (M) which are attached firmly to the bottom of the cilia. SEM. X 2,500.

Figure 3.6 The bronchial surface epithelium in an early pneumatic lesion. The large holes (G) are goblet cell openings and the small holes (L) may be due to damage to the cell membrane. Large amounts of cellular debris (arrows) and neutrophils (n) are present on the surface. SEM. X 660. The inset figure illustrates a focal area of squamous cell metaplasia (arrows). SEM. X 300.

Figure 3.7 A large number of microorganisms attached to the top of the bronchial cilia in an early pneumatic lesion (large arrows). The morphological features of many of the attached organisms were similar to those of mycoplasmas (small arrows). SEM. X 10,000.

Figure 3.8 A submucosal gland opening denuded of cilia and showing squamous metaplasia. The secretions which are fixed in situ contain a large number of neutrophils (n) mixed with mucosal strands and granules (g). SEM. X 700.

Figure 3.9 An affected bronchiole which has a thickened wall (arrows). Its lumen contains large amounts of necrotic exudate consisting mainly of neutrophils (n). SEM. X 400.

Figure 3.10 The luminal surface of an affected bronchiole showing a few macrophages (arrow) attached to epithelial cells. SEM. X 1600.

Figure 3.11 (Left) High magnification of the bronchiolar epithelial surface from a severely affected area. The surface consists of ciliated, brush and Clara-like cells. SEM. X 5080.

Figure 3.12 (Right) High magnification of the bronchiolar epithelial surface from a less severely affected area. Clara cells (A) exhibit small microvillus-like projections (arrows). SEM. X 6000.

Figure 3.13 The alveolar region in early pneumonia showing thickening of the alveolar septa (arrows) and
accumulation of neutrophils (n) and a few macrophages (MA) within the alveolar space. SEM. X 1,470.

Figure 3.14 The tracheal epithelium from an early pneumonic lesion showing electron-dense material (arrows) covering the surface. A few microorganisms (O) are embedded in the amorphous material. There is neutrophil infiltration between underlying cells. TEM. X 7,640.

Figure 3.15 High magnification of the surface material showing the bacteria (O) which are not in direct contact with cilia. TEM. X 31,800.

Figure 3.16 The bronchial epithelium in an early pneumonic lesion showing neutrophils (n) between the epithelial cells. TEM. X 5,660.

Figure 3.17 The bronchial epithelial surface showing a large number of mycoplasmas (M) and bacteria (b) between cilia. TEM. X 14,900. The inset micrograph illustrates the tubular organelles (arrows) which may be the means of attachment between mycoplasmas and cilia. TEM. X 72,100.

Figure 3.18 Ultrastructure of the bronchiolar epithelium in an early pneumonic lesion. Both ciliated (C) and non-ciliated cells (N) are present together with distinct basal cells (B). TEM. X 4,300.

Figure 3.19 High magnification of the distinct basal layer (arrows) which was observed in some pneumonic bronchioli. TEM. X 5,200.

Figure 3.20 A severely affected bronchiole showing ciliated cells (C) which have lost their cilia and show rupture of the apical plasma membrane (arrows). TEM. X 7,800.

Figure 3.21 The bronchiolar luminal contents in early pneumonic lesion. Cellular debris, neutrophils and macrophages are present. TEM. X 7,800. The inset micrograph shows a mycoplasma-like organism observed inside the digestive vacuole of a neutrophil. TEM. X 103,600.

Figure 3.22 Tracheal epithelial surface of an advanced pneumonic lesion showing severe squamous
metaplasia. H&E. X 312.

Figure 3.23 The bronchial epithelial surface of an advanced pneumonia lesion showing moderate hyperplasia (large arrows) and metaplasia (white arrows). Large numbers of neutrophils are present between the epithelial cells (small arrows). The lumen of the submucosal gland (S), is full of mucus mixed with large numbers of neutrophils and macrophages. H&E. X 156.

Figure 3.24 The epithelium of a small bronchus from an advanced pneumonia lesion showing moderate hyperplasia. The lymphoid aggregations in the submucosa are more extensive than those seen in early lesions. The lumen of the submucosal gland contains small numbers of neutrophils and macrophages. H&E. X 312.

Figure 3.25 A bronchiole from an advanced pneumonia lesion showing marked epithelial hyperplasia and peribronchiolar lymphoid aggregations. Large numbers of neutrophils and mononuclear cells are found within the epithelium (arrows). The lumen contains mucus, neutrophils and macrophages. H&E. X 156.

Figure 3.26 Tracheal luminal surface from an advanced pneumonia lesion showing single non-ciliated cells (arrows) together with irregular areas of cilia loss. SEM. X 600.

Figure 3.27 High magnification of the ciliated surface showing a large number of mixed microorganisms (arrows) between the cilia. Many of the cilia are entangled and shortened. SEM. X 1,300.

Figure 3.28 The bronchial luminal surface from an advanced lesion. Ciliated cells predominate over other types which are mostly goblet cells (arrows). A large number of neutrophils (n) and macrophages (MA) were observed on the epithelial surface. SEM. X 680.

Figure 3.29 A bronchiole covered by cells with a large number of microvillus-like projections. Clara cells (A) and a small number of ciliated cells (C) were also
common. SEM. X 1980.

Figure 3.30 Another type of bronchiole containing no ciliated cells. An occasional single cilium can be observed (arrow) attached to a Clara-like cell. SEM. X 2,860.

Figure 3.31 A bronchiole from a severely affected part of the lung showing a markedly thickened wall (arrow) and lumen occluded by neutrophils and macrophages. SEM. X 500.

Figure 3.32 High magnification of the luminal contents of the above showing numerous neutrophils (n) and macrophages (MA) together with mucous granules and strands. SEM. X 2,400.

Figure 3.33 Alveoli from a severely affected part of lung showing marked thickening of the alveolar septa (arrows). Alveolar spaces contain both neutrophils and macrophages. Increased numbers of type II epithelial cells can be seen in some alveoli (II). SEM. X 1000.

Figure 3.34 High magnification of two, type II alveolar epithelial cells. The one on the left shows a secretory bubble or cytoplasmic bleb (arrow) protruding from its surface and the one on the right shows an evacuated bubble (arrow). SEM. X 5,800.

Figure 3.35 Ultrastructure of the tracheal epithelium from an advanced pneumonic lesion showing variable degrees of metaplastic change. Most of cells in this region were irregular columnar in shape. TEM. X 6,930.

Figure 3.36 Another area of tracheal epithelium from an advanced lesion showing severe metaplastic change. Most of cells in this region are squamous in shape. TEM. X 8,150.

Figure 3.37 The cytoplasmic contents of damaged tracheal epithelial cells. The mitochondria were either small, electron-dense or clear with disrupted cristae. Observe the neutrophil between the cells (arrows). TEM. X 10,400.

Figure 3.38 The bronchial epithelium from an advanced
pneumonic lesion showing flattened, compressed epithelial cells. Goblet cells are empty. A few bacteria (arrows) are closely associated with cilia. A macrophage (MA) can be seen between the cells. TEM. X 6,880.

Figure 3.39 The epithelium from a severely affected bronchiole showing differentiation of the first layer into typical columnar epithelial cells. The majority of cells have a vacuolated cytoplasm and some ciliated cells have lost their cilia. The apical plasma membrane of some cells has ruptured (arrows), releasing cell contents into the lumen. Mycoplasma-like organisms (M) can be observed in close association with ciliated cells. TEM. X 138,100.

Figure 3.40 Bronchiolar epithelium from severely affected lung showing a cytoplasmic projection (arrow) protruding from the luminal surface of a ciliated cell. TEM. X 10,670.

Figure 3.41 The lumen contents of a severely affected bronchiole. It consists of amorphous material, cellular debris, neutrophils. TEM. 4,700.

Figure 3.42 Microorganisms resembling mycoplasmas (arrows) closely associated with the cilia of affected bronchiolar epithelial cells. TEM. X 38,160.

Figure 4.1 The mean thicknesses (with standard errors) of the tracheobronchial epithelium, submucosa and mucosa at six levels of normal lungs (A), early pneumonic lesions (B) and advanced pneumonic lesions (C).

Figure 4.2 Differences in the mean thickness of the epithelium (A), submucosa (B) and mucosa (C) between normal, early pneumonic and advanced pneumonic groups.

Figure 4.3 The percentage increase in mean thickness of epithelium, submucosa and mucosa in early and advanced pneumonic groups compared to normal lungs.

Figure 4.4 The parameters of the submucosal glands in normal (A), early pneumonic (B) and advanced pneumonic (C) groups.

Figure 4.5 A comparative statistical analysis of submucosal gland parameters of normal and early pneumonic
groups at the upper trachea (A), lower trachea (B), extrapulmonary bronchi (C) and intrapulmonary bronchi (D).

Figure 4.6 A comparative statistical analysis of submucosal gland parameters of normal and advanced pneumonic groups at the upper trachea (A), lower trachea (B), extrapulmonary bronchi (C) and intrapulmonary bronchi (D).

Figure 5.1 Statistical analysis of the different types of glycoprotein at each level (A) and between levels (B) of normal tracheobronchial airways. In C the categories of glycoprotein are simplified into mixed, acid and neutral.

Figure 5.2 Statistical analysis of the different types of glycoprotein at each level (A) and between levels (B) of the tracheobronchial airways of the early pneumonic group. In C the categories of glycoprotein are simplified into mixed, acid and neutral.

Figure 5.3 Statistical analysis of the different types of glycoprotein at each level (A) and between levels (B) of the tracheobronchial airways of the advanced pneumonic groups. In C the categories of glycoproteins are simplified into mixed, acid and neutral.

Figure 5.4 A comparative statistical analysis of the different types of glycoprotein in normal, early pneumonic and advanced pneumonic groups at upper trachea (A), lower trachea (B), extrapulmonary bronchi (C) and intrapulmonary bronchi (D).

Figure 6.1 Epithelium of uninoculated control cultures maintained for 13 hrs in FM4 medium. The cells are healthy and well differentiated. H&E. X 312.

Figure 6.2 Epithelium of tracheal organ culture infected with \( +10^2 \) CFU/ml *M. ovipneumoniae* in FM4 medium for 13 hrs. There is epithelial cell cytoplasmic vacuolation, nuclear swelling and chromatin margination. H&E. X 312.

Figure 6.3 Epithelium of tracheal organ culture inoculated with \( +10^4 \) CFU/ml *M. ovipneumoniae* in FM4 medium
for 13 hrs. There is cytoplasmic vacuolation and moderate loss of cilia. Metaplastic change is evident in some areas (arrow). H&E. X 312.

Figure 6.4 A tracheal organ culture inoculated with \( +10^6 \) M. ovipneumoniae and maintained in FM4 medium for 13 hrs. The epithelium in disorganised with severe epithelial cell exfoliation and loss of cilia. The remaining epithelium has a squamous-like appearance (arrows). H&E. X 312.

Figure 6.5 Tracheal organ culture infected with \( +10^8 \) CFU/ml M. ovipneumoniae and maintained in FM4 medium for 30 min. The epithelial architecture is slightly disrupted. H&E. X 312.

Figure 6.6 Tracheal culture inoculated with \( +10^8 \) CFU/ml M. ovipneumoniae and maintained in FM4 medium for 13 hrs. The epithelium is severely damaged and the nuclei appear pyknotic. H&E. X 312.

Figure 6.7 A different area from the above culture showing a squamous, bistratified epithelium. H&E. X 312.

Figure 6.8 Uninfected tracheal epithelium maintained in FM4 medium for 36 hr. It is composed almost entirely of ciliated cells. SEM. X 2000.

Figure 6.9 Tracheal epithelium 13 hr after inoculation with \( +10^2 \) CFU/ml of M. ovipneumoniae. There is loss of cilia and ciliated cells and a large number of mycoplasmas (M) are attached to the cilia. SEM. X 4000. The inset micrograph shows the mycoplasmas at high magnification attached by fine projections (arrow). SEM. X 16,000.

Figure 6.10 The surface epithelium of a tracheal organ culture infected with \( +10^4 \) CFU/ml of M. ovipneumoniae for 13 hr. It shows exfoliation of ciliated cells (C) and fragments (F). A large number of mycoplasmas (M) are distributed over and between the tips of the cilia. SEM. X 2000.

Figure 6.11 Tracheal epithelium infected with \( +10^6 \) CFU/ml of M. ovipneumoniae for 13 hr. There is loss of a large number of ciliated cells. Numerous mycoplasmas (arrows) are distributed between the cilia. SEM. X 4000. The inset micrograph shows
the firm attachment of mycoplasma to cilia. SEM. X 20,000.

Figure 6.12 Tracheal epithelial surface 30 min post-inoculation with $10^8$ CFU/ml of _M. ovipneumoniae_. It shows severe damage and most of the ciliary carpet is covered by a single layer of mycoplasmas (arrow). SEM. X 4000.

Figure 6.13 One hr post-inoculation with $10^8$ CFU/ml of _M. ovipneumoniae_. The epithelial surface shows severe sloughing of ciliated cells (arrow). Mycoplasmas (M) are visible between the tips of the remaining cilia. SEM. X 1600. The inset micrograph shows a higher magnification of attached mycoplasmas. SEM. X 4000.

Figure 6.14 A tracheal organ culture 13 hr post-inoculation with $10^8$ CFU/ml of _M. ovipneumoniae_. There is very severe damage and most of the ciliary carpet has disappeared. The surface is covered with extruded cells and cellular fragments with only a few ciliated cells remaining (arrows). SEM. X 1000.

Figure 6.15 Higher magnification of the surface of the above culture showing attachment of numerous _M. ovipneumoniae_ organisms to cilia on the remaining cells. SEM. X 8000.

Figure 6.16 Uninoculated tracheal epithelium maintained for 36 hr in FM4 medium. There is good preservation of the ultrastructure and differentiation into ciliated cells. TEM. X 5,000.

Figure 6.17 A tracheal organ culture maintained in FM4 medium infected with $10^2$ CFU/ml of _M. ovipneumoniae_ showing vacuolation of some cells. TEM. X 6,300. The inset micrograph shows higher magnification of the large vacuoles which contain amorphous material and mitochondria. TEM. X 6,300.

Figure 6.18 A tracheal culture maintained for 13 hr in FM4 medium and infected with $10^4$ CFU/ml of _M. ovipneumoniae_. The epithelial cells are disorganised and show marked destruction of subcellular organelles. TEM. X 5,000.
Figure 6.19 The luminal surface of a tracheal culture infected with $10^8$ CFU/ml of *M. ovipneumoniae*. There is very severe damage including the formation of a large number of cytoplasmic vacuoles (V). Electron-dense material has been deposited on the luminal surface (arrows). A few *M. ovipneumoniae* organisms (M) are in intimate contact with the remaining cilia. TEM. X 8,200.

Figure 6.20 The epithelial surface of an organ culture infected with $10^8$ CFU/ml of *M. ovipneumoniae* for 30 min. The luminal surface has less cilia than the controls and the epithelial cell cytoplasm contains a moderate number of vacuoles. TEM. X 8,200.

Figure 6.21 Flattened surface epithelial cells from a similar culture to the above. These cells had an extended cytoplasm and contained flattened nuclei (N), laminated inclusions (L) and numerous microvilli (MV). TEM. X 19,200.

Figure 6.22 *Mycoplasma ovipneumoniae* organisms (arrow) in close contact with the cilia of a tracheal organ culture infected with $10^8$ CFU/ml for 30 min. TEM. X 19,200.

Figure 6.23 High magnification of an *M. ovipneumoniae* organism from the above culture revealing a distinctive pili-like structure (arrow) attaching it to the cilia. TEM. X 160,100.

Figure 6.24 A tracheal culture infected with $10^8$ CFU/ml of *M. ovipneumoniae* for 13 hr exhibiting very severe disorganisation and intracellular change. TEM. X 6,300.

Figure 6.25 *Mycoplasma ovipneumoniae* growth curves in three different culture systems.

Figure 6.26 The growth curves of four titres of *M. ovipneumoniae* maintained in liquid FM4 medium. Multiplication of the microorganisms only occurred if the inoculum was below a titre of $10^6$ CFU/ml.

Figure 6.27 A tracheal organ culture inoculated with $10^1$ CFU/ml *B. parapertussis* for 1 hr and maintained in T199 medium. The epithelial cells show mild
cytoplasmic vacuolation and loss of cilia from their luminal border. H&E. X 312.

Figure 6.28 An organ culture inoculated with $+10^5$ CFU/ml B. parapertussis for 1 hr and maintained in T199 medium. There is complete loss of cilia from many epithelial cells (arrows). H&E. X 312.

Figure 6.29 A tracheal culture infected with $+10^7$ CFU/ml B. parapertussis in T199 medium. The entire epithelial layer is disorganised and shows severe loss of ciliated cells. The remaining cells showing severe cytoplasmic vacuolation and the nuclei appear pyknotic. H&E. X 312.

Figure 6.30 A tracheal culture inoculated with $+10^1$ CFU/ml of B. parapertussis for 1 hr. The epithelium is largely intact although a few cells show extrusions. SEM. X 1,200. The inset micrograph shows a high magnification of structures recognisable as coccobacilli (arrows) which are attached to the lower portion of cilia. SEM. X 12,000.

Figure 6.31 A tracheal organ culture infected with $+10^3$ CFU/ml of B. parapertussis for 1 hr showing many epithelial cell extrusions. SEM. X 1000.

Figure 6.32 The epithelium of a tracheal culture inoculated with $+10^5$ CFU/ml of B. parapertussis for 1 hr showing moderate extrusion of epithelial cells (arrows). SEM. X 1,200.

Figure 6.33 Bacterial microcolonies observed on the top of the ciliary carpet of tracheal cultures inoculated with $+10^5$ CFU/ml of B. parapertussis for 1 hr. SEM. X 8000.

Figure 6.34 The mucus membrane of a tracheal culture inoculated with $+10^7$ CFU/ml of B. parapertussis for 1 hr showing severe loss of cilia. SEM. X 400.

Figure 6.35 High magnification of the epithelium of a tracheal culture infected with $+10^7$ CFU/ml of B. parapertussis. Most of the non-ciliated cells are swollen and protrude into the lumen. SEM. X 2000. The inset micrograph illustrates the close
association of B. parapertussis organisms (arrows) with the cilia. SEM. X 10,000.

Figure 6.36 The epithelium of an organ culture infected with +10^3 CFU/ml of B. parapertussis for 1 hr. It exhibits mild cellular degeneration including slight swelling of the nucleus (N) and margination of chromatin. Occasionally bacteria (arrows) were found among the cilia. TEM. X 6,300.

Figure 6.37 A large number of B. parapertussis organisms (Arrows) are present among the cilia of tracheal cultures infected with +10^5 CFU/ml for 1 hr but cell damage is minimal. TEM. X 5,000.

Figure 6.38 High magnification of a B. parapertussis organism showing the dense, fuzzy, pili-like structures on its surface. TEM. X 85,500. The inset micrograph shows that the extracellular membrane of the cilia at the site of bacterial attachment is absent (arrow). TEM. X 64,100.

Figure 6.39 A tracheal organ culture infected with +10^7 CFU/ml of B. parapertussis for 1 hr showing severe damage and exfoliation of most of the upper and intermediate cells leaving only a layer of basal cells remaining. TEM. X 8,200.

Figure 6.40 Bordetella parapertussis growth curves in tracheal organ culture maintained in T199 medium showing that all titres reached a plateau (lag phase) at the level of +10^6 to +10^8 CFU/ml in the seventh hour post-inoculation.

Figure 6.41 A tracheal organ culture inoculated with +10^3 CFU/ml of P. haemolytica for 3 hrs in T199 medium. The epithelial cells show mild nuclear swelling, vacuolation and loss of cilia. H&E. X 312.

Figure 6.42 An organ culture infected with +10^7 CFU/ml of P. haemolytica for 3 hrs and maintained in T199 medium. There is severe exfoliation of ciliated and non-ciliated epithelial cells (arrows). H&E. X 312.

Figure 6.43 A tracheal organ culture inoculated with +10^9 CFU/ml of P. haemolytica for 3 hrs and maintained in T199 medium. The epithelial layer is severely
disorganised with severe exfoliation of both ciliated and non-ciliated cells. H&E. X 312.

Figure 6.44 The epithelial surface of an organ culture inoculated with $+10^3$ CFU/ml of P. haemolytica for 3 hrs showing moderate loss of cilia. The cilia show an obvious lack of rigidity and some lie across the non-ciliated surface. The non-ciliated surface appears flattened and shows numerous microvilli. SEM. X 4,000.

Figure 6.45 An organ culture infected with $+10^7$ CFU/ml of P. haemolytica for 3 hrs showing severe epithelial damage. Exfoliated cells cover the entire luminal surface. SEM. X 400. The inset micrograph shows the plasma membrane of an exfoliated cell which is roughened and contains numerous holes and pits (arrows). SEM. 2,000.

Figure 6.46 A tracheal organ culture examined 3 hrs post-inoculation with $+10^9$ CFU/ml of P. haemolytica showing extensive and severe exfoliation of the epithelium with complete loss of cilia. SEM. X 400.

Figure 6.47 Exfoliated cells from organ cultures infected with $+10^9$ CFU/ml of P. haemolytica for 3 hrs. They were characterised by a smooth-surfaced membrane which is severely damaged by holes and pits (arrows). Capsulated bacilli-like organisms were observed in small microcolonies (MC) between the fragments of cellular debris. SEM. X 4,000.

Figure 6.48 The ultrastructure of a tracheal culture infected with $+10^3$ CFU/ml of P. haemolytica and examined 3 hrs post-inoculation. The epithelial cells are swollen and their cytoplasm contains numerous empty vacuoles (EV). Some vacuoles are large and contain an amorphous, dark material (DV). TEM. X 5,000.

Figure 6.49 A tracheal organ culture infected with $+10^5$ CFU/ml of P. haemolytica for 3 hrs. Subnuclear vacuolation (arrows) was the most prominent change observed in the epithelial cells. TEM. X 5,000.

Figure 6.50 Epithelial cells of a tracheal organ culture infected with $+10^7$ CFU/ml of P. haemolytica for 3
hrs. There is severe loss of cilia (arrows) and nuclear enlargement (N). The subnuclear cytoplasm is translucent having lost most of its ultrastructural constituents. TEM. X 5,000.

Figure 6.51 The luminal surface of a tracheal culture infected with $10^9$ CFU/ml of P. haemolytica for 3 hrs. It is markedly uneven due to cytoplasmic budding and swelling of microvilli. The cytoplasm contains numerous vacuoles in both supranuclear and subnuclear areas. TEM. X 2,000.

Figure 6.52 Pasteurella haemolytica growth curves in tracheal organ culture maintained in T199 medium showing that all titres reached a plateau of growth at a level of $10^8$ to $10^9$ CFU/ml 7hrs post-inoculation.

Figure 6.53 A tracheal ring infected with $10^2$ CFU/ml N. catarrhalis for 4 hrs and maintained in T199 medium. There is moderate loss of cilia and mild epithelial cell exfoliation. H&E. X 312.

Figure 6.54 An organ culture infected with $10^4$ CFU/ml N. catarrhalis for 4 hrs and maintained in T199 medium. The cytoplasmic vacuolation of epithelial cells and early squamous metaplasia (arrows) are the main features. H&E. X 312.

Figure 6.55 An organ culture inoculated with $10^6$ CFU/ml N. catarrhalis for 4 hrs and maintained in T199 medium. The epithelial layer is disorganised and there is moderate loss of cilia as well as mild epithelial exfoliation. H&E. X 312.

Figure 6.56 A tracheal culture infected with $10^8$ CFU/ml N. catarrhalis for 4 hrs and maintained in T199 medium. There is severe loss of cilia and ciliated cells. In some areas the remaining epithelium shows squamous metaplasia. H&E. X 312.

Figure 6.57 The epithelium of an organ culture infected with $10^2$ CFU/ml N. catarrhalis for 4 hrs showing mild loss of cilia and sloughing of a few epithelial cells. SEM. X 2,000.

Figure 6.58 The luminal surface of a tracheal organ culture inoculated with $10^4$ CFU/ml of N. catarrhalis for 4 hrs showing numerous bulbous projections. The
surface of these varies from rough to microvillus-like in appearance. SEM. X 2,000.

Figure 6.59 The surface epithelium of an organ culture infected with \(10^6\) CFU/ml of _N. catarrhalis_ for 4 hrs. Although there is an increase in non-ciliated cells (arrows) the number of bulges and protruding cells in less than at lower titres. SEM. X 2,000.

Figure 6.60 The epithelial surface of a tracheal culture infected with \(10^8\) CFU/ml of _N. catarrhalis_ for 4 hrs showing severe loss of cilia. The non-ciliated surface in uneven and rough with numerous knob-like projections. Other cells are enlarged and swollen and many have a ruffled plasma membrane. SEM. X 4,000.

Figure 6.61 An organ culture infected with \(10^2\) CFU/ml of _N. catarrhalis_ showing mild loss of cilia only. TEM. X 6,300.

Figure 6.62 A tracheal culture infected with \(10^4\) CFU/ml of _N. catarrhalis_ for 4 hrs showing prominent subnuclear vacuolation and nuclear margination of some epithelial cells. TEM. X 5,000.

Figure 6.63 The epithelium of a tracheal organ culture infected with \(10^6\) CFU/ml of _N. catarrhalis_ for 4 hrs. It consists of an upper layer which is composed of flattened cells with few microvilli and a lower layer containing almost cuboidal cells. Both sloughed and intact epithelial cells exhibit severe vacuolation (V) and loss of intercellular digitation. TEM. X 3,200.

Figure 6.64 An epithelial cell undergoing exfoliation from a tracheal culture infected with \(10^6\) CFU/ml of _N. catarrhalis_ for 4 hrs. It has no discrete nuclear membrane and the mitochondria (M) are electron-dense. TEM. X 8,200.

Figure 6.65 A tracheal organ culture infected with \(10^8\) CFU/ml of _N. catarrhalis_ for 4 hrs showing severe damage to its luminal surface with complete loss of microvilli and cilia. TEM. X 8,200.

Figure 6.66 _Neisseria catarrhalis_ growth curves in tracheal organ culture maintained in T199 medium. All
titres reached a plateau of growth at a level of 
$10^8$ CFU/ml 8 hrs post-inoculation.
LIST OF TABLES

TABLE 1.1: NORMAL TRACHEOBRONCHIAL AIRWAY CELL TYPES .................................................. 8
TABLE 1.2: NORMAL SECRETORY CELL TYPES OF THE SURFACE EPITHELIUM OF AIRWAYS (JEFFERY, 1983) .............................................................. 17
TABLE 1.3: SUBMUCOSAL GLAND DISTRIBUTION THROUGHOUT TRACHEOBRONCHIAL AIRWAYS (JEFFERY, 1983) .............................................................. 18
TABLE 1.4: ORGANISMS COMMONLY ISOLATED FROM NORMAL AND PNEUMONIC LUNGS OF YOUNG SHEEP IN NEW ZEALAND .................................................. 23
TABLE 2.1: SUMMARY OF THE ULTRASTRUCTURAL FEATURES OF TRACHEOBRONCHIAL EPITHELIAL CELL TYPES IN NORMAL SHEEP .................................................. 63
TABLE 4.1: THE RATIO BETWEEN THE LAYERS OF THE TRACHEOBRONCHIAL MUCOSA OF NORMAL SHEEP AT DIFFERENT LEVELS .................................................. 103
TABLE 4.2: THE RATIO BETWEEN THE LAYERS OF THE TRACHEOBRONCHIAL MUCOSA OF EARLY PNEUMONIC LESIONS AT DIFFERENT LEVELS .................................................. 103
TABLE 4.3: THE RATIO BETWEEN THE LAYERS OF THE TRACHEOBRONCHIAL MUCOSA OF ADVANCED PNEUMONIC LESIONS AT DIFFERENT LEVELS .................................................. 103
TABLE 6.1: THE RELATION BETWEEN TIME-STOPPING EFFECT AND NUMBER OF M. OVIPNEUMONIAE ORGANISMS (CFU/ML) IN FM4 MEDIUM .................................................. 137
TABLE 6.2: THE EFFECT OF FOUR M. OVIPNEUMONIAE TITRES ON THE CILIARY ACTIVITY OF TRACHEAL ORGAN CULTURES IN FM4 MEDIUM .................................................. 137
TABLE 6.3: THE EFFECT OF FOUR M. OVIPNEUMONIAE TITRES ON THE CILIARY ACTIVITY OF TRACHEAL ORGAN CULTURES IN T199 MEDIUM .................................................. 139
TABLE 6.4: CYTOPATHOLOGICAL CHANGES IN EPITHELIAL CELLS OF TRACHEAL ORGAN CULTURES INOCULATED WITH M. OVIPNEUMONIAE .................................................. 139
TABLE 6.5: THE RELATION BETWEEN TIME-STOPPING EFFECT AND NUMBER OF B. PARAPERTUSSIS ORGANISMS (CFU/ML) .................................................. 144
TABLE 6.6: EFFECT OF FOUR B. PARAPERTUSSIS
TITRES ON THE CILIARY ACTIVITY OF OVINE FOETAL TRACHEAL ORGAN CULTURE

TABLE 6.7: CYTOPATHOLOGICAL CHANGES IN EPITHELIAL CELLS OF TRACHEAL ORGAN CULTURES INOCULATED WITH B. PARAPERTUSSIS

TABLE 6.8: THE RELATION BETWEEN TIME-STOPPING EFFECT AND NUMBER OF P. HAEMOLYTICA ORGANISMS (CFU/ML)

TABLE 6.9: EFFECT OF FOUR P. HAEMOLYTICA TITRES ON CILIARY ACTIVITY OF TRACHEAL ORGAN CULTURES

TABLE 6.10: CYTOPATHOLOGICAL CHANGES IN EPITHELIAL CELLS OF TRACHEAL ORGAN CULTURES INOCULATED WITH P. HAEMOLYTICA

TABLE 6.11: THE RELATION BETWEEN TIME-STOPPING EFFECT AND NUMBER OF N. CATARRHALIS ORGANISMS (CFU/ML)

TABLE 6.12: EFFECT OF FOUR N. CATARRHALIS TITRES ON THE CILIARY ACTIVITY OF TRACHEAL ORGAN CULTURES

TABLE 6.13: CYTOPATHOLOGICAL CHANGES IN EPITHELIAL CELLS OF TRACHEAL ORGAN CULTURES INOCULATED WITH N. CATARRHALIS