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

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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 pneumonic 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 pneumonic 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 pneumonic lesions. The most significant changes in submucosal gland parameters of early pneumonic sheep occurred in the intrapulmonary bronchi. In sheep with advanced pneumonic lesions changes were most severe in both intrapulmonary and extrapulmonary bronchi. Enlargement of the submucosal glands in pneumonic 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.

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titres reached a plateau of growth at a level of $\pm 10^8$ CFU/ml 8 hrs post-inoculation.

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