AN ULTRASTRUCTURAL AND HISTOLOGICAL STUDY OF THE EQUINE RESPIRATORY TRACT IN HEALTH AND DISEASE

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by

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C O N T E N T S

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DECLARATION

The work described in this thesis was carried out by myself except for the technical assistance received and for the clinical examination of the horses with respiratory disease, both of which have been acknowledged.

The results presented in Chapter 4 and Chapter 6 have recently been published:

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S U M M A R Y

There were two main aims in the work presented in this thesis. First, to carry out a detailed scanning electron microscopic (SEM), histological and, where appropriate, transmission electron microscopic (TEM) study of the surface features of the entire respiratory tract of normal horses. The second aim was to investigate the effects of respiratory disease (Streptococcus equi infection or Strangles and chronic obstructive pulmonary disease) on equine respiratory tract surfaces.

From the review of the literature in Chapter 1 it became apparent that, despite the importance of respiratory disease in horses, there was little information available concerning the histological and ultrastructural features of the respiratory tract either in healthy horses or in horses affected with disease of their respiratory systems. There was no previous description of the morphological features of the complete respiratory tract from the nasal vestibule to the alveolar membrane.

Because the structure of the mammalian respiratory tract is complex and there are a number of features specific to Equidae, in Chapter 2 the gross anatomical features of the equine respiratory tract were outlined as a prelude to the detailed microscopic study.

In Chapter 4 of the work, 21 horses of various types, sex and age (2 days to over 20 years) and 2 female donkeys were used in a study of the upper respiratory

tract. Samples were taken for SEM, histological and histochemical studies from 11 sites, from the basal fold of the ventral nasal concha to the infraglottic cavity of the larynx.

Because relatively large areas are available for examination by SEM, this proved to be the most useful method for defining the nature of the epithelial surfaces. The light microscopic appearance generally confirmed that of SEM.

Epithelial surfaces were similar in all animals regardless of type or age. There was an abrupt junction between the stratified squamous epithelium of the nasal vestibule and the nonciliated stratified cuboidal epithelium of the mucosa of the rostral nasal cavity. In contrast, there was a gradual change from the latter to the well ciliated mucosa of the caudal nasal cavity. Between the rostral nonciliated and caudal well ciliated surfaces there was an extensive partially ciliated transitional zone which extended caudally to at least the level of the first cheek tooth, thus covering a considerable area of the rostral nasal cavity.

Nasopharyngeal surfaces were covered by patches of ciliated and nonciliated cells and the guttural pouch epithelium was well ciliated. Stratified squamous epithelium merged with stratified cuboidal epithelium on the epiglottis and the ventral larynx, caudal to the glottis, was clothed

with a luxuriant carpet of cilia.

Many mucus-secreting surface cells and mucosal glands were present in the upper respiratory tract. The former were present in both ciliated and nonciliated surfaces and were observed in various stages of their secretory cycle. The glands were of a serous type rostrally and sero-mucous in nature caudally. A histochemical analysis using AB-PAS stain showed that they contained mucosubstances of both surface cells and glands were predominantly mixed or acidic in composition.

The upper respiratory tract study was completed by sampling the mucosa of the ethmoidal conchae of 2 horses for SEM and histology. The olfactory epithelium present in this most caudo-dorsal area of the nasal cavity resembled that of other mammals.

In the study of the upper respiratory tract the number of sample sites from each area was necessarily limited. The few samples taken from the long equine nasal cavity did not give a complete picture of the epithelial surfaces, particularly the extent of the partially ciliated transitional zone. Thus Chapter 5 was devoted to a more detailed study of the nasal cavity in 4 horses. Samples from the dorsal and ventral nasal conchae and the nasal septum were taken from each of 8 precise anatomical levels (24 samples per horse), for SEM and light microscopy. In addition, samples for TEM were taken from the 4 rostral

levels (12 samples per horse) from 2 horses. The latter samples confirmed the stratified cuboidal nature of the epithelium in the rostral nasal cavity.

This detailed study mapped out more clearly the patterns of ciliation in the nasal cavity and showed that ciliated cells first appeared most rostrally in the dorsal nasal concha, then the ventral nasal concha and, finally, on the nasal septum. On the latter it was found that small patches of nonciliated cells were still present as far caudally as the level of the sixth cheek tooth. This more comprehensive study also revealed that the partially ciliated transitional zone covered an area representing approximately 35% of the total length of the nasal cavity.

In Chapter 6, the lower respiratory tract surfaces were examined by SEM and light microscopy and TEM was used to give a more detailed picture of the smallest airways and the alveolar membrane. Samples were taken from the trachea and lobar bronchi and, after airway perfusion, from small bronchi and the lung. In the latter, bronchioles, alveolar ducts and alveoli were visualized.

Viewed with the light microscope, the epithelium in the trachea and bronchi was pseudostratified columnar ciliated, i.e., respiratory epithelium. Mucous cells containing mixed and acidic mucosubstances were numerous. Sero-mucous mucosal glands were less numerous in the trachea and bronchi than in the upper respiratory passages and were

sparse in small bronchi. SEM revealed a thick carpet of cilia with interspersed mucous cells. The latter became more obvious in the small bronchi and gave a "moth-eaten" appearance to the surface.

A simple cuboidal epithelium of ciliated cells and nonciliated bronchiolar epithelial (Clara) cells covered the surface of the bronchioles. As the bronchioles decreased in size the ciliated cells decreased in number, while the Clara cells became more numerous and were the predominant cells in the terminal bronchioles. However, both cell types were present at the abrupt junction with alveolar ducts and were found lying adjacent to the cells of the alveolar membrane. TEM confirmed the presence of ciliated cells and mucous cells in the small bronchi. These resembled the ciliated cells and mucous cells of mammals in general. In bronchioles the Clara cells were distinguished by the presence of large, electron-dense cytoplasmic granules and abundant smooth endoplasmic reticulum.

Alveolar pores were a feature of the lung parenchyma in all the animals and their number increased with age.

Type I and Type II pneumocytes were distinguished with SEM and TEM. The former covered most of the alveolar surface, possessed a few small microvillous processes and distinct cell boundaries. Type II pneumocytes protruded

into the alveolar space and microvilli were clustered at the periphery of the cells while the smoother central area often contained small pores. Alveolar macrophages were observed in all the animals. These distinctive cells varied in shape and size, had uneven or ruffled surfaces and long, slender cytoplasmic processes. They often appeared to be passing through alveolar pores.

Mucosal lymphoid tissue was observed in both upper and lower respiratory tracts.

Studies of the normal equine respiratory tract led to an investigation of respiratory disease in horses. In Chapter 7 a spontaneous outbreak of Streptococcus equi infection or Strangles was studied in a group of 19 young ponies. A systematic ultrastructural and histological study of the entire respiratory tract was carried out in 10 of these ponies. The sample sites were the same as those used in Chapters 4 and 6 with additional samples from the nasal and oral surfaces of the soft palate. The ponies were divided into 4 groups, each of which represented a stage in the course of the disease from day 4 to day 90 of the outbreak. In addition, 2 adult ponies, in the acute stage of Strangles, were included in the study.

Inflammation of the nasopharyngeal region and associated lymphadenitis with abscess formation was the main necropsy finding in acute Strangles, while inspissated pus in the guttural pouches and slight enlargement of the

retropharyngeal lymph nodes were found in clinically recovered animals. None of the animals showed any evidence of disease in the lungs at necropsy, histologically or with SEM or TEM.

The necropsy findings were confirmed histologically but damage to epithelial surfaces was more easily detected by SEM which revealed loss of cilia from the guttural pouches, larynx and the larger respiratory passages. These changes were more widespread in clinically recovered animals which also had loss of cilia from nasal cavity surfaces.

A few Gram positive cocci were observed histologically on the oral surface of the soft palate in acute Strangles. Adherence of cocci to nonciliated surfaces in the same site and in the nasal septum was clearly demonstrated by SEM in the early stages of the disease and persisted on the soft palate in recovered animals.

In Chapter 8 another spontaneous respiratory disease was investigated in a study of 10 horses affected with chronic obstructive pulmonary disease. Samples for light microscopy, SEM and TEM were taken from the entire respiratory tract, as in Chapters 4 and 6, with additional samples from segmental bronchi and caudal lobes of the lung.

The gross pathological and microscopic findings generally reflected the severity of the clinical signs.

The upper respiratory tract surfaces were normal except for a few small patches of deciliation in the guttural pouches of 6 horses and the larynx of 4 horses. Nonciliated patches became increasingly frequent distally in the lower respiratory passages in all the horses.

The main histopathological lesion was focal bronchiolitis with peribronchiolar mononuclear cell infiltration and fibrosis. Epithelial hyperplasia, with surface cells containing acidic and mixed mucosubstances was a feature of affected bronchioles which were often occluded with mucus and many neutrophils. Alveolar overinflation was present and areas of emphysema occurred in 6 horses.

Although SEM revealed flattened or collapsed Clara cells and sparsely ciliated cells, there was no evidence of obvious mucus-secreting cells in the bronchiolar epithelium. However, mucus was present on the surface of many bronchioles often associated with large numbers of degenerate inflammatory cells. With TEM the changes in bronchiolar epithelial cells included loss of cilia, cytoplasmic vacuolation and glycogen deposits in ciliated cells, while partial or complete loss of granules was a feature of Clara cells. In some bronchioles, cells containing mucous granules were present.

Overinflation of alveoli with loss of normal structure and a great increase in the number of alveolar

pores was well demonstrated by SEM, while TEM showed some evidence of focal alveolar fibrosis.

An increase in the number of Type II pneumocytes, some of which appeared degenerate with many enlarged pores and vacuoles, were observed with SEM and TEM. There was an increased number of alveolar macrophages which were often in the vicinity of Type II pneumocytes. The Type I pneumocytes appeared normal.

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LIST OF ABBREVIATIONS

HE	:	Haematoxylin and eosin stain
AB-PAS	:	Alcian blue - periodic acid Schiff stain
SEM	:	Scanning electron microscope (or microscopy or microscopical)
TEM	:	Transmission electron microscope (or microscopy or microscopical)
COPD	:	Chronic obstructive pulmonary disease

CHAPTER 1

INTRODUCTION AND REVIEW OF THE LITERATURE

GENERAL INTRODUCTION

The problem of respiratory disease in the horse has been recognised for centuries and over the years has continually stimulated the interest of horse owners, clinicians and other workers in the field of equine medicine. Many of the diseases described in the early literature are even now still a significant cause of morbidity and mortality in the equine population and thus continue to be of great economic importance to the horse industry. In a survey of post-mortem findings in 480 horses, Baker and Ellis (1981[a,b]) found that 8.5% died as a direct result of respiratory disease and that a further 15.4% were affected with disease of the respiratory system. The numerous pathogens, including viruses, bacteria and parasites, which can invade the respiratory system and the various clinical syndromes which they cause have been reviewed by a number of workers (Fitzwygram, 1903; Mahaffey, 1962; Powell, 1975; Clayton and Murphy, 1980; Mair, 1989; Mair and Lane, 1989). These workers have shown that equine respiratory disease occurs in a number of different forms :

(1) Chronic respiratory disease of horses has always been important to man and the clinical syndrome known as "broken wind" or "heaves", with its characteristic abdominal expiratory effort, was probably first described by Aristotle as early as 333 B.C. (Smith, 1919). The

symptoms of broken wind were first linked with pulmonary emphysema by Floyer in 1698 and the functional implications of the condition were recognised in the 18th century (Gibson, 1751). Fitzwygram (1903) wrote at length of the possible aetiology of the condition suggesting that faulty management and, in particular, the feeding of mouldy hay could lead to the development of the disease. Since then there have been numerous reports and reviews (Alexander, 1959; Gillespie and Tyler, 1969; Littlejohn, 1979; Sasse et al, 1986; Derksen, 1987; Clarke, 1987; Winder and von Fellenberg, 1987, 1988; Thomson and McPherson, 1988; Robinson, 1989).

(2) Parasitic infection can also be a cause of chronic coughing in horses (Nicholls et al, 1978; Clayton and Murphy, 1980). Infection with lungworm (Dictyocaulus arnfieldi) has been described in horses and donkeys (Round, 1976; Nicholls et al, 1979; Clayton and Duncan, 1981) and damage to the lungs by migrating larvae of Parascaris equorum may cause coughing in young animals (Clayton and Duncan, 1977, 1978; Clayton and Murphy, 1980).

(3) Virus infection often causes acute respiratory disease in horses. Epizootics of influenza were common in the 17th and 18th centuries (Wood, 1988) and the disease was described in 1903 by Fitzwygram. However, the causal organism, equine influenza virus A/equi/1/Prague, was only isolated in 1958 by Sovinova et al. A second

equine influenza virus A/equi/2/Miami was isolated in 1963 by Waddell and his co-workers. The two strains of equine influenza virus were implicated in upper respiratory disease of thoroughbred horses in 1965 by Ditchfield et al and shortly afterwards a complete description of the clinical signs of equine influenza and its epidemiology was reported (Gerber, 1969). Many reports in the literature have since appeared (Powell et al, 1974; Rose et al, 1974; Sherman et al, 1977; Clayton and Murphy, 1980; Thorsen et al, 1983; Wood, 1988) and these indicated that the disease continues to be a problem although practical vaccination procedures were available as early as 1965 (Gerber, 1969).

Infection with equine herpes virus Types 1 and 2, equine rhinovirus 1 and adenovirus have all be described in outbreaks of respiratory disease in horses (Ditchfield et al, 1965; Powell et al, 1974; Rose et al, 1974; Sherman et al, 1977; Clayton and Murphy, 1980). While all of these can cause some degree of illness and coughing, vaccination is not generally available. The only vaccines to be developed so far are those for equine herpes virus Type 1 and these have had limited success (Blood et al, 1989).

(4) While secondary infection with bacteria may be a sequel to virus infection (Gerber, 1969; Clayton and Murphy, 1980), some bacteria alone are capable of causing respiratory disease and Streptococcus equi infection or

"Strangles" is probably the most notable of these diseases. The contagious nature of Strangles was recognised in the 17th century and the disease was experimentally proved to be contagious by Lafosse in 1790 (Todd, 1910). The streptococcus of Strangles was isolated by Schutz (1888) and a full description of the disease, including early vaccination attempts, was given by Todd in 1910. Investigations by numerous workers have continued to the present day and an effective vaccine has still to be produced (Bazeley, 1943; Mahaffey, 1962; Reif et al, 1981; Clabough, 1987; Wilson, 1988; Timoney, 1988).

A number of other bacteria are known to cause respiratory disease in horses. Corynebacterium equi can cause lung abscesses and bronchopneumonia in foals (Clayton and Murphy, 1980; Hillidge, 1986). Streptococcus pneumoniae and Streptococcus zooepidemicus have been associated with inflammatory disease of the lower respiratory tract (Burrell et al, 1986; Mair, 1989; Mair and Lane, 1989).

Glanders is an infective disease of horses usually involving the respiratory tract. The causal organism, Actinobacillus mallei, is also known by a number of other generic names such as Pfeifferella and Malleomyces. This disease has been known since ancient times and was mentioned in 400 A.D. by Vegetius (Smith, 1919). It was widespread in Europe, Asia and Africa up

to the end of 1918 (Hutra et al, 1949) and is still present in parts of Eastern Europe, Asia and North Africa (Blood et al, 1989); however, the disease was eradicated in Great Britain in 1928 (Davies, 1988).

(5) Fungal infection of the guttural pouches (Guttural pouch mycosis) was first described by Rivolta (1868). After examining a second case in 1873, he concluded that the causal organism was an aspergillus and this organism was also implicated in the disease by later workers (Sequens, 1894; Ries, 1903).

A penicillium was isolated from a case by Andersson (1931) and also by Buer (1942) from one of his cases. By 1966 there had been reports of 12 confirmed cases of guttural pouch mycosis, diagnosed at post-mortem examination (Cook et al, 1968). However, the first complete clinical and endoscopic description of the disease highlighting the serious clinical manifestations, including pharyngeal paralysis and epistaxis, which often proved to be fatal, was established by Cook (1966). The latter worker also recognised the disease as a relatively common occurrence. The causal organism, Aspergillus nidulans, was isolated from affected horses in 1968 by Cook and co-workers. Since then there have been many case reports and descriptions of various forms of treatment (Johnson, 1973; Rawlinson and Jones, 1978; Church et al, 1986; Freeman et al,

1989; Lane, 1989).

(6) Horses occasionally bleed from the nose after exercise (Mahaffey, 1962) and Cook (1974) comprehensively reviewed the disease, describing the detailed findings in 50 horses with epistaxis after exercise. Pascoe and Wheat (1980) linked this clinical sign with haemorrhage from the lung and the condition became knows as "Exercise Induced Pulmonary Haemorrhage" (E.I.P.H.).

An extensive review was carried out in 1985 by Clarke in an attempt to integrate recent findings with the various previous hypotheses. A systematic investigation has since been carried out (O'Callaghan et al, 1987) and Gunson et al (1988), in a retrospective study of sudden death in racehorses, found that 9 out of 11 horses had died of acute pulmonary haemorrhage. Cook et al (1988) have proposed the hypothesis that upper airway obstruction is the possible cause of E.I.P.H. but many questions still remain unanswered.

Despite the importance of respiratory disease in horses, surprisingly few studies of the normal microscopic and ultrastructural features of the respiratory tract have appeared in the literature. Most reports of the general histological and ultrastructural features have been confined to the equine lung, that is, the smaller respiratory passages, the interalveolar septum and the pleura (Gillespie and Tyler, 1967[a]; Nowell and Tyler,

1971; Tyler et al, 1971; Plopper, 1971; Gehr and Erni, 1980; Mair et al, 1987) while more detailed ultrastructural features of the bronchiolar epithelium have been described by Plopper et al (1979, 1980[b]) and Plopper (1983). The upper respiratory tract and, in particular, the nasal cavity have largely been ignored. Mair et al (1987) have described the general light microscopic details of the upper respiratory tract but no reports of the ultrastructural features have appeared in the literature. It seems that the knowledge of the normal appearance of respiratory tract surfaces is fragmentary and a complete study is necessary before attempting to evaluate changes induced by disease processes. The first objective of this study was to carry out a systematic scanning electron microscopic (SEM) survey of the normal equine respiratory tract surfaces from the nasal vestibule to the alveolar membrane with parallel histological studies and also transmission electron microscopy (TEM) of selected sites.

REVIEW OF THE LITERATURE OF THE CELLULAR POPULATION OF THE MAMMALIAN RESPIRATORY TRACT.

The membranous lining of the trachea and bronchi was first mentioned by Laurentius (1602) and later in 1712 Morgagni described more precisely the nature of the respiratory mucosa including the tracheal glands and their ducts. Early descriptions of ciliated cells (Purkinje

and Valentin, 1834; Sharpey, 1836) and of mucous secreting goblet cells (Henle, 1837; Bowman, 1847), have been followed by many reports in the literature of the light microscopic appearance of the mucosal lining of the respiratory tract (Kolliker, 1853; Schulze, 1872; Frankenhaueser, 1879; Bryant, 1916; Miller, 1932; Lucas, 1932; Schaeffer, 1932; Krahl, 1955; Bertalanffy, 1964; Ali, 1965; Pass et al, 1971; Lauweryns and Peuskens, 1971; Hage, 1972; Mariassy and Plopper, 1983; Wilson et al, 1984; Leeson et al, 1985; Tyler et al, 1988).

Transmission Electron Microscopic (TEM) Studies.

Transmission electron microscopy, introduced in the 1950's, opened up a completely new field of study and, in addition to providing further details of cells previously identified by the light microscope, it also revealed other cells of the respiratory tract mucosa which had not previously been recognised. These include the serous cell (Jeffery and Reid, 1975), brush cell (Rhodin and Dalhamn, 1956; Meyrick and Reid, 1968; Baskerville, 1970[a]; Allan, 1978), basal and intermediate cells (Rhodin and Dalhamn, 1956; Rhodin, 1966; Jeffery and Reid, 1975), endocrine cells (Hage, 1973, 1980; Cutz and Conen, 1972; Jeffery, 1983), alveolar type I and type II cells (Low, 1952; Karrer, 1956; Curry et al, 1969; Breeze and Wheeldon, 1977) and alveolar macrophages (Gail and Lenfant, 1983; Burri, 1985). Detailed

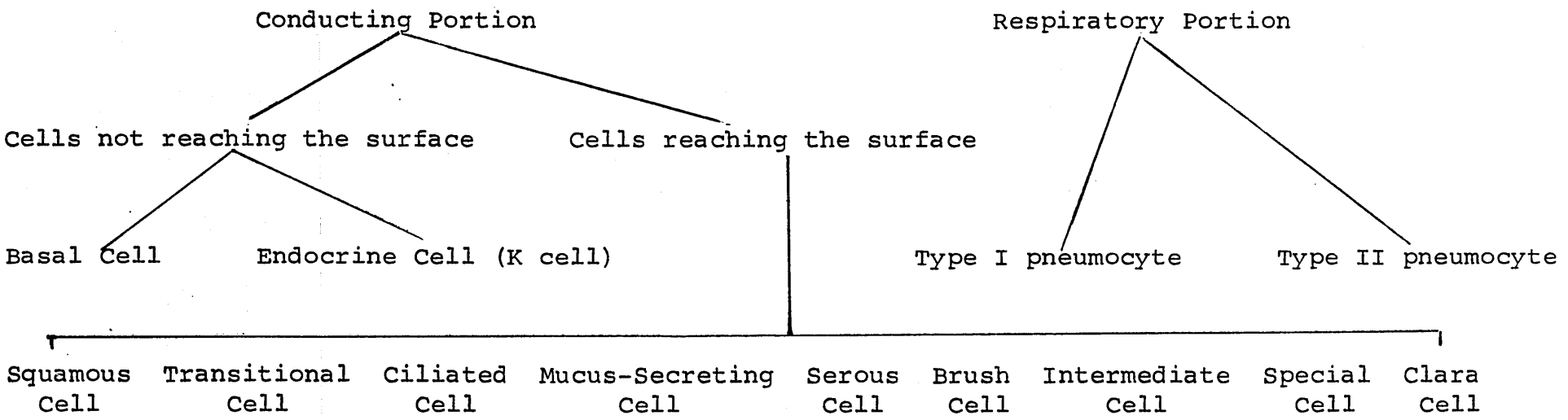
ultrastructural features of cells already well known to light microscopists were now described, namely:- ciliated and nonciliated cells (Rhodin and Dalhamn, 1956; Rhodin, 1966; Friedmann and Bird, 1971), mucus-secreting (goblet) cells (Rhodin and Dalhamn, 1956; Rhodin, 1959; Jeffery and Reid, 1975;. Thaete et al, 1981) and the non-ciliated bronchiolar epithelial (Clara) cell (Cutz and Conen, 1971; Smith et al, 1974; Evans et al, 1978). At the present time at least 11 different cell types populating the epithelium of the adult mammalian tracheobronchial and bronchiolar tree have been described, but not all of these are present in every species (Jeffery and Reid, 1975; Breeze et al, 1976; Breeze and Wheeldon, 1977; Phipps, 1981; Gail and Lenfant, 1983; Jeffery, 1983; Jeffery and Corrin, 1984; Plopper et al, 1986).

An indication of the large number of ultrastructural studies carried out in many different species and the area of the respiratory tract examined by TEM is given in Table 1:1 (Page 28).

Following an exhaustive search of the literature it seemed appropriate at this stage to give a summary of the main morphological features of the various cells which populate the mammalian respiratory tract. The cells of the respiratory tract epithelium are summarised on page 10.

CELLS OF THE MAMMALIAN RESPIRATORY TRACT EPITHELIUM

(excluding the Olfactory Epithelial Cells)



(1) Ciliated Cells

In mammals ciliated cells and mucous cells are the predominant cell types of the respiratory epithelium, there being approximately five ciliated cells to every mucous cell (Rhodin and Dalhamn, 1956; Frasca et al, 1968). In the epithelium of the airways ciliated cells extend from the nasal cavity to the smallest bronchioles.

Detailed descriptions of respiratory tract ciliated cells indicate that there is little species variation in their morphology and they appear very similar in such diverse species as the rat (Rhodin and Dalhamn, 1956; Jeffery and Reid, 1975), dog (Frasca et al, 1968) and man (Rhodin, 1959, 1966; Friedmann and Bird, 1971). Ciliated cells are columnar, measuring approximately 20 µm x 7 µm and taper to 2 µm where they attach to the basement membrane (Frasca et al, 1968). The lateral cell surfaces form complex interdigitations with basal, intermediate and adjacent cells and attachments are made by desmosomes except at the lumenal surface where tight cell junctions are formed.

The cell cytoplasm is more electron lucent than that of the other respiratory epithelial cells. Rough endoplasmic reticulum is present in the lower part of the cell and a well developed Golgi apparatus is situated above the nucleus, which may contain a prominent nucleolus. Many mitochondria are found in the upper part of the cell

just below the apical row of basal bodies, to which the cilia are attached.

The lumenal surface of each cell bears approximately 250 cilia, each approximately 6 µm long and 0.3 µm wide with many microvilli interspersed among them (Rhodin and Dalhamn, 1956; Frasca et al, 1968; Baskerville, 1970[b]). These microvillous projections can be as large as 2 µm long and 0.1 µm in diameter with fine filaments within their cytoplasmic core. Immature cells undergoing cilio-genesis have large numbers of microvilli on their surfaces, with only a few short cilia. The basic structure of cilia with basal bodies and rootlets appears to be common to all mammals. The cell membrane covers the free portion of the cilium which contains longitudinal microtubules, two of which form the central core with nine double tubules arranged in a ring peripherally. The central tubules fuse at the tip of the cilium and do not extend to the basal body as the others do. Atypical cilia, formed by fusion of several cilia, have been observed in the respiratory epithelium in man (Friedmann and Bird, 1971), hamsters (Harris et al, 1974) and dogs (Wheeldon and Pirie, 1974) where they are regarded as a normal feature of the canine bronchial epithelium.

(2) Mucus-Secreting (Goblet) Cells

These cells are found throughout the airway with the exception of the nasal vestibule, the terminal and,

where present, the respiratory bronchioles. In the trachea there are apparently more mucous cells in the cartilaginous wall than in the membranous wall (Rhodin and Dalhamn, 1956; Frasca et al, 1968). The apical cytoplasm of the mucus-secreting cell contains many mucous granules and ribosomes and thus is relatively electron dense compared to ciliated cells. In the mature cell, mucous granules give the cell its characteristic "goblet" shape, with its basal cytoplasm tapering towards the basement membrane. Discharged and immature cells are more columnar in appearance.

The oval nucleus, with a small nucleolus, is found at the base of the cell associated with a few mitochondria. A well developed Golgi apparatus, along with extensive rough endoplasmic reticulum, is situated above the nucleus. An irregular intercellular space surrounds each cell and this is closed at the lumenal surface by tight junctional complexes, while in the lower parts of the cell there are complex interdigitations with adjacent cells and attachment by desmosomes. Mature cells have a bulging surface due to accumulation of mucous granules and often protrude into the lumen of the airway between the ciliated cells. Mucous secretion is extruded through pits or pores on the cell surface. Surface microvilli are peripheral on mature cells but are more numerous and may cover the entire surface of immature or

discharged cells. A small mucous granule cell has been described in the tracheobronchial epithelium of hamsters (Becci et al, 1978) and is considered to represent a developing mucus-secreting cell.

(3) Basal Cell

These oval-shaped electron dense cells form a single row attached to the basement membrane and as their apical borders do not reach the surface, they give rise to the pseudostratified appearance of the epithelium. The large nucleus almost fills the cell and the sparse cytoplasm contains a small Golgi zone, many tonofilaments, ribosomes, a few mitochondria and glycogen granules. A wide irregular intercellular space surrounds the cell and cytoplasmic processes contact adjacent cells, often forming desmosomes (Rhodin and Dalhamn, 1956; Frasca et al, 1968; Baskerville, 1970[b]).

(4) Intermediate Cells

Lying just above the basal cells in a poorly defined layer, these spindle-shaped cells extend towards the surface. There is a large oval nucleus and abundant cytoplasm containing mitochondria and rough endoplasmic reticulum. Cytoplasmic projections extend to adjacent cells and sometimes form desmosomes (Rhodin, 1966; Baskerville, 1970[b]). These cells do not normally reach the surface in the rabbit (Konradova, 1966) or in the pig (Baskerville, 1970[b]) but have been observed at the surface

in the rat with a few microvilli on their apical borders (Jeffery and Reid, 1975). Intermediate cells are generally regarded as differentiating cells and may become ciliated or mucus-secreting cells. They are present in very small numbers in the normal epithelium (McDowell et al, 1983).

(5) Serous Cell

These cells, which are never present in large numbers, were first described in the rat (Jeffery and Reid, 1975) and identification in other species has been confined to the cat, hamster and human foetus (Jeffery, 1983). The cell rests on the basement membrane and extends to the airway lumen where a few microvilli are present on the apical surface. The irregular nucleus is situated basally and the cytoplasm contains abundant rough endoplasmic reticulum. Their characteristic granules are variable in number and are smaller and more electron-dense than those of mucus-secreting cells. The function of the serous cell in unknown but it has been shown that the secretory granules contain neutral glycoprotein and it has been suggested that its secretion may be of lower viscosity than that of mucous cells and that it contributes to the periciliary liquid layer below the tracheobronchial mucus (Jeffery and Reid, 1975).

(6) Brush Cell

This cell was first described in the trachea of

the rat by Rhodin and Dalhamn (1956) but was infrequently observed by these workers. Jeffery and Reid (1975) estimated that brush cells make up approximately 1% of tracheal epithelial cells in the rat. This cell has also been described in the tracheobronchial tree of the mouse, guinea pig, pig and cow (Hama and Nagata, 1970; Inoue and Hogg, 1974; Baskerville, 1970[a]; Allan, 1978). The brush cell has also been identified in the nasal epithelium of the rat (Popp and Martin, 1984; Monteiro-Riviere and Popp, 1984). Cells resembling brush cells have also been observed (but never confirmed by other workers) in the alveoli of rats (Meyrick and Reid, 1968; Dormans, 1985) and were termed type III pneumocytes.

The brush cell is columnar in shape and rests on the basement membrane. The Golgi zone is above the basal nucleus and the cytoplasm contains many free ribosomes and glycogen granules. Small vacuoles and vesicles reside among the numerous apical mitochondria. Cytoplasmic bundles of filaments are a characteristic feature of the cell (Rhodin and Dalhamn, 1956; Baskerville, 1970[a]). The lumenal surface is densely covered by microvilli which are taller, wider and more uniformly arranged than those of ciliated and mucous cells (Andrews, 1974). The microvilli contain fine axial filaments which appear to be continuous with the filament bundles in the cytoplasm. The function of brush cells is unknown but it has been

suggested that they may be absorptive cells (Jeffery and Reid, 1975) or, due to the presence of cytoplasmic filaments, could possibly function as stretch receptors (Meyrick and Reid, 1968). Afferent nerve endings have been observed to contact the lateral cell surfaces and it is possible that this indicates a chemoreceptor role (Breeze and Wheeldon, 1977).

(7) Nonciliated Bronchiolar Epithelial (Clara) Cell

The nonciliated bronchiolar cells were first described by Kolliker (1881) but the earliest detailed study was presented by Clara (1937) after whom the cells were named. They occur in the bronchioles of all mammals (Plopper, 1983) but can extend proximally in the bronchi as far as the hilus in the rat (Jeffery and Reid, 1975) and into the trachea in the mouse (Hansell and Moretti, 1969; Pack et al, 1981). Clara cells are the predominant secretory cell in the tracheobronchial tree of the rabbit (Plopper, 1983).

Columnar in shape, the Clara cell often protrudes into the airway lumen, beyond adjacent cells. The central nucleus is deeply indented and the well developed Golgi apparatus lies above it. One of the most consistent features of the cell is the large number of ovoid, electron-dense secretory granules and the presence of abundant smooth endoplasmic reticulum in the cytoplasm (Jeffery and Reid, 1975; Plopper, 1983). However, a number of species

differences have been observed, for example, in man, pig, rat and mouse, there are many secretory granules present but in the dog and cow these are scanty or absent (Widdicombe and Pack, 1982). Cytoplasmic glycogen deposits are well developed in the dog and cow but are scanty in man, pig and rabbit (Plopper et al, 1980; Plopper, 1983).

There is still dispute about the function of Clara cells but it is generally accepted that they are a source of secretion in bronchioles, contributing to the periciliary fluid. It has also been considered that they play a role in detoxifying processes via the cytochrome P450 mono oxygenase system at bronchiolar levels (Boyd, 1977; Plopper, 1983).

(8) Endocrine Cell

This cell has been found at all levels of the tracheobronchial and bronchiolar tree. Resembling the intestinal Kultchitsky cell, it has sometimes been termed a K cell but is now referred to principally as a pulmonary neuroendocrine cell (Johnson and Georgieff, 1989). It has been described in man (Bensch et al, 1965; Cutz and Conen, 1972; Hage, 1973, 1980) and in a number of laboratory animals (Cutz et al, 1974; Edmondson and Lewis, 1980). Endocrine-like cells have been observed in the human nasal mucosa (Thaete et al, 1981).

The morphology and cytochemical characteristics

of these cells have been described in detail by Breeze and Wheeldon (1977) and, because of their resemblance to some other endocrine cells elsewhere in the body, they are included in the APUD (amine and amine-precursor uptake and decarboxylation) group of cells (Pearse, 1969). Lying adjacent to the basement membrane, the cell is roughly triangular in shape with a round or oval nucleus. The narrow apical portion of the cell may or may not reach the epithelial surface and there are contradictory reports of this between species (Moosavi et al, 1973) or even within species (Moosavi et al, 1973; Cutz et al, 1974). Cells reaching the surface may have luminal microvilli (Hage, 1973). The basal cytoplasm of the cell contains the characteristic granules which have clear haloes between the electron-dense cores and the limiting membranes. A well developed Golgi apparatus, many free ribosomes and abundant smooth endoplasmic reticulum are also present. The lower cell border interdigitates with adjacent cells without attachment by desmosomes. Pulmonary neuroendocrine cells usually occur singly, but may be found in groups of cells which are termed neuroepithelial bodies.

These structures were identified in human infants and named in 1971 by Lauweryns and Peuskens and are usually located close to points of bifurcation in the airways (Edmondson and Lewis, 1980; Wasano and Yamamoto,

1981). Although found throughout the entire tracheobronchial tree and even in alveolar ducts and alveoli, they are most numerous at bronchiolar level at least in the rabbit (Lauweryns and Goddeeris, 1975). Sorokin et al (1983) reviewed the distribution, structure and histochemistry of pulmonary neuroendocrine cells and it was suggested that they could respond to changes in airway gases by releasing vaso-active substances such as serotonin and regulate airflow by influence on airway smooth muscle (Gail and Lenfant, 1983). While there is still great uncertainty regarding the normal functions of these cells in the lung, data available at the present time suggest roles as intrapulmonary chemoreceptors and/or regulators of airway epithelial differentiation (Johnson and Georgieff, 1989).

(9) Special Type Cell

This cell is found infrequently but has been described in the dog (Frasca et al, 1968) and also in the cat and in man (Jeffery, 1983). Resting on the basement membrane, the wedge shaped cell forms cytoplasmic interdigitations with adjacent cells and does not reach the airway lumen. The Golgi zone is situated above the oval nucleus. The characteristic feature of the cell is the presence of intracytoplasmic disc or rod shaped membrane-bound inclusions. The function of the Special type cell remains unknown.

(10) Type I Pneumocyte

This is a highly differentiated cell which has lost its capacity to divide. By far the greatest proportion of the interalveolar septum is covered by type I pneumocytes; approximately 93% of the human lung alveolar surface and 97% of that of the dog (Crapo et al, 1983). The flattened nucleus protrudes slightly into the alveolar air space and the greatly attenuated cytoplasm extends up to 50 µm from the nuclear area. Micropinocytotic vesicles are present in the cytoplasm but few other organelles although the cell has a high metabolic activity (Burri, 1985). A few short microvilli are present on the cell surface and desmosomes bind adjacent cells together.

(11) Type II Pneumocyte

This cell is cuboidal, has no cytoplasmic extensions and lies between type I pneumocytes to which attachment is made by junctional complexes. The apical portion of the cell bulges into the alveolar air space and is surrounded by a rim of numerous, short microvilli. The cytoplasm is rich in organelles which include the highly characteristic osmiophilic lamellated inclusions which are the intercellular storage granules of the pulmonary surfactant (Burri, 1985). The type II pneumocyte is now recognised as the stem cell of the alveolar epithelium (Kaufmann, 1980).

SEM Studies

The introduction of the scanning electron microscope provided yet another method of studying respiratory tract surfaces. Using its wide range of magnification and great depth of focus, a three dimensional picture was now made possible and thereby adding to the knowledge of the respiratory tract mucosa gained by light microscopy and TEM. Early application of the SEM in the study of respiratory tissue was described by Jaques et al (1965). However, it was not until the early 1970's that technology had improved sufficiently to encourage many workers to use SEM and to report their findings in the respiratory tract. As SEM reports of various levels of the respiratory tract are now extensive, these are summarised in Table 1:2 (Page 34).

The cells of the respiratory tract epithelium shown on Page 10 are, of course. not all visible with SEM. The basal, intermediate and endocrine cells which do not reach the airway lumen are hidden from view. With SEM, the ciliated cell is most commonly observed and covers the greater part of the airway in man (Ebert and Terracio, 1975[a]; Greenwood and Holland, 1975), rat (Andrews, 1974; Alexander et al, 1975), mouse (Greenwood and Holland, 1972), hamster (Becci et al, 1978), ox (Mariassy et al, 1975; Iovanitti et al, 1985), pig (Mebus and Underdahl, 1977; Williams and Gallagher, 1978), monkey (Greenwood

and Holland, 1973; Tyler and Plopper, 1985; Tyler et al, 1988), cat (Tandler et al, 1983[a,b]) and dog (Wright et al, 1983; Majid, 1986).

In most mammalian species examined, mature mucus-secreting cells appear dome-shaped as they protrude between the ciliated cells. These cells have been described in man (Greenwood and Holland, 1975), rat (Andrews, 1974), mouse (Greenwood and Holland, 1975), hamster (Becci et al, 1978), guinea pig (Dahlgren et al, 1972), dog (Wright et al, 1983; Majid, 1986), cat (Tandler et al, 1983[a,b]), monkey (Greenwood and Holland, 1973), ox (Iovanitti et al, 1985) as well as in the horse (Tyler et al, 1971).

Brush cells, characterised by numerous thick, blunt microvilli on their free surfaces, have been observed in the airways of the rat (Andrews, 1974; Popp and Martin, 1984; Dormans, 1985). Bronchiolar Clara cells have been described in SEM studies of a number of species including man (Smith et al, 1979), rat (Kuhn et al, 1974), mouse (Smith et al, 1979), guinea pig (Okada, 1969), hamster (Becci et al, 1978), rabbit (Smith et al, 1979), monkey (Castleman et al, 1975), dog (Wright et al, 1983; Majid, 1986), ox (Iovanitti et al, 1985) and horse (Nowell and Tyler, 1971; Tyler et al, 1971).

Neuroepithelial bodies have been observed in the mouse (Hung et al, 1979) and in the rabbit (Cutz et al,

1978) usually near or at airway bifurcations. There have been many SEM observations of type I and type II pneumocytes in various species such as the rat (Andrews, 1979), guinea pig (Davis et al, 1984), mouse (Zitnik et al, 1978), monkey (Castleman et al, 1975), dog (Kondo et al, 1973; Majid, 1986), ox (Iovanitti et al, 1985) and horse (Nowell and Tyler, 1971; Tyler et al, 1971).

Aspects of the Equine Respiratory Tract

As can be seen in the summary of the literature in Tables 1:1 and 1:2, there are relatively few references to the normal ultrastructural features of the equine respiratory tract. General descriptions of the main histological features of the lung, including the intrapulmonary bronchi, the bronchioles and the pleura have been compared with various mammalian species (McLaughlin et al, 1961[a,b]; Tyler, 1983). These studies concluded that, while the general features were similar in the mammals examined, there were some important species differences, for example, the presence of well developed respiratory bronchioles in the dog, cat and monkey and their virtual absence in the ox and the horse.

General histological features of the respiratory tract of horses have been described by Mair et al (1987) and the distribution of lymphatic tissue in the respiratory mucosa has been determined (Mair et al, 1988). A brief description of the histology of the vomeronasal

organ of the horse and donkey has also been given by Lindsay et al (1978).

Histochemical studies of the lower equine airways have shown that the mucous cells of the trachea and bronchi mainly contain acidic mucosubstances with sulphomucins predominating. There were a few groups of cells containing both acidic and neutral mucosubstances and the neutral portion was usually seen in the apical part of the cell, often extending into the airway lumen. Sialomucins were infrequently found in the horse. In the smaller bronchi, mucous cells were sparse and they were never found in the bronchioles. Submucosal glands were few in number in equine lower airways and the acini often had few mucus-secreting cells or none at all. Again sulphomucins predominated with a small neutral mucosubstance component in some cells (Nicholls, 1978).

Histochemical reactions for oxidative enzymes in the interalveolar septum have also been described in horses (Tyler et al, 1971) and they concluded that both the non-ciliated bronchiolar epithelial cells and the type II pneumocytes have the metabolic potential to produce pulmonary surfactant.

Reports on the ultrastructural features of the normal equine respiratory tract are very sparse and are confined to the lower airways and the alveolar membrane, while the upper respiratory tract has been ignored. SEM

has been used to compare the lung structure of the horse with that of the hamster (Nowell and Tyler, 1971) and they found that the surface epithelium of the small bronchi and bronchioles of both was similar to that of other mammalian species. At alveolar level type I and type II pneumocytes and alveolar macrophages were identified and the presence of interalveolar pores was noted. Tyler et al (1971) gave brief descriptions of SEM and TEM findings in horse lung which followed on similar TEM findings of the interalveolar septum in the horse (Gillespie and Tyler, 1967[a]). More recently, detailed ultrastructural studies of the nonciliated bronchiolar epithelial (Clara) cells have been carried out in various species, including the horse (Plopper et al, 1979, 1980[b]; Plopper et al, 1980; Plopper, 1983).

In a comparative SEM and TEM study of the pleura, Plopper (1971) chose the horse as a model for thick pleura and the dog and rat as models for thin pleura. He found that the mesothelial cells of the visceral pleura, in all three species, were covered by dense aggregations of small microvilli and that elastic tissue and densely packed collagen fibres separated the pulmonary capillaries from the surface in the dog and rat, while in the horse there was an additional elastic layer and two other capillary beds.

A study of these reports clearly reveals that

many gaps exist in the knowledge of normal equine respiratory tract surfaces. In Chapters 4, 5 and 6 a combined SEM, histological and TEM study was carried out in a large group of animals in order to characterise, for the first time, the normal surface features of the equine respiratory tract from the nasal vestibule to the alveolar membrane.

As the equine respiratory tract presents some complex anatomical features, particularly in the nasal cavity, the nasopharynx and in the position of the larynx, it was thought that a brief description of the gross anatomy would be a useful prelude to the further detailed study of the ultrastructural, histological and histochemical features of the equine respiratory tract.

TABLE 1:1

TRANSMISSION ELECTRON MICROSCOPY OF THE RESPIRATORY TRACT OF VARIOUS MAMMALIAN SPECIES

Species	Sample Site	Reference
Rat	Lung : Alveolus	Low (1952)
"	Trachea	Rhodin and Dalhamn (1956)
"	Trachea	Rhodin (1959)
"	Lung : Alveolar Brush Cell	Meyrick and Reid (1968)
"	Bronchus : Feyrter Cell	Moosavi et al (1973)
"	Extrapulmonary Respiratory Tract	Andrews (1974)
"	Clara Cell	Kuhn et al (1974)
"	Clara Cell	Smith et al (1974)
"	Trachea and Lung	Jeffery and Reid (1975)
"	Trachea and Bronchi	Alexander et al (1975)
"	Clara Cell	Evans et al (1978)
"	Clara Cell	Smith et al (1979)
"	Trachea : Non-ciliated Cells	Plopper et al (1983)
"	Alveolar Macrophages	Sorokin et al (1984)
"	Nasal Respiratory Epithelium	Monteiro-Riviere and Popp (1984)

TABLE 1:1 (Cont'd)

Species	Sample Site	Reference
Rat	Alveolar Type III Cell	Dormans (1985)
"	Alveolus	Scheuermann et al (1988)
Mouse	Lung : Alveolus	Karrer (1956)
"	Lung : Alveolus	Curry et al (1969)
"	Trachea	Chen and Lin (1972)
"	Nasal Respiratory Epithelium	Matulionis and Parks (1973)
"	Bronchiole	Hung and Loosli (1974)
"	Clara Cell	Stinson and Loosli (1978)
"	Clara Cell	Plopper et al (1980[a])
"	Trachea	Pack et al (1980)
"	Trachea and Bronchi	Pack et al (1981)
"	Nasopharyngeal Epithelium	Nakano (1986)
"	Nasal Septum	Lessner and Rehn (1987)
"	Type II Alveolar Cell	Ten Have-Opbroek et al (1988)
Hamster	Tracheobronchial Epithelium	Harris et al (1971)
"	Tracheobronchial Epithelium	Becci et al (1978)
"	Clara Cell	Plopper et al (1980[a])
"	Trachea	McDowell et al (1983)

TABLE 1:1 (Cont'd)

Species	Sample Site	Reference
Hamster	Alveolar Macrophage	Sorokin et al (1984)
"	Bronchial Epithelium	Christensen et al (1987)
"	Clara Cell	Strum et al (1990)
Guinea Pig	Trachea	Dahlgren et al (1972)
"	Clara Cell	Plopper et al (1980[a])
"	Trachea	Dalen (1983)
"	Distal Airway	Davis et al (1984)
Gerbil	Trachea, Bronchiole	Spicer et al (1990)
Rabbit	Lung : Alveolus	Kisch (1958)
"	Trachea	Hilding and Hilding (1966)
"	Trachea	Konradova (1966)
"	Clara Cell	Cutz and Conen (1971)
"	Neuroepithelial Bodies	Lauweryns et al (1974)
"	Endocrine Cell	Cutz et al (1975)
"	Bronchi	Sturgess (1977)
"	Clara Cell	Plopper et al (1983)
"	Alveolar Macrophages	Sorokin et al (1984)
Monkey	Intrapulmonary Airway	Castleman et al (1975)
"	Trachea	St. George et al (1984)
"	Tracheobronchial Epithelium	Wilson et al (1984)

TABLE 1:1 (Cont'd)

Species	Sample Site	Reference
Monkey	Distal Airway	Tyler and Plopper (1985)
"	Trachea	Plopper et al (1986)
"	Nasal Epithelium	Harkema et al (1987)
"	Respiratory Bronchiole	Tyler et al (1989)
Dog	Bronchus	Frasca et al (1968)
"	Lung : Alveolus	Ortega et al (1970)
"	Lung : Bronchiole, Alveolus	Kondo et al (1973)
"	Interalveolar Pores	Parra et al (1978)
"	Bronchus and Bronchiole	Hyde et al (1978)
"	Clara Cell	Plopper et al (1980^b)
"	Nasal Cavity	Edwards et al (1983)
Cat	Clara Cell	Plopper et al (1980^b)
Ox	Lung : Blood/Air Barrier	Epling (1964)
"	Nasal Mucosa	Bozarth and Strafuss (1974)
"	Lung : Brush Cell	Allan (1978)
"	Clara Cell	Smith et al (1979)
"	Clara Cell	Plopper et al (1980^b)
"	Alveolus	Atwal et al (1988)
"	Alveolus	Atwal et al (1989)

TABLE 1:1 (Cont'd)

Species	Sample Site	Reference
Sheep	Trachea : Endocrine Cell	Cutz et al (1975)
"	Clara Cell	Plopper et al (1980[b])
"	Tracheobronchial Epithelium	Mariassy and Plopper (1984)
"	Tracheobronchial Epithelium	Mariassy et al (1988)
"	Alveolar Type II Cell	Shibamoto (1989)
Pig	Lung : Bronchiole and Alveolus	Baskerville (1970[a])
"	Bronchial Epithelium	Baskerville (1970[b])
"	Clara Cell	Widdicombe and Pack (1982)
"	Lung : Bronchiole and Alveolus	Winkler and Cheville (1984)
"	Nasal Mucosa	Adams (1990)
Goat	Alveolus	Atwal (1988)
Man	Trachea	Rhodin (1959)
"	Trachea and Lung	Rhodin (1963)
"	Bronchus	Watson and Brinkman (1964)
"	Trachea	Rhodin (1966)
"	Upper Respiratory Tract	Friedmann and Bird (1971)

TABLE 1:1 (Cont'd)

Species	Sample Site	Reference
Man	Clara Cell	Cutz and Conen (1971)
"	Lung : Endocrine-like Cells	Cutz and Conen (1972)
"	Small Bronchiole	Rosan and Lauweryns (1972)
"	Bronchus : Endocrine Cell	Hage (1973)
"	Trachea : Endocrine Cell	Cutz et al (1975)
"	Nasal Mucosa	Busuttil et al (1977)
"	Bronchus	McDowell et al (1978)
"	Clara Cell	Smith et al (1979)
"	Clara Cell	Plopper et al (1980)
"	Nasal Epithelium	Thaete et al (1981)
"	Nasal Epithelium	Carson et al (1985)
"	Nasal Epithelium	Rautiainen (1988)
"	Bronchus	Jeffery et al (1989)
Horse	Lung : Alveolus	Gillespie and Tyler (1967[a])
"	Lung : Bronchiole and Alveolus	Tyler et al (1971)
"	Clara Cell	Plopper et al (1980[b])
"	Lung : Alveolus	Kaup et al (1986)

TABLE 1:2

SCANNING ELECTRON MICROSCOPY OF THE RESPIRATORY TRACT OF VARIOUS MAMMALIAN SPECIES

Species	Sample Site	Reference
Rat	Alveolus	Kuhn and Finke (1972)
"	Extrapulmonary Respiratory Tract	Andrews (1974)
"	Trachea and Bronchi	Alexander et al (1975)
"	Bronchioles	Ebert and Terracio (1975[b])
"	Alveolar Brush Cell	Hijiya (1978[a,b])
"	Trachea and Bronchi	Luchtel (1978)
"	Terminal Bronchiole	Lum et al (1978)
"	Clara Cell	Smith et al (1979)
"	Pulmonary Macrophages	Warheit and Hartsky (1988)
"	Alveolus	Scheuermann et al (1988)
Mouse	Clara Cell	Okada (1969)
"	Lung : Bronchiole	Wang and Thurlbeck (1970)
"	Nasal Cavity	Adams (1972)
"	Respiratory Tract Surface	Greenwood and Holland (1972)
"	Lung : Alveolus	Kuhn and Finke (1972)
"	Lung : Bronchiole and Alveolus	Zitnik et al (1978)

TABLE 1:2 (Cont'd)

Species	Sample Site	Reference
Mouse	Lung : Neuro-epithelial Bodies	Hung et al (1979)
"	Clara Cell	Smith et al (1979)
"	Lung : Neuro-epithelial Bodies	Wasano and Yamamoto (1981)
"	Nasopharynx	Nakano (1986)
Hamster	Lung : Alveolus	Kuhn and Finke (1972)
"	Tracheobronchial Epithelium	Becci et al (1978)
Guinea Pig	Clara Cell	Okada (1969)
"	Trachea	Dahlgren et al (1972)
"	Distal Airway	Davis et al (1984)
Rabbit	Lung : Alveolus	Holma (1969)
"	Bronchus	Sturgess (1977)
"	Lung : Neuro-epithelial Body	Cutz et al (1978)
"	Clara Cell	Smith et al (1979)
Monkey	Tracheobronchial Epithelium and Alveolus	Greenwood and Holland (1973)
"	Intrapulmonary Airway	Castleman et al (1975)
"	Lung : Respiratory Bronchiole	Mellick et al (1977)

TABLE 1:2 (Cont'd)

Species	Sample Site	Reference
Monkey	Tracheobronchial Epithelium	Wilson et al (1984)
"	Nasal Epithelium	Harkema et al (1987)
"	Lung : Bronchus	Maina (1988)
"	Lung : Bronchiole	Tyler et al (1988)
Dog	Lung : Parenchyma	Groniowski et al (1972)
"	Lung : Bronchiole and Alveolus	Kondo et al (1973)
"	Trachea, Gland Orifices	Nadel (1977)
"	Lung : Bronchiole, Alveolus	Hyde et al (1978)
"	Interalveolar Pores	Parra et al (1978)
"	Nasal Cavity	Edwards et al (1983)
"	Trachea and Lung	Wright et al (1983)
Cat	Trachea	Tandler et al (1983[a,b])
"	Lung : Bronchiole	Plopper et al (1983)
Ox	Lung : Bronchus, Bronchiole and Alveolus	Mariassy et al (1975)
"	Clara Cell	Smith et al (1979)
"	Lower Respiratory Tract	Iovanitti et al (1985)

TABLE 1:2 (Cont'd)

Species	Sample Site	Reference
Sheep	Lung : Bronchiole, Alveolus	Tyler et al (1971)
Pig	Lung : Bronchiole	Wang and Thurlbeck (1970)
"	Trachea and Bronchi	Mebus and Underdahl (1977)
"	Trachea and Lung	Williams and Gallacher (197
"	Lung : Bronchiole and Alveolus	Winkler and Cheville (1984)
"	Nasal Mucosa	Adams (1990)
Man	Lung : Bronchiole	Wang and Thurlbeck (1970)
"	Bronchus	Ebert and Terracio (1975[a])
"	Trachea and Lung	Greenwood and Holland (1975)
"	Clara Cell	Smith et al (1979)
"	Nasal Mucosa	Boysen (1982)
"	Nasal Mucosa	Winther et al (1984)
"	Nasal Cavity	Moor-Gillon (1985)
Horse	Lung : Bronchiole and Alveolus	Tyler et al (1971)
"	Lung : Bronchus and Alveolus	Nowell and Tyler (1971)

CHAPTER 2

AN OUTLINE OF THE GROSS ANATOMY OF THE EQUINE RESPIRATORY TRACT

GENERAL CONSIDERATIONS

The respiratory system provides a means by which gaseous exchange can take place between the atmosphere and the blood. The respiratory organs regulate the volume of inspired air and modify the airstream by cleansing, warming and humidifying mechanisms. In addition, the respiratory system houses the olfactory system and the structures concerned with phonation. Essentially the respiratory system, common to all mammals, consists of a conducting portion, which conveys air to and from the lungs, and a respiratory portion within the lungs where gaseous exchange takes place.

In close functional association with the respiratory system is the skeleton of the thorax, its associated muscles and the diaphragm which act in unison as a pumping mechanism, regulating inspiration and expiration.

The conducting portion consists of the nostrils or external nares, nasal cavity, nasopharynx, larynx, trachea, extrapulmonary bronchi and, within the lungs, the bronchi and bronchioles.

The respiratory portion consists of the alveolar ducts, alveolar sacs and alveoli. In some mammals, respiratory bronchioles, which are both conducting and respiratory in function, are also present.

THE RESPIRATORY SYSTEM OF THE HORSE

In order to obtain a basic understanding of the gross anatomical features of the equine respiratory tract prior to embarking on histological and ultrastructural studies, this brief review is based on an analysis of the available literature incorporating the following reports:-

Bradley, 1947; McLaughlin et al, 1961[a,b]; Cook, 1966; Hare, 1975; Hillman, 1975; Sisson, 1975; Lindsay et al, 1978; Nickel et al, 1979; King and Riley, 1980; Lindsay and Burton, 1983; Lindsay and Clayton, 1986; Dyce et al, 1987.

The nose:

In the horse the nose is incorporated in the skeleton of the face and extends from the level of the eyes to the rostral extremity of the head (Fig. 2.1).

Enclosed within the nose is the nasal cavity, divided into two compartments by the nasal septum. The caudal bony part of the septum is continued rostrally by a cartilaginous plate, which is supported initially on the vomer and then on a groove between the palatine processes of the incisive bones.

Caudally and ventrally the nasal cavity communicates with the nasopharynx through the choanae. These two openings are bounded by the palatine and pterygoid bones and medially by the vomer (Figs. 2.2 and 2.7).

Rostrally the osseus apperture of the nose is formed by the edges of the nasal and incisive bones, the nasoincisive notch (Fig. 2.1). Beyond this the wall of the nose is supported medially by the cartilaginous septum which splits to form the dorsal and ventral lateral cartilages of the nose. As these are narrow in the horse, the lateral walls of the nose are unsupported beyond the nasoincisive notch (Hillman, 1975).

The two nostrils are situated at the apex of the nose and form the entrance to the nasal cavity. Due to the lack of lateral support the nostrils can vary greatly in shape. When the animal is at rest they are comma-shaped (Fig. 2.3), but can dilate to become almost circular when respiratory volume increases during exercise.

In the horse (and donkey), the external opening of each nostril does not open directly into the nasal cavity but into a large nostril cavity, the nasal vestibule. This is lined by fine skin bearing many hairs and is continuous with the skin on the external surface of the nose. Dorsally the cavity of the nasal vestibule is extended, as far as the nasoincisive notch, by a cutaneous blind pouch, the nasal diverticulum. This is separated from the remainder of the nasal vestibule by a fold of skin and mucosa supported by cartilage, the alar fold. This fold attaches to the

rostral end of the ventral nasal concha.

The nasal vestibule communicates with the common meatus of the nasal cavity by a horizontal, elliptical opening on its medial wall (Figs. 2.5 and 2.6). This opening is bounded dorsally by the alar fold and ventrally by the basal fold of the ventral concha (Lindsay and Burton, 1983).

(1) The nasal cavity:

The nasal septum, described above, separates the cavity into two halves. Most of the space within each half is occupied by the nasal and ethmoidal conchae. The latter occupy a relatively small space caudally and dorsally. The conchae are delicate osseous scrolls which are attached to the caudal and lateral walls of the nasal cavity by shelf-like basal lamellae of bone. They are covered on all surfaces by the nasal mucous membrane (Fig. 2.4).

On each side, the large dorsal and ventral nasal conchae project from the caudal and ventral walls, splitting the nasal cavity into the dorsal, middle and ventral meatuses (Fig. 2.5). These meatuses communicate with the narrow, vertical common meatus on each side of the nasal septum.

Approximately opposite the last two cheek teeth, the middle meatus communicates with the rostral and caudal maxillary sinuses via the narrow, slit-like

nasomaxillary opening. This is the only means by which the paranasal sinuses of the horse can drain into the nasal cavity.

(2) The nasal conchae:

The ethmoidal conchae are numerous and small and occupy the dorso-caudal portion of the nasal cavity. The middle nasal concha, which is small in the horse, is included in this group (Fig. 2.7). The olfactory mucosa covers the surfaces of the ethmoidal conchae, the walls of the nasal cavity and the nasal septum in this dorso-caudal region.

The dorsal nasal concha, which is the largest concha, extends from the cribriform plate of the ethmoid to approximately the level of the first cheek tooth. It is continued rostrally, on the lateral wall of the nasal cavity, as far as the nostril, by a fold of mucous membrane, the straight fold. The rostral portion of this concha is scrolled and separated from the caudal portion by a bony septum, approximately level with the fourth cheek tooth. The caudal part is excavated into a large dorsal conchal sinus which communicates with the frontal sinus. Together they are referred to as the conchofrontal sinus (Fig. 2.8).

The ventral nasal concha is smaller and shorter and continued rostrally into the nostril by the alar and basal mucosal folds. Like the dorsal nasal concha, it is

also divided by a bony septum, approximately at the level of the fourth cheek tooth, into rostral and caudal portions. The caudal ventral conchal sinus communicates with the rostral maxillary sinus to form the conchomaxillary sinus (Fig. 2.8).

(3) The paranasal sinuses:

These arise as diverticula of the nasal cavity, to which they are either directly or indirectly connected. They are lined by respiratory mucosa continuous with that of the nasal cavity. They are named according to the bones within which they are located.

There are six pairs of sinuses in the horse: dorsal, middle and ventral conchal sinuses, maxillary, frontal and sphenopalatine sinuses (Figs. 2.8, 2.9). The maxillary sinus is divided into rostral and caudal parts by a bony septum. Because of communication between the sinuses, clinically the horse can be regarded as having just two cavities, the first incorporating the ventral conchal and rostral maxillary sinuses and the second, the remainder of the sinuses (Cook, 1966).

Several features of the paranasal sinuses are specific to equidae and these are as follows:- the divided maxillary sinuses; the formation of conchofrontal and conchomaxillary sinuses; the wide communication between the conchofrontal and caudal maxillary sinuses through the frontomaxillary opening; the fact that only the rostral

and caudal maxillary sinuses have communication with the nasal cavity through the nasomaxillary opening and this means that all the other sinuses have only indirect communication with the nasal cavity.

(4) Vomeronasal organs and incisive ducts:

The vomeronasal organs are paired submucosal structures lying on the floor of the ventral meatus on each side of the nasal septum. Each consists of a narrow tube or duct, blind-ending caudally, enclosed in a sleeve of cartilage. The ducts are lined by respiratory mucosa with a ridge of olfactory mucosa on the dorso-lateral walls. The organs vary in length from 12-20 cm depending on the size of the animal (Lindsay et al, 1978).

The rostral end of each vomeronasal duct opens into an incisive duct (Fig. 2.5). The latter passes ventrally through the palatine fissure to end blindly in the submucosa of the hard palate. There are no oral openings of the incisive ducts in equidae (Lindsay and Burton, 1983).

(5) The nasopharynx:

The pharynx is a musculo-membranous tube common to both respiratory and digestive tracts. Wide rostrally and joining both nasal and oral cavities, it narrows caudally to join the oesophagus. It is attached by its muscles to the palatine, pterygoid and hyoid bones and to the larynx.

The nasopharynx is the respiratory portion and extends from the choanae to the intrapharyngeal opening. This opening, approximately 5 cm x 5 cm, is formed by the dorsal free border of the soft palate and by the palato-pharyngeal arches. The latter are prominent mucosal folds measuring up to 1 cm in height. They extend from the free border of the soft palate laterally and meet in the midline dorsal to the arytenoid cartilages of the larynx. Parts of these cartilages and the epiglottic cartilage protrude through the intra-pharyngeal opening to lie in the nasopharynx (Figs. 2.7, 2.10). The nasopharynx communicates with the laryngo-pharynx through the intrapharyngeal opening and here the respiratory and digestive pathways intersect (Nickel et al, 1979).

In the horse only the rostral one third of the nasopharynx is attached to the base of the cranium. The caudal two thirds are related dorsally to the guttural pouches. In the most dorsal part of the nasopharynx there is a median cul-de-sac, the pharyngeal recess. This recess is medial to the most rostral portions of the guttural pouches. The floor of the nasopharynx is the dorsal surface of the soft palate (Cook, 1966).

In the horse, the pharyngeal recess is relatively shallow but in the donkey it is deeper and forms a membranous pouch, 2-3 cm in diameter, which

extends caudally for 4-6 cm (Lindsay and Clayton, 1986).

Laterally, the nasopharynx communicates with the middle ear through the openings of the auditory tubes (Fig. 2.10).

(6) The auditory tubes and guttural pouches:

Each auditory tube extends from the tympanic cavity to the nasopharynx, transmitting air from the latter which equalises pressure on either side of the tympanic membrane.

Communication with the tympanic cavity is by the narrow tympanic opening. The auditory tube is then directed rostrally and ventrally to end at the pharyngeal opening. For about half of its length it is a complete tube with a narrow curved lumen. It then expands caudo-ventrally into an extensive mucosal diverticulum, the guttural pouch.

The pharyngeal opening of the auditory tube is a caudo-ventrally sloping slit, about 5 cm in length, located laterally on the nasopharyngeal wall. It is bounded medially by the free edge of the tube (Fig. 2.10). The lateral wall is formed by the nasopharynx (Sisson, 1975).

The guttural pouches, or diverticula of the auditory tubes, are peculiar to equidae (Figs. 2.10, 2.11). Each has a capacity of approximately 300 ml. Lying between the base of the cranium and the atlas dorsally and

the nasopharynx ventrally, the two pouches are more or less in contact medially. On each side the rostral limit is a small blind sac between the pharyngeal end of the auditory tube and the pharyngeal recess. The caudal extremity is level with the joint between the first and second cervical vertebrae.

The stylohoid bone, on each side, protrudes into the pouch which is reflected over its dorsal border, thus forming a large medial compartment and a smaller lateral compartment (Fig. 2.10). The lateral relations of the pouch are numerous and complex. Briefly, they include some of the muscles of mastication, muscles of the soft palate and hyoid apparatus, the parotid and mandibular salivary glands and the retropharyngeal lymph nodes (Fig. 2.11).

Dorsally, a number of important structures of considerable clinical significance are in contact with the pouch (Fig. 2.11). These include the internal carotid artery and ventral cerebral vein, cranial nerves VII, IX, X and XI and the cranial cervical ganglion (Cook, 1966).

The mucosa of the auditory tubes and guttural pouches is continuous with that of the nasopharynx and tympanic cavities.

(7) The larynx:

The larynx is a short muscular and cartilaginous tubular organ connecting the pharynx to the trachea (Fig. 2.7).

The main function of the larynx is to protect the lower airway from the entry of foreign material, especially during deglutition. This is accomplished by closure of the glottis and also of the rostral opening of the larynx, the aditus laryngis. The calibre of the airway within the larynx can be altered considerably by muscular action. The larynx is also the organ of phonation.

In the horse, the caudal border of the soft palate and the palato-pharyngeal arches fit tightly round the aditus which is thus, more or less, fixed within the nasopharynx (Fig. 2.10). Thus the horse is an obligatory nasal breather. Although the soft palate is raised during deglutition, this elevation cannot be sustained for oral breathing (King and Riley, 1980).

Dorsally, the larynx is related to the laryngo-pharynx, the first part of the oesophagus and to the base of the cranium, to which it is indirectly attached by the basihyoid and thyrohoid bones.

Ventrally, the larynx is quite superficial and is covered by the sternohyoideus and omohyoideus muscles, fascia and skin. The lateral relations vary with the position of the head, but at least part of the larynx is intermandibular even when the head is extended.

(a) The cartilages of the larynx:

A number of cartilages form the skeleton of the

larynx. These are connected by joints, ligaments and membranes and are moved by muscles both extrinsic and intrinsic.

There are three unpaired cartilages, the epiglottic, thyroid and cricoid and three paired cartilages, the arytenoid, corniculate and cuneiform. The corniculate and arytenoid cartilages are fused. The cuneiform cartilages join the epiglottis.

The epiglottic cartilage is the most rostral (Fig. 2.12). Its pointed apex projects into the nasopharynx. It is attached caudally to the thyroid cartilage. The latter is the largest of the cartilages and forms a deep, medial trough-like structure which is open dorsally. The arytenoid cartilages are roughly triangular in shape and lie partly within the thyroid cartilage. The ring-shaped cricoid cartilage is the most caudal. It is attached rostrally to the thyroid cartilage and caudally to the first tracheal ring.

(b) Articulations, ligaments and membranes of the larynx:

The cricothyroid, cricoarytenoid and thyrohyoid articulations are all synovial joints.

The cricotracheal and cricothyroid ligaments are elastic in nature. The ventral part of the latter expands to fill the wide caudal thyroid notch and is known as the cricothyroid membrane.

The vestibular ligaments extend dorsocaudally

from the epiglottic and cuneiform cartilages to the arytenoid cartilages and are formed of loose fibrous tissue.

The vocal ligaments are thin and elastic. They extend ventrally from the vocal processes of the arytenoid cartilages and converge medially to attach to the thyroid cartilage and adjacent cricothyroid ligament.

(c) Muscles of the larynx:

These are skeletal muscles and can be divided into two groups, extrinsic and intrinsic. The former are the strap-like ventral muscles of the neck and, together with the muscles of the hyoid apparatus, they move the larynx as a whole, especially during swallowing.

There are eight pairs of intrinsic muscles which pass from one laryngeal cartilage to another. They function to open and close the glottis and in vocalization.

The cricoarytenoideus dorsalis and the transversus arytenoideus muscles abduct the vocal processes of the arytenoid cartilages and the vocal folds and thus widen the rima glottidis. The former of these muscles is the most important. All the other muscles act to narrow the rima glottidis and to vary the tension in the vocal folds (Hare, 1975).

(d) The mucous membrane of the larynx:

The cavity of the larynx is lined by mucous membrane continuous with that of the pharynx rostrally and the trachea caudally. It is firmly attached over the

epiglottic cartilage, vocal ligaments and inner surface of the cricoid cartilage. Elsewhere the attachment to underlying structures is loose.

On each side of the epiglottis, the mucous membrane is reflected laterally to form the aryepiglottic folds. Vestibular and vocal folds are formed over the respective ligaments. Between these two folds, on each side, is a deep, elongated depression termed the lateral ventricle. This is the entrance to a large mucosal pouch, about 2.5 cm deep, extending to the medial aspect of the thyroid cartilage (Fig. 2.13). In some horses there is also a median ventricle, formed by a depression of the mucous membrane at the base of the epiglottis (Hare, 1975).

(e) The cavity of the larynx:

Communicating rostrally with the pharynx and caudally with the trachea, the cavity of the larynx resembles an hourglass, with expanded rostral and caudal portions, the vestibule and infraglottic cavity respectively. These are joined by a narrow middle part, the rima glottidis (Nickel et al, 1979).

The aditus laryngis is bounded by the epiglottic cartilage ventrally, the aryepiglottic folds laterally and the corniculate cartilages dorsally. Held in the nasopharynx, it faces rostrally and dorsally in the horse but is tipped more caudally in the donkey (Lindsay and

Clayton, 1986).

The vestibule is that part of the cavity between the aditus and the rima glottidis. The vestibular folds project laterally into the vestibule and caudal to them are the lateral ventricles. The floor of the vestibule contains the median ventricle, when present.

The narrow rima glottidis is bounded ventro-laterally by the vocal folds and dorsally by the vocal processes of the arytenoid cartilages.

The caudal compartment is the infraglottic cavity and is contained within the cricoid cartilage.

(8) The trachea:

The trachea is a flexible cartilaginous and membranous tube extending from the larynx into the thoracic cavity. Dorsal to the base of the heart, it bifurcates into right and left principal bronchi. It is essentially a midline structure, but is displaced to the right in its terminal portion by the aorta.

The relations of the trachea are numerous. Briefly, it is related dorsally to the oesophagus for most of its cervical portion. In the distal one third of the neck, the oesophagus deviates to the left, leaving the trachea in contact with the ventral vertebral muscles. Dorsolaterally are the common carotid arteries and vago-sympathetic trunks enclosed within the carotid sheaths and the recurrent laryngeal nerves. Ventrally and

laterally the trachea is covered by the strap-like cervical muscles passing from the sternum to the head. Just caudal to the larynx the two lobes of the thyroid gland are loosely attached to the trachea with the narrow thyroid isthmus joining the lobes ventrally.

The thoracic part of the trachea lies in the cranial mediastinum. Here the oesophagus resumes its midline position, dorsal to the trachea. Ventrally there are the great vessels entering and leaving the heart and laterally the right and left lungs.

The wall of the trachea is composed of four main tissues. From within out are the mucosa and submucosa, the musculo-cartilaginous layer and the adventitia.

There are approximately 48-60 hyaline cartilaginous plates which form incomplete rings, open dorsally. This dorsal gap is filled by the smooth trachealis muscle and connective tissue. Contiguous cartilage plates are joined by fibroelastic tissue.

(9) <u>The lungs</u>:

The lungs are the paired respiratory organs located in the thoracic cavity (Fig. 2.14). Each lung lies within its pleural sac formed by the parietal and mediastinal pleura. This membrane covers the surface of the thoracic cavity and is reflected from the dorsal and ventral walls, more or less in the midline, to cover the mediastinum and its contained organs. Each lung is

covered closely by the visceral pleura which is continuous with the mediastinal pleura at the hilus. Here the lung is anchored by the principal bronchus, pulmonary vessels and nerves but elsewhere is freely moveable within the pleural sac. A small amount of pleural fluid separates the visceral and parietal pleura where they are in contact.

The lungs of the horse are not clearly subdivided into lobes. However, the left lung is considered to have cranial and caudal lobes (Fig. 2.14). The right lung, which is the larger, has cranial, caudal and accessory lobes (Fig. 2.15).

Each lung is roughly cone-shaped. The apex is narrow and projects towards the cupula pleura at the thoracic inlet. The base is wide and applied to the strongly curved surface of the diaphragm, which bulges into the thoracic cavity. The large costal surface of the lung is convex and in contact with the ribs and intercostal muscles. The smaller medial surface is irregular and cranially bears a large concave area for the heart, the cardiac impression.

(10) The bronchial tree:

The trachea bifurcates into right and left principal bronchi, dorsal to the base of the heart, at the level of the fifth or sixth intercostal space. The branching of the bronchial tree is more or less the same

on the right and left sides (Fig. 2.16). On each side the principal bronchus enters the lung and gives off the cranial lobar bronchus laterally. The latter is short. Bending cranially, it gives off a large dorsal branch which passes to the caudodorsal part of the cranial lobe. The lobar bronchus then continues into the cranial part of the cranial lobe. The two branches of the lobar bronchus are termed the caudal and cranial segmental bronchi of the cranial lobe.

The accessory lobar bronchus arises just distal to the cranial lobar bronchus, on the right side. It terminates by dividing into dorsal and ventral segmental bronchi of the accessory lobe.

The continuation of the principal bronchus into the caudal lobe of the lung is termed the caudal lobar bronchus. A series of six segmental bronchi are given off and the termination is the seventh segmental bronchus of the caudal lobe (Hare, 1975).

The segmental bronchi ventilate relatively large, cone-shaped, self-contained portions of the lung, the broncho-pulmonary segments.

Further branching of the segmental bronchi eventually give rise to the bronchioles, less than 1 mm in diameter. The most distal airways are the terminal bronchioles. Poorly developed respiratory bronchioles are seldom found (McLaughlin et al, 1961[a,b]).

The walls of the principal bronchi, outside the lungs, resemble that of the trachea. Within the lungs there are some changes in the bronchial walls; the cartilage forms irregular plates instead of incomplete rings and the smooth muscle is in the form of a double spiral. There is no cartilage in the walls of bronchioles.

Each bronchiole ventilates a lung lobule. In the horse, the lung lobules are incompletely separated but the interlobular septae that are present are well defined and thick. The pleura is thick and vascular.

As a rule, the branches of the pulmonary artery and vein follow the bronchial tree to the alveolar capillary bed. However, in the more central areas of the lung, the pulmonary vein pursues an independent course to the hilus.

The branches of the bronchial arteries closely follow the pulmonary arteries and supply the bronchial tree as far as the terminal bronchioles. They also supply the pleura via interlobular and hilar vessels which anastomose. The termination of the bronchial arteries is in the alveolar capillary bed in common with the pulmonary arteries (McLaughlin et al, 1961[a,b]).

CHAPTER 3

GENERAL MATERIALS AND METHODS

SOURCE OF ANIMALS

The normal animals used were horses of various types and sex and included two female donkeys. Their ages ranged from two days old to over 20 years. All were free from clinical respiratory disease and showed no abnormalities of their respiratory systems at post-mortem and histological examinations. They were obtained through Glasgow University Veterinary School and were destined for slaughter for humane or economic reasons. The sources and clinical background of the horses used in other sections of the work are described in the respective chapters.

POST-MORTEM TECHNIQUES

The animals were killed either by shooting or by an overdose of pentobarbitone sodium (Euthatal, May and Baker, Dagenham), administered intravenously via the left external jugular vein. In both methods exsanguination followed by severing the same vein. The tongue, larynx, trachea, heart and lungs were removed intact. The head was severed at the atlanto-occipital joint and then sectioned sagittally using a band saw to allow access to the nasal passages and the nasopharynx.

HISTOLOGICAL AND STAINING METHODS

(a) Fixation, embedding and sectioning :-

Tissues for examination with the light microscope

were fixed in neutral buffered formalin for seven days then trimmed and post-fixed for two days in mercuric chloride formol. These fixatives were prepared as follows:

Neutral Buffered Formalin

Formaldehyde (40%)	200 ml
Sodium chloride	10 g
Sodium sulphate	30 g
Distilled water	1800 ml

Mercuric Chloride Formol

Saturated aqueous mercuric chloride	900 ml
Formalin	100 ml

After fixation, tissues were dehydrated, cleared and impregnated with paraffin wax. Paraffin embedded sections were cut at 3 µm with a Leitz Rotary Microtome and mounted on glass slides.

(b) Staining :-

Mounted sections were routinely stained with standard haematoxylin and eosin (HE) and Alcian blue-periodic acid-Schiff (pH 2.5) method (AB-PAS) for acidic and neutral mucosecretory units according to a modification of the method of Mowry (1956) as detailed below:

Solutions:

(1) 1% Alcian blue in 3% acetic acid (pH 2.5)

(2) 1% Periodic acid

(3) Schiff's reagent

Steps:

(1) Hydrate sections

(2) Solution (1) for 4 mins.

(3) Wash in distilled water

(4) Solution (2) for 2 mins.

(5) Wash in distilled water

(6) Solution (3) for 8 mins.

(7) Wash in running water for 10 mins.

(8) Mayer's haematoxylin for 4 mins.

(9) Wash in running water

(10) Differentiate in acid alcohol for 10 seconds

(11) Wash in running water

(12) Blue nuclei in Scotts tap water substitute

(13) Wash in running water

(14) Dehydrate, clean and mount

Results:

Acidic mucosubstances	-	blue
Neutral mucosubstances	-	red
Mixed mucosubstances	-	purple

SCANNING ELECTRON MICROSCOPIC METHODS

Tissue samples with a surface area of not more than 1 cm square and less than 2 mm thick were immersed in Karnovsky's fixative (2.5% paraformaldehyde/2% glutaraldehyde) for 24 hours. Those contaminated with surface blood were gently washed in 0.2M cacodylate buffer

first. Specimens were then rinsed in 0.2M cacodylate buffer for 4 hours before dehydration in a series of acetones.

Specimens from the rinse solution were passed through graded acetones during which process they were gradually dehydrated. To avoid shrinkage the time specimens were kept in the acetone was strictly controlled. The dehydration schedule used is given below :-

70% acetone	:	4 hours
90% acetone	:	2 hours
100% acetone	:	2 hours

The specimens were then critical point dried in liquid CO_2 (Polaron, Watford, U.K.), orientated and mounted on aluminium stubbs and sputter-coated with gold paladium.

<u>0.2M Cacodylate Buffer (500 ml)</u>

0.4M sodium cacodylate	-	250 ml
0.2M hydrochloric acid	-	40 ml
Distilled water	-	210 ml

<u>Karnovsky's Fixative (500 ml)</u>

Dissolve 10g paraformaldehyde in 100 ml distilled water at 60°C together with 10 drops of NaOH and the mixture added to the stock solution.

<u>Stock Solution</u>:

0.2M cacodylate buffer	-	250 ml
25% glutaraldehyde	-	50 ml
Distilled water	-	100 ml

TRANSMISSION ELECTRON MICROSCOPIC METHODS

Small portions of the right cranial lobe of the lung and nasal cavity mucosa from selected sites were minced into pieces less than 0.5 mm thick in a Petri dish. These small pieces of tissue were transferred into Bijou bottles containing Karnovsky's fixative and left for 24 hours at 4°C. The fixative was drained and replaced by 0.1M cacodylate buffer for 1 hour. The specimens were post-fixed for 1 hour in 1% osmium tetroxide before washing three times in distilled water.

A graded series of acetones (as used for SEM) was used to dehydrate the specimens which were then placed in two changes of propylene oxide for 20 minutes, then 1:1 mixture of propylene oxide and Emscope Emix resin (Emscope, Ashford, Kent) for 1 hour and finally neat resin for 3 hours. Finally, the tissue was embedded in neat resin in plastic mounting moulds and left in an oven at 60°C to effect polymerisation.

Using glass knives prepared by an LKB 780 knife maker (LKB, Croydon, Surrey), the blocks of embedded tissue were trimmed by means of an LKB Pyramitome and sections cut at 1 μm. The sections were collected from the knife edge using a pair of fine forceps and placed in a droplet of water on a glass slide. The sections were stained with toluidine blue (see below) and examined with a Leitz Laborlux II microscope. Suitable portions were

selected for ultrathin sectioning. Each block was finally trimmed and then placed in an LKB Mk III ultramicrotome. Sections of a silver or pale yellow colour (60-90 nm thick) were collected in a water chamber. They were then flattened using chloroform vapour, picked up on Polaron 300 mesh grids, stained with uranyl acetate and lead citrate (see below) and viewed with a Joel 100 CX electron microscope.

Toluidine Blue:

1% Borax (sodium tetraborate)	1 g
1% Toluidine blue	1 g
Distilled water	100 ml

Staining time : 15 seconds

Uranyl Acetate:

0.2g of uranyl acetate was dissolved in 10 ml of distilled water providing a saturated solution.

Staining time : 5 minutes

Lead Citrate:

1.33g of lead citrate and 1.76g of sodium citrate were dissolved in 30 ml of distilled water and shaken for 30 minutes.

8 ml of 0.1M NaOH were added followed by distilled water to give a final volume of 50 ml.

Staining time : 5 minutes.

PHOTOGRAPHIC METHODS

(a) Light Microscopy:

A Leitz Laborlux 12 microscope equipped with a Wild MPS45 Photoautomat Unit was used.

For black and white photography, Agfa PAN 35mm film (12 ASA) was employed. For colour transparencies Kodachrome 25 (25 ASA) film was used. For black and white prints Agfa-Gevaert Rapitome Photographic paper P1-P4 using an Agfa-Gevaert Rapidoprint PD 3700 automatic processor was employed.

(b) Scanning Electron Microscopy:

All the specimens were examined by means of a Philips 501B SEM at an operating kilovoltage of 15 kv and using spot series ranging from 200 to 1000. An automatic Rolliflex camera using Ilford FP4 (125 ASA) film was employed. Black and white prints were prepared as for light microscopy.

(c) Transmission Electron Microscopy:

Electron micrographs were taken using Ilford Technical E M plates (3.1/4" x 4.3/4"), developed in PQ Universal and fixed in Ilford Ilfospeed fixer. Black and white prints were prepared as above.

CHAPTER 4

A SCANNING ELECTRON MICROSCOPIC AND HISTOLOGICAL STUDY OF THE EQUINE UPPER RESPIRATORY TRACT

INTRODUCTION

In the review of the literature it was emphasised that, despite the clinical importance of the region, there are remarkably few reports of the normal features of the equine upper respiratory mucosa. While the general histological features of the equine upper respiratory tract have been described in one recent report (Mair et al, 1987), there is no information available concerning the ultrastructural appearance of the region. This is particularly surprising considering the importance of clinical respiratory disease in horses and the frequent involvement of the upper respiratory tract in diseases such as Strangles.

The purpose of this section of the work was to carry out a detailed scanning electron microscopical (SEM) and histological study of the equine upper respiratory tract, from the nasal vestibule to the larynx.

MATERIALS AND METHODS

(1) Animals

Twenty one horses and two donkeys were used in this study. The source of these animals, the method of destruction and the post-mortem procedures carried out are described in Chapter 3. The horses were of various types and sex and for the purpose of this study they were divided into four age groups (Table 4.1).

TABLE 4.1

DETAILS OF THE ANIMALS EXAMINED IN THE SEM STUDY OF THE EQUINE UPPER RESPIRATORY TRACT

Group	Age	Type	Sex	Case Number
Birth - 1 year	2 days	Thoroughbred	M	PH 4
	6 months	Shetland Pony *	M	PH 25
	9 months	Shetland Pony *	M	PH 27
	1 year	Shetland X Pony *	M	PH 32
	1 year	Shetland X Pony *	M	PH 33
2 - 5 years	2 years	Anglo-Arab	G	PH 7
	2 years	Shetland X Pony *	F	PH 22
	4 years	Thoroughbred	G	PH 6
	4 years	14 hh Highland Pony	F	PH 11
	5 years	14 hh Pony	F	PH 3
6 - 10 years	6 years	Show Jumper	F	PH 10
	6 years	15 hh Trotter	F	PH 31
	7 years	13.2 hh Pony	F	PH 1
	7 years	Riding Horse	G	PH 12
	7 years	Shetland Pony *	F	PH 13/PHN 1
Over 10 years	12 years	Thoroughbred	G	PH 17/PHN 3
	13 years	Cob	G	PH 9
	13 years	Shetland Pony *	F	PH 23
	13 years	12 hh Pony	F	PH 14/PHN 2
	20 years	Thoroughbred	F	PH 21
	20 + years	12 hh Pony	G	PH 24
	13 years	Donkey	F	PH 2
	20 + years	Donkey	F	PH 5

M = Male, F = Female, G = Gelding, *Shetland Ponies 9-10.2 hh

(2) Sample Sites

Samples for SEM and histology were taken from the following sites:-

(1) Nasal septum at the level of the rostral edge of the first cheek tooth, midway between the dorsal and ventral borders (Fig. 4.1).

(2) Ventral nasal concha at the level of the rostral edge of the first cheek tooth (Fig. 4.2).

(3) Basal fold of the ventral nasal concha, 1cm rostral to the angle between the basal and alar folds (Fig. 4.2).

(4) Dorsal nasal concha at the level of the rostral edge of the fifth cheek tooth (Fig. 4.2).

(5) Conchofrontal sinus at the level of the rostral edge of the fifth cheek tooth (Fig. 4.2).

(6) Nasopharynx, 1cm cranioventral to the pharyngeal opening of the auditory tube (Fig. 4.2).

(7) Lateral free border of the pharyngeal opening of the auditory tube (Fig. 4.2).

(8) Medial free border of the pharyngeal opening of the auditory tube (Fig. 4.2).

(9) Dorso-caudal wall of the medial compartment of the guttural pouch (Fig. 4.2).

(10) Epiglottis, 1cm caudal to the tip (Fig. 4.2).

(11) Ventral larynx caudal to the vocal folds (Fig. 4.2).

In order to give some indication of the distance between each anatomically related sample site, specific

distances were measured in an adult horse of average size (Table 4.2).

For SEM examination, small pieces of tissue, not more than 1 cm square and less than 2 mm thick, were immersed in Karnovsky's fixative (2.5% paraformaldehyde/ 2% glutaraldehyde in 0.1M cacodylate buffer) for 24 hours. Tissues were rinsed in 0.2M cacodylate buffer for 4 hours before dehydration through a series of acetones, then dried in liquid CO_2 in a Polaron critical point dryer. The dried tissues were then mounted on aluminium stubbs and coated with gold paladium in an Emscope sputter coater. Specimens were examined by means of a Philips 501B SEM at 15 Kv and spot sizes varying from 1000 to 200.

For histological examination, small blocks of tissue were fixed in 10% neutral buffered formalin for 7 days, trimmed and post-fixed in mercuric chloride formol for 2 days, dehydrated, cleared and impregnated with paraffin wax. Sections, 3 µm thick, were cut and routinely stained with haematoxylin and eosin and alcian blue-periodic acid-Schiff stains.

To complete the study of the nasal cavity, samples of olfactory mucosa were taken, for SEM and histology, from the ethmoidal conchae (Fig. 4.2) of 2 animals, PH13/PHN1 and PH14/PHN2 (Table 4.1 on page 65).

TABLE 4.2

MEASUREMENTS MADE IN THE UPPER RESPIRATORY TRACT OF A 14.2 hh ADULT HORSE

1.	Total length of the nasal septum	= 30cm
2.	Rostral end of the nasal septum to the angle between the alar and basal folds of the ventral nasal concha	= 4cm
3.	From the angle between the alar and basal folds of the ventral nasal concha to the caudal end of the accessory cartilage of the alar fold	= 2cm
4.	Caudal end of the accessory cartilage of the alar fold to the rostral edge of the first cheek tooth	= 2cm
5.	Distances between the rostral edges of adjacent teeth	= 2.5cm
6.	Choanae to the aditus laryngis	= 10cm
7.	Choanae to the pharyngeal openings of the auditory tubes	= 6cm
8.	Tip of the epiglottis to the caudal extremity of the larynx	= 10cm

RESULTS

SEM Findings:

(1) Nasal septum:

The surface was usually smooth but occasionally appeared wrinkled or undulating. Mucus was a constant feature with strands often trapping red blood corpuscles (RBC) and other debris. Duct orifices of the underlying mucosal glands were an obvious feature (Fig. 4.3). The epithelial cells were almost all nonciliated. Individual cells had distinct boundaries and many were rounded in outline and bulged slightly from the surface. This gave an overall "cobblestone" appearance to the epithelium at this site. Other cells varied in size and were often larger, flatter and polygonal in outline and occasionally appeared depressed below the level of surrounding cells (Fig. 4.4). The cell surfaces were studded with numerous microvillous processes which were usually short and stubby. Scattered among these microvillous cells a second cell type was seen (Fig. 4.5). This appeared to be a mucus-secreting cell and various stages of development were noted. Initially it was dome-shaped and microvilli were more numerous at the periphery of the cell. Mucous droplets were visible through the surface cell membrane of the central part of the cell (Fig. 4.6). The second stage was seen to be discharging mucus

(Figs. 4.7, 4.8), while a third stage had a disrupted surface with pores or craters in the central part of the cell and probably represented a discharged cell (Fig. 4.9). A final stage had a central depressed area surrounded by a smooth raised edge which resembled a "doughring". While microvilli were numerous on the periphery of the cell, they were often sparser in the central area (Fig. 4.10). This latter stage appeared to be a cell in the process of regeneration.

Although nonciliated cells were the predominant type, in all animals a few ciliated cells were seen. These occurred singly or in small groups and the cilia were often sparse and poorly developed (Fig. 4.11). One animal, PH 10, had a particularly large patch of well ciliated cells.

(2) Ventral nasal concha:

The surface was either flat or had shallow folds. Mucus was usually abundant in sheets and strands which appeared to trap debris and RBCs. Both microvillous cells and ciliated cells were present, the former generally being more numerous except in one animal, PH 10, where most of the cells were ciliated. Microvillous cells had distinct boundaries and many were rounded giving the "cobblestone" appearance described previously, while others were flatter, larger and polygonal. Mucus-secreting cells, in various stages of development, as

described in the nasal septum, were observed among the microvillous cells, as were a few cells with sparse or poorly developed cilia. Where ciliated cells occurred in groups, the cilia were well developed and long (Fig. 4.12). Gland duct orifices were observed, particularly in the nonciliated areas.

(3) Basal fold of the ventral nasal concha:

Under low magnification, three distinct areas were distinguished (Fig. 4.13). The most rostral was the hairy skin of the nasal vestibule where squamous cells with surface microplicae or short microvilli were the main feature (Fig. 4.14) and were often seen desquamating from the surface of the epithelium. Numerous hairs protruded from among these cells (Figs. 4.15, 4.16). There was an abrupt transition to a narrow middle zone which was hairless but presented similar squamous surface cells to those described above. Caudally, there was another abrupt transition to the nasal mucosa proper which was deeply folded (Fig. 4.17). The rounded, bulging surface cells were covered with short microvillous processes and gave the characteristic "cobblestone" appearance to the epithelium similar to that described in the nasal septum and ventral nasal concha. Few mucous cells were seen but the duct orifices of underlying mucosal glands were present in considerable numbers.

(4) Dorsal nasal concha:

At this more caudal site in the nasal cavity the surface was either smooth or had slight folds. Mucus was a usual feature and often partially obscured the surface. Most of the cells were well ciliated but among them were many nonciliated cells which gave rather a "moth-eaten" appearance to the surface (Fig. 4.18). At higher magnification some of the latter cells were seen to contain submembranous mucous droplets or were actively discharging mucus (Fig. 4.19), others appeared flatter and covered with sparse microvilli. These nonciliated cells usually occurred singly but were occasionally seen in small groups. Gland duct orifices were not an obvious feature, presumably obscured by the ciliated cover.

(5) Conchofrontal sinus:

The surface was smooth and mucus was always present, often in large amounts. The appearance was very similar to that of the dorsal nasal concha with well ciliated cells interspersed with nonciliated cells many of which contained mucous droplets. In one animal, PH 3, larger patches of nonciliated cells were present. These cells varied in shape and size; some protruded from the surface while others were flatter and polygonal in outline. The cell surfaces were studded with microvillous processes which varied in length from cell to cell (Fig. 4.20).

(6) Nasopharynx:

Deep, parallel folds, transected by smaller folds and clefts, were a usual surface feature (Fig. 4.21), as was the presence of considerable amounts of surface mucus. Ciliated epithelium was a predominant feature in 6 of the 23 animals (PH 2, PH 5, PH 7, PH 9, PH 10, PH 12). The cilia were well developed and were particularly long in the depths of the folds. A few cells discharging mucus were seen protruding between the cilia (Fig. 4.22). Groups of nonciliated cells were always present and were particularly obvious in 7 of the 23 animals (PH 1, PH 3, PH 4, PH 6, PH 11, PH 13 and PH 7). The latter cells varied greatly in size and shape and numerous microvillous processes studded the cell surfaces (Fig. 4.23). These nonciliated nasopharyngeal cells resembled those described in the rostral nasal cavity, on the nasal septum and the ventral nasal concha. Occasional ciliated cells were seen in the predominantly nonciliated areas, often with sparse, poorly developed cilia. Mucus-secreting cells were also observed (Fig. 4.24).

(7 and 8) Lateral and medial surface of the pharyngeal opening of the auditory tube:

Both the lateral and medial surfaces were clothed with similar epithelium. Large amounts of mucus were present often obscuring surface detail. Well ciliated cells were the predominant feature with many

discharging mucous cells protruding among them (Fig. 4.25). However, there were always a few patches of nonciliated microvillous cells, similar to those seen in the nasopharynx (Fig. 4.26).

(9) Guttural pouch:

The surface presented deep, parallel folds transected by smaller folds and clefts (Fig. 4.27). Surface mucus was usually present. In all animals except 4 (PH 1, PH 5, PH 11, PH 12) well ciliated cells predominated with many mucus-secreting cells protruding between the cilia (Fig. 4.28). A few nonciliated cells occurred, in small groups, in the four animals mentioned above. In one animal (PH 17) several well defined rounded areas were seen bulging above the surrounding folded surface (Fig. 4.29). These rounded areas were smooth and covered predominantly by ciliated cells, but a few nonciliated microvillous cells were present, usually on the most protruberant parts of these structures (Fig. 4.30).

(10) Epiglottis:

Smooth areas and shallow folds were present on the surface which was often partially covered with mucus. Microvillous cells covered most of the surface. All had distinct boundaries and the smaller, rounded cells gave the "cobblestone" appearance to the epithelium which was observed elsewhere in the upper respiratory tract (Fig. 4.31).

Flat, polygonal cells occurred in other areas and a few mucus-secreting cells, as described in the nasal septum, were also seen (Fig. 4.32). The presence of a few ciliated cells was a constant feature. These usually occurred as single cells (Fig. 4.33), however, in 2 animals (PH 4, PH 13) ciliated cells were arranged in small groups. Numerous gland duct orifices opened onto the surface.

(11) Ventral larynx:

The surface was deeply and regularly folded (Fig. 4.34) and in all but 2 animals (PH 3, PH 7) was covered with a luxuriant carpet of cilia. A few cells secreting mucus were observed protruding between the ciliated cells (Fig. 4.35). In PH 3 and PH 7, nonciliated microvillous cells were present either in small patches or covering larger areas. In these latter areas the microvillous cells were usually mixed with a few ciliated cells (Fig. 4.36).

Histological Findings:

(1) Nasal septum:

The epithelium was of a stratified cuboidal type. In most of the animals the outer cells were rounded and bulged from the surface while in 7 animals (PH 2, PH 5, PH 9, PH 11, PH 12, PH 24, PH 25) there were a few ciliated cells. Surface mucus-secreting cells were fairly

numerous and gave a mixed or acid staining reaction with AB-PAS. Numerous serous secreting mucosal glands were present and their ducts were lined by simple epithelium which usually contained mixed or neutral staining mucous cells, as they approached the surface (Figs. 4.37, 4.38).

(2) Ventral nasal concha:

In most of the animals the epithelium was of a stratified cuboidal type, similar to that described in the nasal septum. In 7 animals (PH 2, PH 4, PH 5, PH 17, PH 24, PH 25, PH 33) small numbers of ciliated cells were observed while in animal PH 6 the surface was completely covered with a pseudostratified columnar ciliated epithelium. Generally surface mucous cells were few in number. They gave a mixed or acid staining reaction with AB-PAS and tended to concentrate close to the duct openings of the numerous serous secreting mucosal glands.

(3) Basal fold of the ventral nasal concha:

This sample site included the skin of the nasal vestibule and three distinct zones could be distinguished. Rostrally, keratinized stratified squamous epithelium covered the surface, with numerous hair follicles, hairs and associated sebaceous and sweat glands (Fig. 4.39). This was succeeded by a narrow hairless zone. There was an abrupt junction with the mucosa of the nasal cavity which was covered by a thick stratified cuboidal type of epithelium with distinctly rounded surface cells (Fig. 4.40).

Surface mucus-secreting cells were sparse and often ill-defined. They contained mostly mixed mucosubstances when stained with AB-PAS. Numerous serous-secreting glands packed the lamina propria and their coiled ducts were initially lined by a simple epithelium, which became double layered and usually contained mixed or neutral staining mucous cells close to the surface openings.

(4) Dorsal nasal concha:

At this more caudal level, the surface was covered by a thick respiratory type of epithelium, i.e., pseudostratified columnar ciliated with mucous cells (Fig. 4.41). The latter contained mixed and neutral mucosubstances (Fig. 4.42). Sero-mucous mucosal glands were present in considerable number and the mucous component gave a staining reaction to AB-PAS similar to that of the surface mucous cells.

(5) Conchofrontal sinus:

The mucosa was often thin and closely applied to the underlying bone forming a mucoperiosteum. The epithelium was pseudostratified columnar ciliated with mucous cells and was generally thinner than that of the dorsal nasal concha. Glands were absent in the mucoperiosteum but where the mucosa was thicker, a few glands similar to those of the dorsal nasal concha, were seen.

(6) Nasopharynx:

The surface was usually uneven and frequently dipped down forming clefts. Two distinct types of epithelium were seen. In 15 of the 23 animals pseudostratified columnar ciliated epithelium (see Fig. 4.41) and a stratified cuboidal type of epithelium (Fig. 4.43) alternately clothed the surface, although the former type predominated. The outer cells of the latter type were rounded and this epithelium resembled that of the rostral nasal cavity on the nasal septum and ventral nasal concha. In 5 animals (PH 2, PH 5, PH 6, PH 7, PH 11) the entire surface was ciliated while 3 animals (PH 12, PH 13, PH 22) had a completely nonciliated epithelium. Patches of mucous cells were often concentrated in the surface clefts and they contained both acid and neutral mucosubstances. Sero-mucous mucosal glands were numerous, the mucous component giving a mixed or acidic reaction to AB-PAS stain. Subepithelial aggregations of lymphatic tissue (Fig. 4.43), occasionally forming nodules, were seen in most animals.

(7 and 8) Lateral and medial surfaces of the pharyngeal opening of the auditory tube:

The appearance of both lateral and medial surfaces were similar. The epithelium was generally of a pseudostratified columnar ciliated type although groups of nonciliated surface cells were observed in 7 animals

(PH 2, PH 3, PH 11, PH 14, PH 21, PH 31, PH 32) while in 4 animals (PH 13, PH 22, PH 25, PH 27) the epithelial surface cells were mostly nonciliated. Mucous cells were more numerous in the ciliated epithelium and their staining reaction with AB-PAS was generally mixed although some neutral cells were present. There were a few sero-mucous mucosal glands with mixed or acidic mucous acini. Sub-epithelial lymphatic aggregations, similar to those in the nasopharynx, were usually present.

(9) Guttural pouch:

The epithelium was of a respiratory type, i.e., pseudostratified columnar ciliated with mucous cells. Numbers of the latter varied from animal to animal and they contained either acid or a mixture of acid and neutral mucosubstances. A few sero-mucous glands were present and the mucous component gave a similar staining reaction with AB-PAS as the surface mucous cells. Sub-epithelial lymphatic aggregations were observed in 6 of the 23 animals (PH 1, PH 5, PH 17, PH 27, PH 32, PH 33) and these occasionally caused the surface to bulge outwards but in no case was there any alteration in the epithelium covering these areas.

(10) Epiglottis:

The epithelium of the dorsal surface of the epiglottis was thick and of a mixed character. A stratified squamous non-keratinizing type merged with a

stratified cuboidal type of epithelium. In the latter surface mucous cells were sparse and ill-defined and gave a mixed staining reaction with AB-PAS. Mucosal glands were numerous, mostly mucous secreting and contained both acidic and neutral mucosubstances.

(11) Ventral larynx:

In contrast to the epiglottis, this surface of the larynx was covered by pseudostratified columnar ciliated epithelium with mucous cells, albeit few in number. A few nonciliated surface cells were seen in 2 animals (PH 1, PH 27). Very few sero-mucous glands were present in the mucosa. Acid or neutral mucosubstances were present in the glands and in the surface mucous cells when stained with AB-PAS.

Viewed with SEM the surface of the olfactory epithelium was covered by a dense, tangled mass of long, slender cilia (Fig. 4.44). Many duct openings of the underlying olfactory glands were observed and in some areas numerous secretory droplets lay on the surface (Fig. 4.45). With higher magnification olfactory vesicles with shorter, thick cilia could be seen protruding from the surface (Fig. 4.46). The sustentacular cells were not obvious as their microvillous surfaces were obscured by the dense cilia and secretions.

Histologically the olfactory mucosa consisted of thick pseudostratified columnar epithelium (6-7 nuclei deep)

with many underlying branched tubular olfactory glands which contained alcianophilic secretory granules (Fig. 4.47).

DISCUSSION

From the review of the literature it can be seen that there have been virtually no comprehensive reports of the normal surface structure of the equine upper respiratory tract. Some general histological features have been described by Mair et al (1987) but there are no descriptions of the ultrastructural appearance. For this reason the present study was undertaken to determine the surface features of the respiratory tract, from the nasal vestibule to the larynx, in normal horses of various types and age. Light microscopy alone did not give a complete picture of the surface topography and the findings in this study indicated the excellent surface structural information that only SEM can provide.

The histological features of the normal equine upper respiratory tract detected by light microscopy in this study, in general, resembled those of other mammalian species (Bryant, 1916; Schaeffer, 1932; Ali, 1965; Pass et al, 1971; Phipps, 1981; Adams and Hotchkiss, 1983; Leeson et al, 1985; Nakano and Muto, 1987). They were also in accord with the findings of Mair et al (1987) in the horse.

Excluding the olfactory epithelium, which

occupies the most caudal and dorsal part of the nasal cavity, three main types of epithelium were found in the upper respiratory tract of the horse. Respiratory epithelium, i.e., pseudostratified columnar ciliated with mucous cells, covered the greatest area. This included the more caudal regions of the nasal cavity, the guttural pouches and the larynx caudal to the vocal folds. Stratified squamous epithelium, either keratinizing or non-keratinized, was found in the nasal vestibule and on the epiglottis. Epithelium of a stratified cuboidal type was present in the rostral nasal cavity and also on parts of the nasopharyngeal surfaces where patches were present merging with the mainly respiratory type of epithelium. This stratified cuboidal epithelium was also present on the epiglottis merging with the non-keratinized stratified squamous epithelium.

Unlike other domestic species, the horse has unmodified integument around its nostrils and this fine, hairy skin is continued into the nasal vestibule where it meets the nasal mucosa at a sharply defined line over the alar and basal folds of the ventral nasal concha. The apparent abrupt junction, observed in gross specimens, was in fact composed of three distinct zones when viewed microscopically. The most rostral zone was stratified squamous keratinizing epithelium with hair follicles and their associated sebaceous and sweat glands. SEM

presented a dramatic picture of desquamating squamous cells with a forest of numerous hairs projecting from the surface, trapping debris and providing an obstacle to the entrance, of at least the larger foreign particles, into the nasal cavity. The presence of hairs, with their associated glands, in the outer part of the nasal vestibule was described in man by Schaeffer as early as 1932, but has not been described in other animals, although stratified squamous keratinized epithelium has been noted in the vestibular region of the mouse (Greenwood and Holland, 1972), rat (Andrews, 1974), monkey (Harkema et al, 1987) and pig (Adams, 1990). In the horse a narrow zone of stratified squamous keratinized epithelium without hairs was sandwiched between the rostral hairy vestibular epithelium and the stratified cuboidal type of epithelium of the nasal cavity proper. It was this junction between the two latter types of epithelium that was so obvious in the gross specimens and also sharply defined with SEM and the light microscope. This abrupt junction has also been observed in the rat (Andrews, 1974) and monkey (Harkema et al, 1987), while in the mouse it has been noted that the stratified squamous epithelium extended into the ventral nasal cavity for a considerable distance and that there was a gradual transition of this epithelium to the more caudal ciliated respiratory surfaces (Adams, 1972; Greenwood and Holland, 1972). A similar situation

exists in the pig where the stratified squamous epithelium extends for approximately 15% of the length of the nasal cavity (Adams, 1990).

In the horse, a large zone of nonciliated epithelium covered the rostral nasal cavity to about the level of the first cheek tooth; a distance of 8 cm in an adult 14.2 hh animal and approximately 25% of the total length of the nasal cavity (see Table 4.2, page 68). In the rostral part of this zone, when viewed with SEM, the rounded microvillous cells, with their distinct boundaries, gave a characteristic "cobblestone" appearance to the surface while more caudally the cells were often varied in shape and size.

Openings from the ducts of underlying mucosal glands were a conspicuous surface feature. Many mucus-secreting cells were present among the microvillous cells and their appearance varied with the stage of development of the cell. Dome-shaped cells, with peripheral microvilli bulged with mucous droplets which were clearly visible through the surface membrane. These cells preceded a secretory stage with discharge of mucus from the surface of the cell. At a later stage, after discharge, the cell surface was disrupted with the appearance of pores or craters and this was followed by a final stage where the depressed central area of the cell was surrounded by a smooth raised edge resembling a

"doughring". Microvilli were numerous peripherally and were also present within the "doughring". This seemed to be an end stage in the secretory cycle and possibly represented a cell in the process of regeneration. Some similar stages in the secretory cycle of mucous cells in the nonciliated epithelium of the nasal cavity have been described in the rat (Andrews, 1974; Popp and Martin, 1984) and monkey (Harkema et al, 1987), although the final regenerative stage, noted in this study of the horse, has not been described before.

At the level of the first cheek tooth, caudal to the nonciliated area, a few ciliated cells made an appearance on the surfaces of the nasal septum and ventral nasal concha and were more frequently seen on the latter site. Indeed, in one animal most of the surface of the ventral nasal concha was well ciliated. However, the ciliated cells usually occurred singly or in small groups and the cilia appeared short or they varied in length.

The caudal nasal cavity was covered by a well ciliated epithelium with mucous cells and it extended into the conchofrontal sinus. There was obviously a gradual increase in the number of ciliated cells from rostral to caudal parts of the nasal cavity and this appears to be common to mammals in general and has been described in the rat (Andrews, 1974; Popp and Martin, 1984), dog (Adams and Hotchkiss, 1983; Majid, 1986) and monkey (Harkema

et al, 1987).

In the horse, where the ciliated cells appear at first among the mainly microvillous cells of the rostral nasal cavity, it was noted that many of the former cells possessed short cilia or cilia of various lengths and it would seem reasonable to suppose that these could be developing ciliated cells. Similar types of cells have been observed by other workers in the rat (Popp and Martin, 1984), in calves (Adams, 1986) and pigs (Adams, 1990), and it was suggested by these workers that these cells were developing ciliated cells.

A nasal epithelial brush cell has been described in the rat (Popp and Martin, 1984) but has not been observed with SEM by other workers although Adams (1986) in his study of calves, described a brush cell in the nasal mucosa which he found with TEM (but not with SEM). No brush cells were found in the equine upper respiratory tract in the present study.

The presence of a substantial area of nonciliated epithelium covering the rostral part of the nasal cavity, between the nasal vestibule and the well ciliated caudal surfaces, has been described as a normal feature in several species other than the horse. It has been found in domestic animals such as the dog (Adams and Hotchkiss, 1983; Majid, 1986), calf (Adams, 1986) and pig (Adams, 1990), in laboratory animals such as the mouse (Adams, 1972; Greenwood

and Holland, 1972), the rat (Andrews, 1974; Popp and Martin, 1984) and monkey (Harkema et al, 1987) and also in man (Mygind, 1975; Moore-Gillon, 1985). Adams (1986) referred to this area as the transitional zone of the nasal mucosa.

Histologically this thick, nonciliated epithelium was of a stratified cuboidal type in the horse and the presence of surface mucous cells was confirmed by AB-PAS stain. Majid (1986), in the dog, referred to this type of thick stratified epithelium as transitional epithelium, similar to that which lines the urinary tract. It has also been described as transitional epithelium, again in the dog, by Adams and Hotchkiss (1983). However, Adams (1986) in his study of calves later referred to the whole nonciliated area as the transitional zone and described it as being lined by stratified cuboidal epithelium.

In the present study, this epithelium in the horse did not conform to descriptions of transitional epithelium of the urinary tract (uroepithelium) either with the light microscope (Leeson et al, 1985) or with SEM (Kessel and Kardon, 1979) but seemed to be similar to that described in calves (Adams, 1986).

It has been mentioned earlier that in the horse the stratified squamous epithelium of the nasal vestibule abruptly joined the stratified cuboidal epithelium of the

rostral nasal cavity and that this latter type of epithelium only covered the nonciliated zone. Although this latter zone has certainly been described in other species, it was not always made clear whether the epithelial junction was at the level of the nasal vestibule or more caudally. However, Adams and Hotchkiss (1983) have stated that the stratified squamous epithelium extends caudally from the nasal vestibule into the more rostral part of the nasal cavity in the dog and this was also the case in the mouse (Adams, 1972; Greenwood and Holland, 1972) and the pig (Adams, 1990). From his histological, TEM and SEM studies of the nasal mucosa in man, Boysen (1982) concluded that the nonciliated zone was due to squamous metaplasia and that it appeared as early as 4 years of age and continued to develop thereafter. In the present study of horses of all ages, there was no indication that this zone was due to squamous metaplasia, or that the zone increased to any extent with age.

There appears to be species variation in the extent of the nonciliated zone. In the dog, Adams and Hotchkiss (1983) stated that up to 50% of the nasal cavity surface was covered by nonciliated epithelium. In young calves the rostral 40% was covered by stratified cuboidal epithelium (Adams, 1986), while in pigs, epithelium containing few ciliated cells covered the rostral 50% of the nasal cavity (Adams, 1990). A smaller nonciliated

zone was found in the monkey by Harkema et al (1987), who indicated that the nonciliated epithelium covered approximately the rostral 25% of the nasal cavity. The findings in the horse in this present study appeared to be similar to the situation in the monkey. In a study of human patients, who had undergone laryngectomy, Moore-Gillon (1985) found that the nonciliated transitional zone normally present in the human nose, had been transformed to a densely ciliated surface. This change was attributed to lack of airflow through the nose after removal of the larynx and diversion of respiratory airflow through an end tracheostome. It was suggested that in normal individuals, inspired air causes drying of the nasal mucous layer and some destruction of cilia in the anterior part of the nose. This is an interesting observation but would seem to imply that the newborn should have complete ciliation of the nasal cavity. However, this has not yet been established in human infants at birth although Boysen (1982) stated that the main type of epithelium in young children was ciliated. However, it has been established in the rat that, while the caudal nasal cavity surfaces were ciliated, the rostral surfaces were nonciliated in prenatal animals at 20 days gestation, i.e., 3 days before birth and that this clear distinction in the nasal cavity surfaces was maintained postnatally and into adult life (Menco and

Farbman, 1987).

In the present study, one very young animal (a 2 day old foal) had a nonciliated zone in the rostral nasal cavity similar to that found in adult horses. Adams (1986) suggested that the nonciliated transitional zone in the rostral nasal cavity of 7 day old calves was comparable to a similar zone in adult dogs and was probably a normal functional entity. Thus in the rat, calf and dog it would appear that the rostral nasal cavity is normally covered by a nonciliated epithelium which is present at a young age, in the rat even before birth, and that this epithelium is comparable to that found in older animals (Adams, 1986; Menco and Farbman, 1987). The findings in the present study, in the horse, seem to be in accord with the findings of these workers.

The nasopharyngeal surface of the horse was very irregular with numerous folds and deep clefts. The surface of the epithelium was characterised by mixed patches of ciliated cells and microvillous cells. The latter, which resembled the nonciliated microvillous cells of the rostral nasal cavity, were a consistent feature and did not seem to be associated with subclinical disease. There was a degree of individual variation in the proportion of ciliated to nonciliated patches. In 6 of the 23 animals the former predominated while in 4 others nonciliated patches were more noticeable. These variations

were not related to type and age. References in the literature to SEM features of the nasopharynx in other mammals are few but Andrews (1974), in the rat, described patches of ciliated and nonciliated cells in the nasopharynx, and stated that the proportions of these patches varied from one rat to another. More recently Majid (1986) described similar nasopharyngeal epithelium in the dog. The present findings in the horse are in accord with these studies.

The surfaces of the lateral and medial edges of the pharyngeal openings of the auditory tube in general presented a similar picture to that of the nasopharynx. Although ciliated cells predominated, patches of non-ciliated microvillous cells were always observed.

The SEM findings were confirmed histologically in the nasopharyngeal surface although in 5 out of the 23 animals the entire surface was ciliated while in a further 3 animals it was completely nonciliated. This was perhaps to be expected, given the variability of the epithelial surface cells in different animals when viewed with SEM and the much smaller area available for examination in a histological section compared with the larger area of the SEM specimens. However, in most of the animals ciliated cells predominated in the epithelium covering the openings of the auditory tubes when viewed with the light microscope. Similar epithelial types were recorded by Mair et al (1987)

in a general histological study of equine respiratory tract surfaces, but they inferred that the ciliated epithelium occurred rostrally in the nasopharynx while the nonciliated epithelium was present more caudally, which was not the case in the present study where the two types were mixed. However, the well ciliated epithelium covering the auditory tube openings observed in this study of horses was also noted by Mair et al (1987).

The appearance of an intermediate or transitional type of stratified cuboidal epithelium, with imperfect or few surface cilia, has been described in the nasopharynx in several species. It occurred between the rostral ciliated epithelium and the caudal stratified squamous epithelium at the boundary with the oropharynx in the rabbit, guinea pig, monkey, cat and man (Bryant, 1916) and in the mouse (Nakano, 1986). Because the horse nasopharynx is long (see Table 4.2, page 68) and the samples taken in the present study were from an area about midway between the rostral and caudal boundaries, they were, therefore, a considerable distance from the oropharynx. Thus the boundary between the naso- and oropharynx, described by the workers above, was not examined. However, the stratified cuboidal type of epithelium, which was found in patches in the more rostral area of the nasopharynx in the present study, appeared similar to that described by Bryant (1916) and

it would seem that this type of epithelium becomes predominant in the caudal nasopharynx, as suggested by Mair et al (1987).

Guttural pouch surfaces, which are continuous with those of the nasopharynx through the pharyngeal openings of the auditory tubes, always appeared regularly folded and covered by well ciliated epithelium with numerous mucous cells. Small groups of nonciliated cells were observed in 4 animals only. In one other animal several well defined rounded areas bulged from the surrounding surface and, although mainly covered by ciliated cells, a few microvillous cells were present on the most protruberant parts of these structures. Histologically the guttural pouch surface epithelium was of a respiratory type, i.e., pseudostratified columnar ciliated with mucous cells and this has also been described by other workers (Sisson, 1975; Mair et al, 1987). The prominant bulging areas, observed in one animal with SEM, proved to be subepithelial lymphoid nodules when examined with the light microscope. Histologically 5 other animals had similar lymphoid deposits but these were not large enough to be observed with SEM.

The presence of mucosal lymphatic tissue in the respiratory tract of the horse has been studied by Mair et al (1987, 1988). They observed isolated lymphocytes in the lamina propria, migrating through the epithelium

and free in the lumen of the respiratory passages. In addition there was subepithelial lymphoid tissue in nodules and dense aggregations of small lymphocytes often, but not always, associated with the nodules. With appropriate planes of section the lymphoid nodules were seen to be overlaid with a modified lympho-epithelium which was thinner than that of adjacent areas, nonciliated and contained no mucous cells. The lymphoid nodules were identified in the nasal cavity, nasopharynx, auditory tubes and larynx and were more prominent in the latter three sites. In the present study mucosal lymphatic tissue was also observed histologically in the upper respiratory tract of the horse. Aggregations of lymphocytes and lymphoid nodules were most numerous in the nasopharynx and associated structures but usually did not appear to be covered by a modified epithelium. On the other hand, with SEM the prominent guttural pouch nodules, observed in one animal, did have nonciliated cells present on the most protruberant parts and this probably represented the lympho-epithelium described by Mair et al (1987) in their histological study.

The epithelium of the dorsal surface of the epiglottis of the horse was mostly nonciliated. With SEM microvillous cells of varied shape and size covered the surface. A few ciliated cells were always present, usually singly, but in 2 animals small groups of these cells were

observed. Histologically the epithelium was of a stratified cuboidal type although in some of the animals this merged with stratified squamous epithelium rostrally. Surface cilia were not observed with the light microscope. In contrast the ventral larynx was clothed with a luxuriant carpet of ciliated cells in all the animals except 2, where small groups of microvillous cells were found (but only with SEM).

The surface of the epiglottis is subject to considerable wear and tear during swallowing and in mammals in general the covering epithelium is of a robust type, similar to that found in other exposed situations. It has been described in domestic animals in general (Nickel et al, 1979), in the dog (Majid, 1986), in the rat (Andrews, 1974) and in the mouse (Nakano and Muto, 1987). However, there seemed to be some species variation in the laryngeal epithelium caudal to the epiglottis. The complete ciliation, caudal to the vocal folds, found in the present study of the horse differed from that of the rat where large patches of microvillous cells throughout the larynx were described as a normal feature (Andrews, 1974). Majid (1986) in his SEM study of 18 dogs, found that only 5 animals had complete ciliation of the ventral larynx, while in 6 animals only squamous microvillous cells were present. In the remaining dogs a "cobblestone" type of epithelium covered the surface between the ciliated

laryngo-tracheal junction and the rostral larynx. It would appear from this study of normal dogs that there was considerable individual variation in the laryngeal epithelium in this species which was not the case in the horse.

Defence of the respiratory system against inhalation of harmful particles is of prime importance in the maintenance of health and the airway mucociliary system is one of the most important mechanisms in this war against a hostile external environment (Phipps, 1981). Epithelial mucous cells and submucosal glands synthesize and secrete the mucosubstances which form part of this mucociliary system. It is obvious that the nasal cavity is in the front line of this defence, a fact which has long been recognised by workers in this field (Schaeffer, 1932; Hilding, 1963; Proctor, 1977; Morgan et al, 1984).

Up to the present time the mucous secreting cells of the equine upper respiratory tract have received little attention although Mair et al (1987) briefly mentioned the presence of epithelial mucous cells and seromucous glands in the nasal cavity, nasopharynx and larynx. In the few reports of the histochemistry of nasal cavity mucus in other species, it has been stated that human nasal mucous cells and glands contained acid mucopolysaccharides (Bang and Bang, 1977). In the dog the nasal cavity mucus-secreting cells were found to be acidic while those of the

nasopharynx and larynx were acidic or mixed in their staining reaction (Majid, 1986).

The previous findings in the horse nasal cavity were confirmed and extended in the present investigation which was confined to assessing the distribution of acidic and neutral mucosubstances and did not attempt to classify the acidic component further into sulphomucins and sialomucins, as has been done in the equine lower respiratory tract (Nicholls, 1978). It was established that epithelial mucous cells and mucosecretory units in the mucosal glands contained predominantly acidic or mixed acidic and neutral mucosubstances, although occasional cells secreting only neutral mucosubstances were also present. Surface mucous cells were present on the non-ciliated epithelium of the rostral nasal cavity albeit rather sparsely distributed in the most rostral area at the junction of the nasal vestibular skin and the nasal mucosa. The muscosal glands were particularly numerous in the nasal cavity rostrally and were of a serous secreting nature. Further caudally the glands were of a sero-mucous secreting type and became less numerous in nasal cavity surfaces and were practically absent from the guttural pouch and ventral larynx. However, the nasopharynx and epiglottis possessed numerous glands. The numerous mucus-secreting cells in the ciliated epithelium of the upper respiratory tract contained mostly acidic or mixed mucosubstances although

patches of neutral staining cells were seen in all sites.

It has been suggested that nasal cavity mucus formed a continuous layer on the surface (Hilding, 1963; Morgan et al, 1984). Ciliary action moved mucus continually towards the pharynx where it was normally swallowed and the mucus on the nonciliated rostral parts of the nasal cavity was dragged caudally by traction (Hilding, 1963; Morgan et al, 1984). It would seem, from the present study, that the horse has an ample number of cells and glands to provide such a mucous blanket in the upper respiratory tract.

Because the olfactory mucosa is relatively inaccessible in the horse, mainly lying in the extreme caudo-dorsal area of the nasal cavity with a very small component in the vomeronasal organ, it was not included in the main upper respiratory tract study. However, to complete the study of the nasal mucosa, samples from the caudal olfactory region were taken from 2 horses and its histological and SEM appearance was similar to that described in other mammalian species (Allison, 1953; Greenwood and Holland, 1972; Andrews, 1974; Kessel and Kardon, 1979).

In this investigation, the upper respiratory tract of a large group of horses has been studied in some detail and there appeared to be no substantial differences in the epithelial surfaces observed in the animals of

various ages and types examined. However, it can be seen from a study of the literature that there are differences in the proportions and distribution of epithelial types both between and within species, particularly in the rostral nasal cavity (Greenwood and Holland, 1972; Adams, 1972, 1986; Andrews, 1984; Adams and Hotchkiss, 1983; Popp and Martin, 1984; Harkema et al, 1987). In the present study it became obvious that in the horse also there were individual differences in epithelial type distribution, especially in the more rostral parts of the nasal cavity. Thus it was thought that it would be worthwhile to try to determine the pattern of ciliation in the equine nasal cavity by serial sampling of multiple sites for examination with SEM, TEM and light microscopy. The results of this study are described in Chapter 5.

CHAPTER 5

AN ULTRASTRUCTURAL AND HISTOLOGICAL STUDY OF THE EQUINE NASAL CAVITY

INTRODUCTION

The presence of rostral nonciliated and caudal ciliated surfaces in the nasal cavity have been described in several mammalian species such as the rat (Andrews, 1974; Popp and Martin, 1984; Menco and Farbman, 1987), mouse (Greenwood and Holland, 1972; Adams, 1972), dog (Adams and Hotchkiss, 1983; Majid, 1986), calf (Adams, 1986), monkey (Harkema et al, 1987), pig (Adams, 1990) and man (Mygind, 1975; Boysen, 1982; Moore-Gillon, 1985). It became clear in Chapter 4 that the nasal cavity surfaces of the horse were also nonciliated rostrally and ciliated caudally. However, it was also evident that from rostral-caudal areas the ciliated cells appeared gradually with some variation in individual horses. This gradual appearance of nasal ciliated cells has been described in other species; in the mouse (Greenwood and Holland, 1972), calf (Adams, 1986), monkey (Harkema et al, 1987), pig (Adams, 1990) and man (Mygind, 1975).

Apart from a very brief histological description of the equine nasal cavity by Mair et al (1987), a search of the literature revealed that there were no SEM reports of the nasal cavity surfaces in the horse. In the histological and SEM study of the equine upper respiratory tract, described in Chapter 4, only a limited number of sites were sampled due to the length

and large area of the nasal mucosal surfaces. It was concluded, therefore, that only by incorporating a large number of samples could a much more comprehensive picture of the nasal epithelium be established and its overall pattern of ciliation determined. Thus it was decided to carry out a systematic SEM and histological study using multiple sample sites from the basal fold of the ventral nasal concha to the level of the sixth (and last) cheek tooth.

Excluding the stratified squamous keratinized epithelium of the nasal vestibule, the most rostral non-ciliated area of the nasal cavity epithelium has been variously described as being transitional in the dog (Adams and Hotchkiss, 1983; Majid, 1986) and stratified cuboidal in calves and pigs (Adams, 1986, 1990). In Chapter 4, this latter type of epithelium was also observed histologically in the rostral nasal cavity of the horse. However, observations with the light microscope are limited and it was decided to take a number of further samples from the most rostral part of the nasal cavity for examination with TEM in order to characterise more completely the nature of this most rostral nonciliated epithelium.

MATERIALS AND METHODS

(1) Animals:

The 4 horses used in this study are listed below:-

(i) (PHN 1): 10.2hh Shetland pony female aged 7 years

(ii) (PHN 2): 13hh pony female aged 13 years

(iii) (PHN 3): Thoroughbred gelding aged 12 years

(iv) (PHN 4): 12hh Welsh pony female aged 15 years

The first three animals were included in the study of the upper respiratory tract in Chapter 4 and are also listed in Table 4.1, page 65.

The source of these animals, the method of destruction and the post-mortem procedures carried out are described in Chapter 3.

(2) SEM and Histological Studies:

Samples were taken from the following levels in the nasal cavity:-

Level 1 : 1 cm rostral to the angle between the basal and alar folds of the ventral nasal concha (Fig. 5.1).

Level 2 : At the caudal border of the accessory cartilage supporting the alar fold of the ventral nasal concha (Fig. 5.1).

Levels 3 - 8. At the level of the rostral edge of the first to sixth cheek tooth respectively (Fig. 5.1).

At each of these levels samples were taken from three sites (Fig. 5.2):

A. The nasal septum

B. The ventral nasal concha

C. The dorsal nasal concha.

(3) TEM Studies:

Samples were taken from levels 1 - 4 (as indicated above) from each of three sites, A, B and C (Figs. 5.1 and 5.2) from 2 animals (PHN 3 and PHN 4).

In total 24 samples were taken for SEM and histology from each of the 4 horses and 12 samples for TEM were taken from 2 horses. The samples for SEM and TEM were immersed in Karnovsky's fixative (2.5% paraformaldehyde/ 2% glutaraldehyde) and for histological examination in neutral buffered formalin. Subsequent processing, staining and examination was carried out as described in Chapter 3.

RESULTS

(1) SEM and Histological Findings:

Level 1 : a) SEM

Surfaces of all 3 sample sites, A, B and C were covered by nonciliated microvillous cells of varied shape and size. Small round cells, bulging from the surface, with distinct cell borders gave the characteristic "cobblestone" appearance to the epithelium (Fig. 5.5), while other flatter cells were larger and polygonal in outline. Numerous duct openings of the underlying glands were also an obvious surface feature (Fig. 5.6). Mucous cells in their various stages of development were observed, as described in Chapter 4 (Figs. 5.7, 5.8).

b) Histology

The epithelium was of a stratified cuboidal type with surface mucous cells in groups often associated with the openings of ducts from the numerous underlying serous glands. Mucous cells lined the ducts close to the surface. These latter cells and the surface mucous cells gave a mixed or neutral staining reaction with AB-PAS (see Figs. 4.37, 4.38).

Level 2 : a) SEM

The surface in sample sites A and B appeared similar to that described above. In sample site C, the dorsal nasal concha, ciliated cells were present, either singly or in small groups (Fig. 5.9). Gland duct openings were conspicuous in all 3 sites.

b) Histology

The epithelium was of a stratified cuboidal type and ciliated cells were not seen on the surface. Mixed and acidic staining mucous cells were in groups and numerous serous glands were present in the lamina propria. Small subepithelial aggregations of lymphocytes (Fig. 5.10) were noted in the nasal septum of one animal (PHN 3). Scattered individual lymphocytes were present in the lamina propria and within the epithelium in all sites and, indeed, were a constant feature of the nasal cavity epithelium at all levels in all the horses.

Level 3 : a) SEM

Microvillous cells were the predominant surface cell type but in all 3 sample sites ciliated cells were present, often with sparse or short cilia (Fig. 5.11). These ciliated cells were more numerous in sample sites B and C in 3 of the horses (PHN 1, PHN 2, PHN 3). Many mucous cells in various stages of development (Fig. 5.12) were present as well as numerous duct openings.

b) Histology

Stratified cuboidal epithelium was present in sample sites B and C with a few ciliated surface cells. Mucous cells were found to contain mixed and acidic mucosubstances when stained with AB-PAS. Glands in the lamina propria were again of a serous type but were fewer in number than in levels 1 and 2.

Level 4 : a) SEM

At level 4, sample sites A, B and C all had surface microvillous cells and ciliated cells (Fig. 5.13). In 2 horses (PHN 1 and PHN 4) microvillous cells predominated and ciliated cells occurred singly or in small groups in all 3 sample sites. There was individual variation in the other animals. In one horse (PHN 3) a similar pattern, to that described above, was present in sample sites A and B while sample site C was well ciliated. The remaining horse (PHN 2) resembled the others in sample site A but in the other sample sites

ciliated cells were the predominant type. Mucous cells were present in both ciliated and nonciliated surfaces.

b) Histology

Patches of stratified cuboidal and pseudostratified columnar ciliated epithelium covered the mucosal surface. Mixed and acidic staining mucous cells were more numerous in the latter type of epithelium. Sero-mucous mucosal glands were present in moderate numbers and contained a few weakly neutral staining acini.

Level 5 : a) SEM

Site A now had an equal population of microvillous cells and ciliated cells (see Fig. 4.23). The 2 cell types were mostly evenly distributed but there were large patches of completely ciliated cells in 2 horses (PHN 1, PHN 2). In one horse (PHN 1), the surface of sample sites B and C resembled that of A. In the remaining 3 horses (PHN 2, PHN 3, PHN 4), the surface was almost all well ciliated in sample sites B and C. Cells secreting mucus were a constant feature among the microvillous cells and the ciliated cells. Only a few gland duct openings were observed.

b) Histology

The epithelial pattern was similar to that of level 4. Patches of surface mucous cells gave a mixed and acidic staining reaction with AB-PAS stain. A few sero-mucous glands were present in the lamina propria and

contained a few neutral staining acini.

Level 6 : a) SEM

At this level, the surface in site A was similar to that of level 5 in 3 horses (PHN 1, PHN 3, PHN 4) but was well ciliated in the fourth animal (PHN 2). The ventral nasal concha, sample site B, was almost completely ciliated in 3 horses (PHN 2, PHN 3, PHN 4) while the remaining animal (PHN 1) had mixed groups of ciliated cells and microvillous cells. Sample site C was well ciliated in all the animals (see Fig. 4.18). Mucous cells were always present, sometimes in large numbers. Few gland duct openings were observed.

b) Histology

The epithelium was of a respiratory type, i.e., pseudostratified columnar ciliated with numerous mucous cells containing mixed and acidic mucosubstances (see Figs. 4.41, 4.42). A few sero-mucous glands in the lamina propria were similar to those of level 5. Small subepithelial lymphatic deposits were noted in sample sites A and B in one horse (PHN 3).

Level 7 : a) SEM

In 3 horses (PHN 1, PHN 3, PHN 4) sample site A had mixed groups of microvillous cells and ciliated cells, the latter being more numerous. The surface was almost completely ciliated in the fourth animal (PHN 2). Sample sites B and C were well ciliated in all the

horses (Fig. 5.14). Mucous cells were a constant feature but gland duct openings were few in number.

b) Histology

The appearance at this level was similar to that described for level 6.

Level 8 : a) SEM

At this most caudal level ciliated cells predominated in sample site A although groups of microvillous cells were still present in all horses. Sample sites B and C were covered by a well ciliated epithelium interspersed with many mucus-secreting cells (Fig. 5.14). Gland duct openings were not an obvious feature.

b) Histology

A pseudostratified columnar ciliated epithelium covered all surfaces. The numerous mucous cells gave a mixed or acidic staining reaction with AB-PAS. A few sero-mucous glands with neutral staining mucous acini were present in the lamina propria.

(2) TEM Findings:

Levels 1 -4 of the nasal cavity were sampled for TEM in each of the 3 sites; A, nasal septum; B, ventral nasal concha and C, dorsal nasal concha, in 2 animals (PHN 3, PHN 4). The findings in each of the sites were essentially the same in each animal and are described below.

A. Nasal Septum at Levels 1, 2, 3 and 4:

The epithelium of the nasal septum was stratified (up to 5 or 6 cells thick). It consisted of basal cells, intermediate cells and cells which reached the surface. Electron dense basal cells rested on a thin basal lamina which was indented by numerous cytoplasmic processes from the cells extending into the lamina propria (Fig. 5.15). The nuclei were deeply indented and the cytoplasm contained numerous mitochondria, free ribosomes and bundles of tonofilaments. Many fine cytoplasmic processes extended from the lateral and dorsal cell surfaces into the intercellular spaces. Adjacent cells were joined by desmosomes (Fig. 5.16).

The intermediate layer of cells was several cells thick and lay between the basal cells and those cells which reached the surface. They were generally larger and less electron dense than the basal cells (Fig. 5.17). The nuclei of the intermediate cells were usually irregular or indented and cytoplasmic organelles included many mitochondria, a few clear vacuoles and, in some cells, a few small round, or irregularly shaped electron dense granules. In transverse section the cells appeared polygonal in outline. The most striking feature was the presence of numerous finger-like cytoplasmic projections which extended from the surfaces of the cells into the intercellular spaces. Some of these processes in contact

with adjacent cells were joined by desmosomes (Fig. 5.18).

The surface cells were basically of 2 types; agranular cells with apical microvilli and cells with large secretory granules (Fig. 5.19). The microvillous cells had either flat apical surfaces or were distinctly domed (Fig. 5.20), the latter corresponding to the distinct round bulging cells of the "cobblestone" epithelium observed with SEM. The nuclei were oval or indented and the cytoplasm contained many mitochondria and a few clear vacuoles. Only very occasionally were small, round or irregular electron dense granules found in the apical cytoplasm. Fine cytoplasmic processes from the lateral and ventral cell surfaces interdigitated with adjacent cells across the intercellular spaces. Some of these were joined with desmosomes (Fig. 5.20). At the lumenal cell surface, cells were linked by tight junctions. Numerous microvilli projected from the apical surfaces (Fig. 5.21). Most of the microvillous cells were of the type just described but there were also a few cells which differed from the majority. These cells had a smaller apical surface area and appeared wedge-shaped and narrower than the other microvillous cells. The cytoplasm was usually more electron dense and the microvilli were longer and more erect compared with the majority of the microvillous cells (Fig. 5.22).

The secretory surface cells had morphologic

features of typical mucus-secreting cells with characteristic large membrane-bound granules of varying electron density. A few microvilli were always present on the apical surfaces of the cells (Fig. 5.23). The apical portions of some of the cells bulged with secretory granules and projected beyond the surfaces of adjacent cells, while in other cells the plasma membrane had ruptured releasing the granules (Figs. 5.23, 5.24). Other cells were also observed with depressed apical surfaces and only a few residual granules remaining and probably represented discharged cells (Figs. 5.25, 5.26). The mucous cells did not appear to reach the basal lamina. The varied appearance of these mucus-secreting cells corresponded, in many respects, to the stages in mucous secretion observed with SEM in Chapter 4. Intraepithelial mononuclear cells with the morphological characteristics of lymphocytes were observed at all 4 levels (Figs. 5.15, 5.25). The indented nucleus was surrounded by a rim of cytoplasm which was more electron-lucent and contained fewer organelles than that of the surrounding epithelial cells. Cytoplasmic projections often appeared to be inserting between the latter cells.

B. Ventral Nasal Concha at Levels 1, 2, 3 and 4:

In levels 1, 2 and 3 the epithelium was similar in appearance to that of the nasal septum described above. In level 4 the basal, intermediate, surface microvillous

cells and surface mucous cells were similar to those described in the nasal septum but ciliated surface cells were also present. Due to the thickness of the epithelium it was difficult to determine whether ciliated cells extended from the surface to the basal lamina or not, although they did appear to be elongated with their lightly stained nuclei situated well below the apical surface. The cytoplasm of the apical portion of the cells contained many mitochondria, a variable number of clear vacuoles and occasional small electron dense bodies. A Golgi complex was situated above the nucleus and just below the apical surface basal bodies of cilia were clearly visible. Surface microvilli and cilia were present although the latter were sometimes sparse. Ciliated cells were observed contiguous to both microvillous cells and mucous cells and the apical borders of adjacent cells were linked by tight junctions. The lateral surfaces of ciliated cells were irregular and sometimes cytoplasmic processes extended into the intercellular spaces (Fig. 5.27).

C. Dorsal Nasal Concha at Levels 1, 2, 3 and 4:

At all 4 levels there was a mixed population of surface cells. Microvillous, ciliated and mucus-secreting cells were present (Fig. 5.28) and were similar to those described in the nasal septum and ventral nasal concha, as were basal cells, intermediate cells and

intraepithelial lymphocytes.

DISCUSSION

In Chapter 4, the SEM appearance of nasal cavity surfaces of the horse were described for the first time. It was clearly established that, regardless of age or type of animal, the nasal mucosa was nonciliated rostrally and well ciliated caudally and that between these two surfaces there was an extensive transitional zone where there was a gradual change from one type of epithelium to the other. This gradual transition from rostral nonciliated epithelium to caudal well ciliated surfaces has also been described in the mouse (Greenwood and Holland, 1972), rat (Andrews, 1974), calf (Adams, 1986), monkey (Harkema et al, 1987), pig (Adams, 1990) and man (Mygind, 1975).

The nasal cavity of the horse is of considerable length so in the present study it was clearly important to examine multiple sample sites. Clear anatomical landmarks were used to determine the levels at which the samples were taken. Obviously different types of horses have different lengths of head, thus the length of the nasal cavity must also vary. However, by using known anatomical features as landmarks it seemed reasonable to predict that the relative distances between the sample sites would be similar in the different types of horses

examined.

At each of 8 levels in the nasal study, samples were taken from 3 sites, viz., nasal septum, ventral nasal concha and dorsal nasal concha, in order to give a comprehensive SEM and histological picture of the mucosal surfaces. In addition, samples were taken for TEM from 3 sites in levels 1 - 4 in order to characterise more fully the type of epithelium present in the rostral region of the nasal cavity.

Only at the most rostral level were the surfaces of all 3 sample sites completely nonciliated. Caudally, ciliated cells appeared, albeit sparsely initially, on the dorsal nasal concha. At the level of the first cheek tooth ciliated cells were present in all surfaces, though few in number, often as single cells but sometimes in small groups. Gradually the number of ciliated cells increased, particularly in the nasal conchae. In these sites they were the predominant cell type by level 4, opposite the second cheek tooth and completely covered the surfaces by level 7, opposite the fifth cheek tooth. In the nasal septum ciliated cells were generally less in number than in the nasal conchae. Equal numbers of nonciliated and ciliated cells were present by level 5, opposite the third cheek tooth. The latter cells increased caudally but even by level 8, opposite the sixth cheek tooth, small patches of nonciliated

cells were still present on the surface of the nasal septum. A previous study in the rat has also indicated that patches of nonciliated microvillous cells among the ciliated cells was a normal feature of the epithelium of the caudal nasal cavity (Andrews, 1974).

The histological picture mirrored that of SEM. A stratified cuboidal type of nonciliated epithelium corresponding to the microvillous cells observed with SEM was present in the rostral portion of the nasal cavity. The sparse surface cilia which were noted with SEM were difficult to distinguish histologically but the patches of well ciliated cells, as they increased caudally, were easier to detect. The stratified cuboidal type of epithelium merged with pseudostratified columnar ciliated epithelium at level 4 and caudal to this the latter type became predominant.

Although there was some individual variation in the numbers of ciliated cells present in the more rostral levels, a definite pattern emerged which was more precise that that established in Chapter 4 (Figs. 5.3, 5.4).

The transitional zone of epithelium between the nasal vestibule rostrally (stratified squamous epithelium) and the caudal pseudostratified ciliated (respiratory) surfaces represented approximately 35% of the total length of the nasal cavity of the horse. This percentage was calculated using the measurements made in

the head of an adult animal of average size (Table 4.2, page 68).

Nasal cavity surfaces in other mammalian species have been the subject of several reports (Greenwood and Holland, 1972; Adams and Hotchkiss, 1983; Harkema et al, 1987; Adams, 1972, 1986, 1990) and there is general agreement that a transitional zone of epithelium exists in the rostral cavity. As most of these workers did not describe precisely the exact location of their sample sites or the extent of the transitional zone epithelium, a direct comparison with the present study in the horse could not always be made. In calves, a transitional zone representing 40% of the overall nasal cavity was reported by Adams (1986) and, although ciliated cells were first noted on the ventral nasal concha, the precise location of the first of these cells was not mentioned. In a recent report Adams (1990) described a transitional zone in the pig extending for 40% of the length of the nasal cavity but again gave no indication of the position of the first ciliated cells. In the monkey, a transitional zone has been described extending for 25% of the length of the nasal cavity, with a gradual change to the well ciliated surface of the more caudal areas; however, the location of the first appearance of ciliated cells was not clarified (Harkema et al, 1987). In general, therefore, it seems

that the extent of the transitional zone in the horse nasal cavity is less than in the calf and pig but more than in the monkey (Adams, 1986, 1990; Harkema et al, 1987).

The earliest appearance of ciliated cells in the dorsal region of the nasal cavity was observed in the mouse (Adams, 1972) and, in the present study, a similar situation exists in the horse. However, in contrast to the mouse and the horse, the ciliated cells in calves first appeared on the ventral nasal concha (Adams, 1986). Thus it would seem that there may be wide species differences in the sites at which ciliated cells first appear but the reason for this remains obscure.

The pattern of mucous cells, submucosal glands and mucosubstances was largely similar to that described in Chapter 4.

Lymphoid tissue observed in the upper respiratory tract of the horse was described in Chapter 4. Similar tissue was again noted in this part of the work and the appearance and distribution of this tissue was similar to that described by Mair et al (1987, 1988) in their studies of the immune system of the equine respiratory tract.

To date there have been no TEM studies of the nasal cavity epithelium of the horse. Several workers have, however, reported the TEM appearance in other

species such as the mouse (Matulionis and Parks, 1973), rat (Andrews, 1974; Monteiro-Riviere and Popp, 1984), dog (Okano and Sugawa, 1965), calf (Adams, 1986), monkey (Harkema et al, 1987), pig (Adams, 1990) and man, (Busuttil et al, 1977; Boysen, 1982). Most of the TEM reports of nasal cavity epithelium in the above species showed a covering of ciliated respiratory epithelium but nonciliated rostral surfaces have also been studied in the calf (Adams, 1986), monkey (Harkema et al, 1987), pig (Adams, 1990) and man (Boysen, 1982).

In the present study the stratified cuboidal nature of the epithelium of the transitional zone of the rostral nasal cavity, as observed with the light microscope, was confirmed by TEM.

Basal cells, intermediate cells and cells which reached the surface were all identified. The single row of basal cells, resting on the basal lamina, had cytoplasmic processes extending towards the lamina propria which gave the latter an irregular appearance. Processes from their other surfaces interdigitated with those of adjacent basal cells and overlying intermediate cells. These cells resembled in many respects the basal cells of the epithelium of the transitional zone in the nasal cavity of calves and pigs as described by Adams (1986, 1990). An intermediate layer, several cells thick, lay above the basal cells. Adams (1986) reported similar

intermediate cells in the calf. In the transitional zone of the monkey Harkema et al (1987) described intermediate cells which stained positively with PAS. The intermediate cells in the horse were unstained with AB-PAS. A striking feature of intermediate cells in the horse was the presence of numerous finger-like cytoplasmic processes extending from the cell surfaces into wide intercellular spaces. Many desmosomes joined adjacent cells. Similar cytoplasmic processes and desmosomes have been described in intermediate cells of the monkey (Harkema et al, 1987) and calf and, in the latter, it was suggested that the wide intercellular spaces between adjacent intermediate cells could contribute to fluid movement through the epithelium (Adams, 1986). The surface cells in the horse were basically of three types: nonciliated cells with surface microvilli, secretory cells and ciliated cells. The latter were less common than the others and, although not observed in the nasal septum, were present in the dorsal nasal concha at all levels and in the ventral nasal concha at level 4. This surface appearance with TEM corresponded to that observed with SEM where the microvillous cells appeared either flat or distinctly domed, the latter giving the characteristic "cobblestone" appearance to the epithelium. Similar microvillous surface cells have been described in the calf, pig and the monkey (Adams, 1986, 1990; Harkema

et al, 1987). It has been suggested that the nonciliated nature of the epithelium of the human nasal cavity is the result of squamous metaplasia and the microvillous surface cells were described as modified goblet cells (Boysen, 1982). This was certainly not the case in the horse, or in the calf, pig and monkey.

Another smaller microvillous cell, albeit sparsely distributed, was observed in the horse with TEM. This cell was narrower than the other microvillous cells and had a smaller apical surface. The surface microvilli were longer and appeared more erect than those of adjacent microvillous cells and the cytoplasm was distinctly more electron dense. These cells did not have microfilaments and microtubules extending into the rigid microvilli, a characteristic feature of brush cells described in the nasal epithelium of the rat (Monteiro-Riviere and Popp, 1984) and calf (Adams, 1986). These cells have not so far been described in any other species. It was difficult to ascertain whether they were a different cell type or were merely a variation of the other microvillous surface cells. Certainly with SEM the microvillous surface cells in the rostral nasal cavity of the horse showed a remarkable diversity of cell size, shape and length of microvilli.

The secretory surface cells, with prominant large membrane-bound granules of varying density, had the

characteristic morphologic features of mucus-secreting cells described in the nasal cavity of other mammalian species (Okano and Sugawa, 1965; Matulionis and Parks, 1973; Busuttil et al, 1977; Monteiro-Riviere and Popp, 1984; Adams, 1986; Harkema et al, 1987). All the secretory cells possessed a few surface microvilli, often in a peripheral position and the number of secretory granules was variable. The varied appearance of these cells suggested different stages in their secretory cycle. The apical portions of some cells were packed with granules and could sometimes be seen bulging outwards beyond the level of adjacent cells. In others, the cell membrane had ruptured releasing the granules onto the surface. Discharged cells were often depressed centrally with few granules remaining in a crater with the remnants of the cell membrane and a few microvilli still persisting at the lateral edges of the apical surface. Somewhat similar stages in mucous secretion have been described in the monkey (Harkema et al, 1987) and rat (Monteiro-Riviere and Popp, 1984). In the present study there was good correlation in the appearance of these mucus-secreting cells seen with TEM with the stages of mucous secretion described with SEM in this Chapter and in the previous Chapter 4.

With TEM ciliated cells were present in the epithelium of the dorsal and ventral nasal conchae,

particularly in the more caudal samples. They resembled the typical ciliated cells described in the nasal cavity of other species such as the dog (Okano and Sugawa, 1965), mouse (Matulionis and Parks, 1973), rat (Monteiro-Riviere and Popp, 1984), calf (Adams, 1986), monkey (Harkema et al, 1987) and man (Busuttil et al, 1977). The cells appeared elongated but, due to the thickness of the epithelium, it was difficult to determine whether or not they were anchored to the basal lamina. Adams (1986), in his study of calves, described ciliated surface cells in the transitional zone of the nasal cavity but did not state whether they extended to the basal lamina or not. However, similar ciliated cells in the monkey transitional zone appeared to extend between the surface and the basal lamina (Harkema et al, 1987).

With TEM and also with light microscopy, intraepithelial lymphocytes were found in all sample sites in the nasal cavity of the horse and this is in accordance with the work of Mair et al (1987) who described them as a usual feature in the horse. In man Busuttil et al (1977) in their TEM study, concluded that intraepithelial lymphocytes were an integral part of upper respiratory tract epithelium and since then they have also been described as a usual feature in the nasal epithelium of calves (Adams, 1986) and monkeys (Harkema et al, 1987).

The results of this systematic study of nasal cavity surfaces in 4 horses have confirmed and extended the findings of the study of the equine upper respiratory tract described in Chapter 4. Observations with SEM, TEM and light microscopy have combined to give a more complete picture of the epithelium which clothes the equine nasal cavity. It has been demonstrated that an extensive transitional zone, representing approximately 35% of the total length, exists in the rostral nasal cavity of the horse. The epithelium of this zone was of a stratified cuboidal type and was completely nonciliated in the most rostral part. Thereafter, ciliation was gradual until the caudal surfaces were clothed by well ciliated respiratory epithelium. It should be emphasised that the epithelium of the transitional zone is a normal occurrence in all ages and types of horse and is not the result of deciliation or metaplastic change due to injury to the mucosa and this must be taken into account in the study of animals with respiratory disease where alterations in the epithelial surfaces might be encountered.

CHAPTER 6

AN ULTRASTRUCTURAL AND HISTOLOGICAL STUDY OF THE EQUINE LOWER RESPIRATORY TRACT

INTRODUCTION

The problem of the coughing horse is well recognised by owners and veterinary clinicians alike. The various manifestations of respiratory tract disease in the horse and the aetiologic agents involved have been reviewed many times in the present century and range from the early report of Fitzwygram in 1903 to the recent works of Mair (1989) and Mair and Lane (1989).

Despite the obvious significance of equine respiratory disease there are very few reports in the literature of the normal histological and ultrastructural features of respiratory tract surfaces in the horse. Only recently have the surface features of the equine upper respiratory tract been described in detail (Chapters 4 and 5). There have, of course, been scattered reports of some of the structural features of the equine lower respiratory passages and lung (Gillespie and Tyler, 1967[a]; Nowell and Tyler, 1971; Tyler *et al*, 1981; Plopper, 1971, 1983; Nicholls, 1978; Plopper *et al*, 1980; Plopper *et al*, 1980[b]; Mair *et al*, 1987). However, most of these workers concentrated on the smaller respiratory passages and alveolar surfaces and no systematic study of the surface features of the whole lower respiratory tract of the horse has been carried out.

As a detailed knowledge of the normal surfaces of this area is a necessary prerequisite for an informed

assessment of changes brought about by disease processes, the present study was undertaken to characterise the surface features of the lower respiratory tract in normal horses. A systematic scanning electron microscopical (SEM) and histological study was carried out from the trachea to the alveolar membrane. In addition, transmission electron microscopy (TEM) was used in parallel in order to identify the nature of the epithelial cell types populating the smaller respiratory passages and alveoli.

MATERIALS AND METHODS

Twenty one horses of various types, sex and age and two donkeys were used. All the animals are listed in Table 4.1, page 65 with the following exceptions : in the 6 - 10 year group one horse was added, a 9 years old Hunter Gelding (PH 16), in the over 10 years group, one animal (PH 14/PHN 2) was omitted because the head only was available. The source of the animals, the method of destruction and the post-mortem procedures were carried out as described in Chapter 3.

Samples for SEM were taken from the following sites (Fig. 6.1) :

1. Dorsal trachea midway between the larynx and the tracheal bifurcation

2. Ventral trachea midway between the larynx and the tracheal bifurcation

3. Right cranial lobar bronchus

4. Right caudal lobar bronchus

5. Small bronchus from the right cranial lobe of the lung

6, 7 and 8. Lung slices from 3 different locations in the right cranial lobe.

When samples 1 - 4 had been taken, the right cranial lobar bronchus was cannulated and the right cranial lobe of the lung perfused with Karnovsky's fixative. Samples 5 - 8 were then taken. Small pieces of tissue, not more than 1 cm square and less than 2 mm thick, were immersed in Karnovsky's fixative for 24 hours then rinsed in 0.2M cacodylate buffer for 4 hours. Tissues were dehydrated through a series of acetones before drying in liquid CO_2 in a Polaron critical point dryer. The dried tissues were mounted on aluminium stubbs and coated with gold paladium in an Emscope sputter coater. Specimens were examined by means of a Philips 501B scanning electron microscope at 15 Kv and at various spot sizes from 1000 - 200.

For TEM examination of small bronchi, bronchioles, alveolar ducts and alveoli, small portions of tissue, less than 0.5 mm thick, from the perfused right cranial lobe of the lung, were immersed in Karnovsky's

fixative for 24 hours. Tissues were post fixed in osmium tetroxide and embedded in Emscope Emix resin. Ultrathin sections were stained with uranyl acetate and lead citrate and viewed with a Joel 100 CX11 transmission electron microscope.

For histological examination, the left cranial lobar bronchus was cannulated and the left cranial lobe of the lung was perfused with 10% neutral buffered formalin. Small blocks of lung tissue from the left cranial lobe, together with blocks from mid trachea, left cranial lobar bronchus and left caudal lobar bronchus (Fig. 6.1) were fixed in 10% neutral buffered formalin for 7 days, trimmed and post fixed in mercuric chloride formol for 2 days, dehydrated, cleared and impregnated with paraffin wax. Two 3 µm sections were cut from each site and routinely stained with HE and AB-PAS stains.

RESULTS

SEM Findings

Sample sites 1 and 2. Dorsal and ventral trachea:

The surfaces of these were essentially the same. Deep, regular, parallel folds were prominent (Fig. 6.2) and mucus was always present, often in sufficiently large amounts to obscure some of the surface cells. In 13 of the 23 animals the surface cells were well ciliated (Fig. 6.2) and protruding from among the cilia there were

moderate numbers of discharging mucous cells, either singly or in small groups (Figs. 6.3, 6.4). Mucous cells were more numerous in the ventral trachea. In the remaining animals a few small patches of nonciliated cells were observed. The latter had surface microvilli and distinct cell borders and a few ciliated cells were usually interspersed among them (Fig. 6.5). Five horses (PH 13, PH 16, PH 23, PH 25, PH 27) had nonciliated patches in both the dorsal and the ventral trachea while 3 animals (PH 1, PH 9, PH 32) had patches only in the dorsal trachea and 2 animals (PH 5, PH 7) in the ventral trachea.

Sample sites 3 and 4. Cranial and caudal lobar bronchi:

The lobar bronchi had regularly folded surfaces with mucus present, often in substantial amounts (Fig. 6.6). Well ciliated surface cells with numerous intervening mucous cells were present in most animals (Fig. 6.7). A few small nonciliated patches, similar to those described in the trachea (Fig. 6.5), were present in both cranial and caudal lobar bronchi in 2 horses (PH 23, PH 27). In a further animal (PH 9) nonciliated patches were present only in the cranial lobar bronchus while in another horse (PH 25) they were confined to the caudal lobar bronchus.

Sample site 5. Small bronchus:

The surface had either shallow folds or

undulations and patches of surface mucus were a common feature. Ciliated cells predominated although the numerous nonciliated cells among them gave the surface a rather "moth-eaten" appearance under low power (Fig. 6.8). The surface of the nonciliated cells varied; some were flat with sparse microvilli, others were dome-shaped with a smooth central area surrounded by a few microvilli while others bulged with mucous droplets and some cells appeared to be discharging mucus (Figs. 6.9, 6.10).

Sample sites 6, 7 and 8. Lung:

Small bronchial surfaces were similar to that described above. The pleural surface, interlobular septae, bronchioles, alveolar ducts and alveoli were also examined (Figs. 6.11, 6.12).

(a) Bronchioles: These were identified by the absence of cartilage plates in their walls. The larger bronchioles had shallow surface folds while the smaller passages were smooth (Fig. 6.12). Ciliated cells and nonciliated bronchiolar epithelial (Clara) cells were present on the surface. The number of ciliated cells decreased and their cilia became progressively more sparse as the bronchioles reached their termination. On the other hand, the nonciliated bronchiolar epithelial cells gradually became more numerous until they were the dominant cell type in the terminal bronchioles. These latter cells, which were often dome-shaped and projected

into the bronchiolar lumen, had wrinkles or clefts on their surfaces and stubby microvillous processes, most numerous at the periphery of the cells (Figs. 6.13, 6.14). Mucous secreting cells could not be distinguished in the bronchioles with SEM. The junction of the terminal bronchioles with the alveolar ducts was abrupt (Fig. 6.15) with ciliated cells or nonciliated bronchiolar epithelial cells lying adjacent to the cells of the alveolar membrane. At this level the cilia were sparse and often appeared tangled and coated with secretion, presumably surfactant (Fig. 6.16). No respiratory bronchioles were seen.

(b) Alveolar membrane: Fixation by airway perfusion simplified identification of alveolar ducts and alveoli. At the abrupt junction with the terminal bronchioles, type I and, less commonly, type II pneumocytes were found in contact with the cells of the bronchiolar epithelium. Underlying capillary loops often bulged into the alveolar space and profiles of their red blood cells could sometimes be seen through the thin alveolar membrane. Type I pneumocytes covered most of the alveolar surface which was uneven, with numerous fine wrinkles and small microvillous processes. Distinct cell boundaries were commonly seen (Figs. 6.17, 6.18). Type II pneumocytes usually occurred singly although occasionally groups of 2 or 3 cells were seen (Fig. 6.19). They stood out distinctly from type I cells, were

irregular in outline and protruded slightly into the alveolar space. Numerous stubby surface microvilli were concentrated round the periphery of each cell while the smoother protruding central zone often had small pores or depressions (Figs. 6.20, 6.21). Alveolar macrophages, although not numerous, were frequently observed in all the animals. They varied in size and shape, often had uneven or ruffled surfaces and numerous long slender filamentous processes extending from the cell borders. They were sometimes seen apparently migrating through the interalveolar pores (Figs. 6.22, 6.23, 6.24). The diversity of shapes and surface features of alveolar macrophages can be appreciated by comparing Figs. 6.19, 6.20, 6.22, 6.24 and 6.24. Interalveolar pores were an obvious feature in all the animals examined. Varying in size and shape, they were fewer in number in the younger horses where 1 - 6 pores per exposed portion of an alveolus were usual (Fig. 6.25), although they were not present in every alveolus. In animals over 6 years of age most alveoli had numerous pores in their exposed portions with as many as 20 in some instances (Fig. 6.26).

(c) Pleura: The pleural surface was uneven and wrinkled (Fig. 6.27) and often obscured by secretion. The surface cells were flat, had indistinct borders and were covered by a velvety mat of short microvillous

processes (Fig. 6.28).

Histological Findings

Sample sites 1 and 2. Dorsal and ventral trachea:

The tracheal surface was lined by a thick pseudostratified columnar ciliated epithelium (approximately 6 nuclei thick). The many mucous cells present contained either mixed or acidic mucosubstances (Figs. 6.29, 6.30). A few sero-mucous glands were present in the lamina propria, the cells of the mucous component containing mixed or acidic mucosubstances.

Sample sites 3 and 4. Cranial and caudal lobar bronchi:

The epithelium was pseudostratified columnar ciliated but was thinner than that of the trachea (up to 4 nuclei thick). Numerous mixed or acidic staining mucous cells were present. Sero-mucous glands were sparse and their weak staining reaction was mixed or acidic (Fig. 6.31).

Sample site 5. Small bronchus:

The surface was lined by low pseudostratified columnar ciliated epithelium which eventually became a simple columnar ciliated epithelium in the smaller bronchi. The many mucous cells were mixed or acidic in their staining reactions. Glands were extremely sparse and were often absent from the smaller passages, but when present were similar to those described in the lobar bronchi.

Sample sites 6, 7 and 8. Lung:

The pleura consisted of a thick fibro-elastic connective tissue covered by a single layer of squamous cells. The interlobular septae were of a similar connective tissue and divided the lung parenchyma into incomplete lobules. The appearance of the small bronchi is described above. Bronchiolar epithelium was simple columnar or cuboidal in type (Figs. 6.32, 6.33) with ciliated and nonciliated cells. The latter, which bulged into the lumen, were the most numerous cells in the terminal bronchioles. Almost all of these cells gave a negative staining reaction for mucosubstances. Only in the largest bronchioles was there an occasional cell containing mixed or acidic mucosubstances. There were no glands in the bronchiolar walls. The surface of the alveolar membrane consisted of a single layer of flattened cells and occasional alveolar macrophages were seen (Fig. 6.33).

Histologically lymphatic tissue was not an obvious feature of the lower respiratory tract but single lymphocytes and small aggregations of lymphocytes were observed in the lamina propria of all the lower respiratory passages (Fig. 6.29).

TEM Findings

(a) Small bronchi:

The presence of ciliated cells and mucus-

secreting cells, observed with SEM and the light microscope, was confirmed with TEM. The former cells had numerous surface cilia, each anchored to a dense basal body in the apical portions of the cell. Many microvilli projected between the cilia. A well developed Golgi complex was situated above the nucleus with most of the mitochondria lying between this and the apical surface. Sparse granular endoplasmic reticulum was mainly found in the lower portions of the cells. The mucus-secreting cells contained large granules of variable electron density occupying the apical portions of the cells which often bulged into the lumen. A few surface microvilli were usually present laterally. A Golgi complex was adjacent to the nucleus and granular endoplasmic reticulum was located in the basal parts of the cells (Figs. 6.34, 6.35). Both ciliated and mucous secreting cells reached the basal lamina (Fig. 6.36) and occasional small basal cells with dense cytoplasm, which did not reach the surface, were sandwiched between them.

(b) Bronchioles:

The simple epithelium of ciliated and non-ciliated cells (Fig. 6.37) rested on a thin basal lamina. The ciliated cells were similar to those described in the small bronchi. The nonciliated bronchiolar epithelial cells were columnar in shape and the rounded apical portions, studded with a few stubby microvillous

processes, usually projected into the lumen well beyond the ciliated cells (Fig. 6.38). The oval nuclei were located towards the base of the cells. The cytoplasm was filled with agranular endoplasmic reticulum and very little granular endoplasmic reticulum was found below the nucleus. Mitochondria were scattered throughout the cytoplasm and the Golgi complex was located close to the nucleus. The most obvious feature was the presence of numerous, large, round or oval electron dense granules in the apical portions of the cells and occasionally lateral to the nucleus (Figs. 6.38, 6.39).

(c) Alveolar membrane:

The type I pneumocytes had attenuated cytoplasm with occasional micropinocytotic vesicles and their surfaces possessed a few stubby microvillous processes. Cytoplasmic extensions continued round the edges of the interalveolar pores from one alveolus to another (Fig. 6.40). The type II pneumocytes projected into the alveolar space and had low surface microvilli (Fig. 6.41). An irregular nucleus, Golgi complex, granular endoplasmic reticulum and mitochondria were present but the distinguishing feature was the presence of osmiophilic lamellar inclusion bodies which could sometimes be seen extruding through the cell membrane into the alveolar space (Figs. 6.42, 6.43). Alveolar macrophages were

irregular in outline and contained numerous lysosomes and vacuoles, some of which contained osmiophilic lamellar material (Fig. 6.44). The macrophages were sometimes seen within the interalveolar pores (Fig. 6.45).

DISCUSSION

It can be seen from the literature that the normal structure of the equine respiratory tract has received relatively little attention. The first detailed ultrastructural and histological study of upper respiratory tract surfaces in the horse were described in Chapters 4 and 5. To complement this and to complete the comprehensive study of the surface features of the entire equine respiratory tract the present study of lower respiratory tract surfaces, from the trachea to the alveolar membrane, was undertaken with SEM and light microscopy. With the latter method it is not always easy to accurately define the surfaces of the smaller respiratory passages and the alveolar membrane, hence the decision to apply TEM to these areas.

In general, the features of the normal equine lower respiratory tract, detailed by light microscopy in the present study, resembled those described in other mammals (Bertalanffy, 1964; Baskerville, 1970[a]; Becci et al, 1978; Kennedy et al, 1978; Mariassy and Plopper, 1983; Tandler et al, 1983[a,b]; Wilson et al, 1984;

Leeson et al, 1985; Tyler et al, 1988; Maina, 1988; Plopper et al, 1989) and were also in accord with the limited observations of Mair et al (1987) in the horse.

In the present study, the SEM appearance of the trachea and lobar bronchi was essentially the same. A luxuriant carpet of cilia covered the surfaces and intervening mucous cells were often observed protruding from among the cilia. These findings were generally similar to those observed with SEM in the trachea and major bronchi in other species such as cattle (Mariassy et al, 1975; Iovannitti et al, 1985), dog (Wright et al, 1983; Majid, 1986), cat (Tandler et al, 1983[a,b]), non-human primates (Greenwood and Holland, 1973; Castleman et al, 1975; Mellick, 1977; Wilson et al, 1984; Plopper et al, 1989) and man (Greenwood and Holland, 1975; McDowell et al, 1978).

There appear to be no reports of tracheobronchial surfaces in newborn and very young horses and in the present study only one neonatal animal was available for examination. In this 2 days old foal the trachea and bronchi were completely ciliated and resembled the adult pattern. Greenwood and Holland (1975) examined tracheobronchial surfaces in human foetuses and premature infants and found that there was only partial ciliation in the former at 12 weeks gestation but complete ciliation in the latter by 34 weeks gestation. On the

other hand, complete ciliation of the tracheobronchial tree was not present at birth in the dog but develops in 2 days in the bronchi and in 5 days in the trachea (Wright et al, 1983). Thus it would appear that the development of ciliation in the lower respiratory passages is subject to species variation.

In the present study, a few small nonciliated patches were observed in the trachea of 10, and in the bronchi of 5, of the 23 animals examined. The presence of nonciliated patches did not appear to be related to the age or type of animal. It seemed reasonable to suppose that these patches were not normal and possibly represented the result of latent infection and similar nonciliated patches have been described by Iovannitti et al (1985) in the trachea and bronchi of adult cattle and were considered to be abnormal in that species. In contrast to the situation in horses and cattle, large nonciliated areas were considered to be a normal feature of tracheobronchial surfaces of the rat, hamster and mouse, indeed in the latter the major proportion of tracheal epithelial cells were nonciliated (Andrews, 1974; Becci et al, 1978; Pack et al, 1980).

Surface mucus was consistently observed with SEM in the trachea and bronchi of the horse but the duct orifices of underlying glands were not seen, presumably hidden by the dense ciliary carpet. In contrast to the

situation in the horse, gland duct openings were prominent in completely ciliated tracheal surfaces in the cat (Tandler et al, 1983[a,b]) and in cattle (Iovannitti et al, 1985). However, in the latter two species submucosal glands are numerous, while in the horse they are few in number (Breeze and Turk, 1984); this might well account for the different SEM appearance of the horse trachea.

Although ciliated cells were the most numerous cell type in the small bronchi of the horse, nonciliated cells progressively became more evident. A similar surface appearance of small bronchi in the horse has also been described by Nowell and Tyler (1971) and Nicholls (1978) and in cattle by Iovannitti et al (1985). In the present study the nonciliated cells varied in appearance, some were flat with microvilli covering the entire surface, some bulged from the surface with a central smooth domed area and peripheral microvilli, while others were discharging mucus. In many respects these nonciliated cells resembled the mucous secreting cells described in the trachea and bronchi of the rat (Andrews, 1974, 1979), hamster (Becci et al, 1978) and dog (Wright et al, 1983; Majid, 1986). The presence of many mucous cells among the ciliated cells in the small bronchi was confirmed histologically and with TEM.

Brush cells have been described in TEM studies

in the trachea of rats, but occurred infrequently (Rhodin and Dalhamn, 1956; Jeffery and Reid, 1975). These cells were also observed in the tracheobronchial epithelium of some other species such as the mouse (Hama and Nagata, 1970), guinea pig (Inoue and Hogg, 1974), pig (Baskerville, 1970[a]) and calf (Allan, 1978). However, in the present study no brush cells were observed, either with SEM or TEM, in the epithelium of the lower airways of the horse.

In the bronchioles, ciliated cells predominated in the larger passages but gradually became less numerous towards their terminations and the cells possessed fewer cilia. Conversely, the nonciliated bronchiolar epithelial (Clara) cells increased in number towards the smaller passages and became the predominant cell type in the terminal bronchioles. This is in accord with the work of Plopper (1983) who stated that the Clara cells formed between 55% and 75% of the bronchiolar epithelial population in the horse. The junction between the terminal bronchioles and the alveolar ducts was abrupt. Both ciliated cells and Clara cells were seen to be contiguous with the cells of the alveolar membrane at the junction. A similar situation exists in some laboratory rodents (Tyler, 1983) and cattle (Iovannitti et al, 1985). In contrast, Clara cells only extend to the alveolar membrane in the dog (Wright et al, 1983;

Majid, 1986) and in some species of monkey (Castleman et al, 1975; Plopper et al, 1989). Mucus secreting cells were described in the terminal bronchioles of the Rhesus monkey by several workers (Castleman et al, 1975; Mellick et al, 1977; Tyler et al, 1988; Plopper et al, 1989). However, in the present study in the horse, only in the largest bronchioles were a few cells containing mucosubstances demonstrated histologically and none were identified with SEM or TEM. This is in agreement with the work of Nicholls (1978) who found few, if any, mucous cells in horse bronchioles. The presence of respiratory bronchioles in the horse, albeit poorly developed (Nowell and Tyler, 1971; Tyler et al, 1971) was not confirmed in the present study.

Nonciliated bronchiolar epithelial cells or Clara cells have been found in the bronchioles of all mammals (Plopper, 1983). Only in some rodents and the rabbit have they also been found in the proximal airways (Hansell and Moretti, 1969; Jeffery and Reid, 1975; Pack et al, 1980; Plopper, 1983). In the present study the Clara cells characteristically projected into the bronchiolar lumen and had numerous fine wrinkles or clefts on their uneven apical surfaces. These cells were similar to bronchiolar Clara cells observed with SEM in other species such as the hamster (Nowell and Tyler, 1971), rat (Ebert and Terracio, 1975[b]), cattle

(Mariassy et al, 1975; Iovannitti et al, 1985) and dog (Wright et al, 1983; Majid, 1986). There is no doubt, however, that there are distinct species variations in the morphology of Clara cells when viewed with TEM and those of the horse have been compared with those of the ox, sheep, dog and cat (Plopper et al, 1980[b]). In the present study the Clara cells were similar to those described by Plopper (1983) in the horse which, in common with laboratory animals, sheep and pig were distinguished by the presence of abundant agranular endoplasmic reticulum and numerous dense cytoplasmic granules. In contrast, the Clara cells of the ox, cat and dog have a quite different appearance with cytoplasmic glycogen the predominant feature and few, if any, dense granules (Plopper, 1983; Majid, 1986). Thus it is interesting to note that although the Clara cells of the horse appeared very like those of cattle and the dog when viewed with SEM (Iovannitti et al, 1986; Wright et al, 1983; Majid, 1986), the TEM appearance was quite different (Plopper, 1983; Majid, 1986).

The secretory nature of Clara cells was proposed by Ebert and Terracio (1975[b]) in their SEM study of the rat and secretory granules were observed with TEM in the mouse (Stinson and Loosli, 1978). The fluid lining layer of the bronchioles is continuous with that of the bronchi proximally and the alveolar

membrane distally and it is now generally agreed that Clara cells are the source of secretory material for at least part of the bronchiolar fluid layer (Widdicombe and Pack, 1982; Plopper, 1983). As a result of studies in the rabbit, Boyd (1977) and Serabjit-Singh et al (1980) have suggested that the Clara cell is involved in metabolism of noxious compounds via the cytochrome P450 monooxygenase system and it is now generally accepted that Clara cells are involved in detoxification processes (Plopper, 1983). The Clara cell is also thought to be the progenitor cell of the bronchiolar epithelium (Evans et al, 1978; Plopper, 1983). Further study, however, is necessary to fully elucidate the nature and function of Clara cells in health and disease and they remain "the mystery cells of the lung" (Widdicombe and Pack, 1982; Plopper, 1983).

The cells of the alveolar membrane were readily studied and easily identified by SEM. The type I pneumocytes had uneven surfaces with many wrinkles and small microvillous processes and junctions between adjacent cells were distinct. Type II pneumocytes protruded into the alveolar space, had numerous peripheral microvilli and smooth central areas which often possessed small depressions or pores. With TEM the attenuated cytoplasm, with few organelles, of the type I pneumocytes was easily distinguished from the cuboidal type II cells with their prominent nuclei,

mitochondria and characteristic osmiophilic lamellar inclusions, the latter occasionally seen extruding through the apical cell membrane. The ultrastructural appearance of both type I and type II pneumocytes in the present study was similar to earlier descriptions of these cells in the horse (Gillespie and Tyler, 1967[a]; Nowell and Tyler, 1971; Tyler et al, 1971) as well as in many other species such as cattle (Mariassy et al, 1975; Iovannitti et al, 1985), goat (Atwal, 1988), pig (Baskerville, 1970[b]), dog (Majid, 1986), hamster (Nowell and Tyler, 1971; Kennedy et al, 1978), rat (Andrews, 1979; Scheuermann et al, 1988), mouse (Zitnik et al, 1978), guinea pig (Davis et al, 1984), non-human primates (Castleman et al, 1975; Maina, 1987, 1988) and man (Burri, 1985). So it would appear that, across the species, the morphology of the type I and type II pneumocytes is remarkably similar. An alveolar type III cell or alveolar brush cell has been described in the rat (Meyrick and Reid, 1968; Hijiya, 1978[a,b]; Dormans, 1985) and it was suggested by Nowell and Tyler (1971) that an alveolar brush cell was also present in the horse but their evidence, as indicated in photomicrographs, was unconvincing. Cells resembling the alveolar brush cells of the rat were not observed in the horse in the present study.

The type I pneumocytes cover by far the greatest

area of the alveolar membrane accounting for 92-97% of the total surface area, while the type II pneumocytes make up the remainder (Crapo et al, 1983; Burri, 1985). The former cells are highly specialized and are probably unable to undergo cell division while the latter cells produce, store and secrete the pulmonary surfactant and, according to the present state of knowledge, represent the stem cell population of the alveolar epithelium (Burri, 1985).

SEM demonstrated the presence of alveolar macrophages in the lungs of horses in all the age groups. Although not numerous these large distinctive cells were easily seen adhering to the surface of the alveolar membrane. Their cell surfaces were usually uneven or ruffled, long filamentous cytoplasmic processes extended peripherally and the cells were frequently observed in the process of migrating through alveolar pores. Their presence in the alveoli was confirmed by TEM. The ultrastructural features of alveolar macrophages have been reported in the horse (Nowell and Tyler, 1971; Tyler et al, 1971) and in several other species such as the mouse (Curry et al, 1969), hamster (Nowell and Tyler, 1971), rat (Scheuermann et al, 1988), non-human primates (Greenwood and Holland, 1973; Maina, 1987, 1988) and cattle (Mariassy et al, 1975; Iovannitti et al, 1985). Some authors commented on the rarity of

these cells (Greenwood and Holland, 1973; Mariassy et al, 1975) and they were only observed with TEM in the dog and not with SEM in spite of diligent searching (Majid, 1986). The current accepted view is that alveolar macrophages develop by two clearly defined mechanisms: either by the differentiation of monocytes which migrate into the alveolar space or by the division of existing alveolar macrophages (Furth, 1985; Evans and Shami, 1989).

Interalveolar pores are a feature of mammalian lungs in general and have been described in a number of species including the horse. Nowell and Tyler (1971) and Tyler et al (1971), in their SEM and TEM studies, found many pores in the equine lung, unlike the findings of Nicholls (1978) who could demonstrate few pores in the lungs of normal horses. In the present study, there was no difficulty in detecting alveolar pores with SEM and TEM in all the horses examined. Although Mariassy et al (1975) reported that alveolar pores were rarely found in cattle, they were readily observed in that species by Iovannitti et al (1985) in a SEM study of adults and 1 week old calves. Alveolar pores have been recorded in ultrastructural studies of a number of other species including the hamster (Nowell and Tyler, 1971), rat (Andrews, 1979; Scheuermann et al, 1988), dog (Parra et al, 1978; Majid, 1986; Gillett et al, 1989) non-human

primates (Greenwood and Holland, 1973; Shimura et al, 1986; Maina, 1987, 1988) and man (Takaro et al, 1979, 1985; Burri, 1985). It is generally agreed that in life the alveolar pores are bridged by the pulmonary surfactant (Parra et al, 1977; Burri, 1985; Gillett et al, 1989) and it has been suggested that fixation by airway perfusion provides the best demonstration of the pores (Parra et al, 1977). By this method the latter workers counted an average of 19 pores per exposed alveolar surface in dogs but did not give any indication of the age of the animals examined. In the present study in the horse the lungs were also fixed by airway perfusion and the alveolar pores, although present in all the animals, were fewer in number in animals under 6 years of age but increased in older animals with up to 20 per exposed portion of an alveolus being counted in some instances. It has also been recorded in the monkey and the dog that the number of alveolar pores increased with age (Shimura et al, 1986; Gillett et al, 1989). The histogenesis and function of alveolar pores is still poorly understood but currently it is thought that they probably do not have a role in collateral ventilation in normal lung but could be of key importance in lung pathology (Desplechain et al, 1983; Gillett et al, 1989). Alveolar membrane senescence may contribute to the increase in the number

of pores in older animals (Gillett et al, 1989).

The airway mucociliary system is a most important protective mechanism in defence of the lower respiratory system against harmful inhaled particles and the mucous component of the system is secreted by the submucosal glands as well as by cells in the surface epithelium of the tracheobronchial tree (Phipps, 1981). Histochemical analysis has shown that there is some species variation in the composition of the mucosubstances (Breeze et al, 1976) but there have been very few reports of mucous secretion in the tracheobronchial tree of the horse. Goco et al (1963) compared the tracheobronchial mucosa of some laboratory and domestic animals, including the horse, and observed that the latter possessed glands which were unstained with PAS but because of autolysis the presence of surface mucous cells could not be determined. Recently the presence of epithelial mucous cells and muco-serous glands in the equine lower respiratory tract have been briefly mentioned in a histological study (Mair et al, 1987). In a more comprehensive analysis of the histochemistry of mucous cells in the equine tracheobronchial tree, Nicholls (1978) defined neutral and acidic mucosubstances and further categorized the latter into sulphomucins and sialomucins. She found that, although the cells of the horse contained mostly acidic sulphomucins, with neutral mucosubstances

confined to isolated groups of cells, some cells contained a mixture of both acidic and neutral types. Similar histochemical studies have shown that acidic rather than neutral mucosubstances were also more prevalent in the dog (Wheeldon et al, 1976; Spicer et al, 1983), ox (Allan et al, 1977) and monkey (St. George et al, 1984; Plopper et al, 1989). In the present study an assessment of the distribution of neutral and acidic mucosubstances was carried out without further classification of the latter component. Numerous mucous secreting cells were observed in the tracheobronchial epithelium and muco-serous mucosal glands were present but not in large numbers. Epithelial mucous cells were rare in the bronchioles and glands were never found. The distribution of mucus secreting elements in the mucosa of the equine lower respiratory tract in the present study and the histochemistry of the mucosubstances was, in general, similar to that described by Nicholls (1978) and Mair et al (1987).

The distribution and histological features of lymphoid tissue in the equine respiratory tract have been recently reported by Mair et al (1987, 1988) and histological and ultrastructural features in this tissue in the upper respiratory tract of the horse have been described in Chapters 4 and 5. In the present study of the lower respiratory tract the presence of underlying

lymphoid tissue was not detected by SEM nor was it a particularly obvious histological feature. However, isolated lymphocytes and small aggregations of lymphocytes were noted in the lamina propria of the respiratory passages at all levels and these observations were similar to those of Mair et al (1987).

In this systematic study of a large group of normal horses and donkeys, the surface features of the equine lower respiratory tract have been characterised by SEM, TEM and light microscopy. There appeared to be no obvious differences in animals of various types or age and the findings generally conformed to the cellular patterns described in many other mammalian species. In addition, this study has expanded the information previously recorded in the horse by earlier workers and thus provides a useful basis for future investigations in horses suffering from respiratory disease.

CHAPTER 7

AN ULTRASTRUCTURAL AND HISTOLOGICAL STUDY OF *STREPTOCOCCUS EQUI* INFECTION OR STRANGLES IN HORSES

INTRODUCTION

Strangles is an acute contagious disease of horses characterised by inflammation of the upper respiratory tract and lymphadenitis of regional lymph nodes. The causal organism, Streptococcus equi, only affects Equidae and is a beta-haemolytic, gram positive coccus belonging to Lancefield's Group C. The disease is worldwide in distribution and affects all types and ages of horses but young animals are particularly susceptible. Morbidity can be as high as 100% in susceptible populations but mortality is generally low in uncomplicated cases (Todd, 1910; Bazeley and Battle, 1940; Mahaffey, 1962; Wilson and Salt, 1978; Clabough, 1987; Yelle, 1987; Wilson, 1988).

Strangles is one of the first equine diseases to be described in the veterinary literature. As early as the 5th century AD, Vegetius, an adviser to the Roman cavalry, recorded the epizootic character and contagious nature of the disease and recognised that buildings, pasture and water supply used by affected horses were a source of contagion and must be closed to animals free from the disease (Smith, 1919). Solleysel in 1664 described Strangles as a disease which affected most young horses at some stage and he believed that it was commonly spread by drinking containers which had been previously used by affected animals. Over 100 years

later, its contagious nature was demonstrated experimentally by Lafosse (1790) and this was confirmed a few years after by Viborg in 1802 (Todd, 1910). Although Rivolta (1873) observed chains of cocci in pus from an affected horse, Schutz (1888) was the first to isolate and describe in detail the causal streptococcus. In a comprehensive review, Todd (1910) described the epidemiology, clinical signs and pathogenesis of the disease and outlined early attempts to induce immunity by inoculation of dead and living cultures and immune serum. He found that the former methods were ineffective but the latter afforded some degree of protection and aided recovery of sick horses. It was not until 30 years later that Bazeley (1940) successfully produced an experimental vaccine from young cultures of capsulated Streptococcus equi and rightly concluded that the immuno-antigen was associated with the capsule. The first commercial vaccine became available in 1969 and, although many more have been produced since, none have been completely effective (Clabough, 1987). Indeed, epizootic outbreaks of Strangles have occurred in groups of vaccinated horses (Timoney and Eggers, 1985). Recently, it has been proposed that locally produced antibodies could be important in Streptococcus equi infections and that intra-nasal inoculation might be the most effective method of administering a vaccine

(Timoney, 1988[b]).

In spite of widespread use of vaccines, Strangles is still one of the most common infectious diseases of horses and, although rarely fatal, it is of great economic importance to the horse industry because of the prolonged course and recovery period and possible serious complications (Yelle, 1987; Timoney, 1988[a]). While there have been many reports and reviews of the epidemiological, clinical and immunological aspects of Strangles, there have been very few descriptions of necropsy findings. The gross pathological and histological findings in 20 experimentally infected ponies were briefly recorded by Knight et al (1975), while Reif et al (1981) and Jubb et al (1985) have given general descriptions of the post-mortem features of the disease, excluding the histopathology, and these reports concentrated on the pathology of the pharynx and drainage lymph nodes. However, up to the present time there have been no reports of the ultrastructural features of the recorded pharyngeal lesions nor indeed of the respiratory tract as a whole of horses affected with Strangles. It would appear, therefore, that there is an obvious gap in the knowledge of the disease. Thus it seemed appropriate to carry out a detailed ultrastructural and histological study of the respiratory tracts of affected horses in order to establish the presence, or otherwise,

of any changes in the surface mucosa of the pharynx and elsewhere in the respiratory tract brought about by infection with Streptococcus equi.

MATERIALS AND METHODS

(1) Natural History of a Spontaneous Outbreak of Strangles

In 1988 an opportunity arose to study an outbreak of Strangles in a group of 19 recently weaned, 4 - 5 months old female Dartmoor X ponies which had been transported from the South West of England to the West of Scotland, where they were held in a dealer's yard. Two weeks after arrival they were transferred to Glasgow University Veterinary School and Strangles was diagnosed in a pony and within 5 days of the initial case 15 ponies also developed the disease. Three animals remained unaffected.

The diagnosis of Strangles was based on the following clinical signs which were usually of sudden onset:-

1. Dull demeanour and loss of appetite.
2. Fever (temperature 101-105^{o}F).
3. Bilateral nasal discharge, initially of a serous nature but subsequently mucopurulent.
4. Progressive enlargement of lymph nodes in the head region with associated swelling and pain, particularly noticeable in the mandibular group of nodes.

Other notable features of the disease were conjunctivitis with a mucopurulent ocular discharge, difficulty in swallowing and respiratory stertor. As the disease progressed, abscesses developing in the lymph nodes, ruptured and sinuses formed discharging thick creamy-yellow pus to the exterior, especially in the intermandibular region. Haematology showed that there was a marked leucocytosis with a neutrophilia (total white cell count $\geq (15^{x10^9}/l)$; neutrophils $\geq (5^{x10^9}/l)$). Diagnosis was confirmed by cultures of Streptococcus equi obtained from nasal swabs. Daily clinical examination of each affected animal was carried out during the course of the illness until recovery was complete, usually 17 - 21 days after the onset of clinical signs. Blood samples for routine haematology and serological examination were taken during the course of the disease and during the recovery period. Nasal swabs and swabs from discharging sinuses were also taken for routine bacteriological examination. The disease was allowed to run its course and, although no antibiotic therapy was administered, all the affected ponies were carefully monitored in order to ensure that there was no unnecessary suffering.

Ten of the affected ponies (PH 55 - PH 60, PH 62, PH 63, PH 65, PH 66) were available for a systematic SEM and histological study of both upper and

lower respiratory tracts. In addition TEM was carried out on the lung. The ponies were divided into 4 groups, each of which represented a stage in the development or recovery of the disease (Table 7.1, page 182).

A further 2 ponies were included in the study, making a total of 12 ponies. The latter 2 animals were a 5 year old female Fell X (PH 34) and a 15 year old female Shetland X (PH 42), which were obtained from different sources, and shortly after admittance to Glasgow University Veterinary School developed typical clinical signs of Strangles. Streptococcus equi was cultured from nasal swabs and from discharging abscesses. These 2 ponies were killed when the disease was fully developed; PH 34 on the 8th day after admittance and PH 42 on the 12th day.

(2) Methods of Investigation

The method of destruction and post-mortem procedures carried out are described in Chapter 3. In addition, samples were taken for routine bacteriological examination; swabs from the nasal cavity, nasopharynx, guttural pouches, trachea and lobar bronchi and pieces of tissue from lung and mandibular and retropharyngeal lymph nodes. In addition, swabs were taken from the soft palate of ponies in Group 4 and from the nostrils of PH 34.

Sample sites for SEM, histology and TEM

were the same as those used in the upper and lower respiratory tract studies of normal horses and are described in Chapter 4 (Figs. 4.1, 4.2) and Chapter 6 (Fig. 6.1). Additional samples for SEM and histology were taken from the nasal and oral surfaces of the soft palate (see Fig. 2.7) in the midline, midway between its rostral and caudal borders, from animals in Groups 2 and 4 (Table 7.1, page 182). Airway perfusion of the lungs was carried out as described in Chapter 6, all tissues for SEM and TEM were immersed in Karnovsky's fixative and tissues for histology were fixed in 10% neutral buffered formalin. Subsequent methods of processing, staining and examination were performed as described in Chapter 3. In addition, the Gram-Engbaek technique was used for examination of surface bacteria in paraffin sections of the soft palate (Engbaek et al, 1979).

(3) Samples for Serology

Serum samples were sent to the Department of Infectious Diseases, Virology Section, Animal Health Trust, Newmarket, Suffolk, for routine examination for the presence of antibodies to a standard group of viruses:

i. Influenza A/Equi 1/Prague 56

Influenza A/Equi 2/Miami 63

Influenza A/Equi 2/Recent field isolate

ii Rhinopneumonitis virus EHV-1, Abortion strain and Respiratory strain

iii Equine rhinovirus ERV-1, ERV-2

iv Adenovirus.

At the request of the laboratory, samples collected on days 1, 11, 17, 24 and 40 of the Strangles outbreak were sent for examination. The ponies in the present study which were sampled on each of these days are listed below :-

Day 1 All the ponies in Groups 1 - 4

Day 11 3 ponies in Group 2 (day they were killed)

Day 17 2 ponies in Group 3 and 2 ponies in Group 4

Day 24 2 ponies in Group 3 and 2 ponies in Group 4

Day 40 2 ponies in Group 3 (day they were killed).

RESULTS

(1) Post-mortem findings:

All the ponies had some evidence of disease in the upper respiratory tract. The most striking and consistent feature was enlargement of lymph nodes in the head, particularly the mandibular and retropharyngeal groups of nodes (Fig. 7.1). Two animals in Groups 1 and 2, PH 34 and PH 42, had suppurative lymphadenitis. The abscesses were small ($\leq$ 1 cm) in the early stages of the disease, larger (1 - 4 cm) in the later stages with thick creamy yellow pus and considerable oedema had

developed in the surrounding tissues (Figs. 7.2, 7.3). In one animal (PH 42) abscesses in the mandibular lymph nodes had ruptured and formed discharging sinuses in the intermandibular region and on the right side a retro-pharyngeal abscess had ruptured into the guttural pouch. Other features noted included increased amounts of surface mucus and lymphoid hyperplasia in the nasopharynx, granular appearance of the mucosa of the guttural pouches with the presence of purulent exudate (Fig. 7.4) or plugs of inspissated pus and serous or mucopurulent discharge at the nostrils and eyes. The lower respiratory passages appeared free from gross abnormalities although, in 2 animals (PH 34 and PH 66), there was a small amount of mucopus in the trachea. The lungs appeared normal in all the ponies and there was no evidence of spread of infection to other organs in the body. The post-mortem findings are summarised in Table 7.2, page 183).

(2) Bacteriological findings:

Streptococcus equi and Streptococcus zooepidemicus were cultured from nasopharyngeal swabs taken during the course of the disease and after recovery. The former organism predominated in the early stages of the disease while the latter was the common organism in the later stages and in recovered cases. Streptococcus equi was cultured from swabs and lymph nodes taken after death from animals in Group 1 and Group 2 (except PH 58) and from the

adult ponies PH 34 and PH 42. Streptococcus zooepidemicus was cultured from post-mortem samples from animals in Groups 3 and 4. Streptococci were not isolated from the bronchi and lung samples from any of the animals and only the pony (PH 34) had a positive swab culture of Streptococcus equi from the trachea. The same pony had additional swabs taken from the nostrils and Streptococcus equi was cultured and Gram negative rods. The latter were not considered to be of significance and were not investigated further. The isolation of streptococci from post-mortem samples is summarised in Table 7.3, page 185).

(3) Serology findings:

None of the ponies had antibodies to influenza viruses or adenovirus and there was no evidence of active Equine rhinoviruses in any pony. Five ponies (PH 56, PH 57, PH 62, PH 63, PH 65) also had no antibodies to Rhinopneumonitis virus EHV-1. Four of the remaining ponies (PH 55, PH 58, PH 59, PH 64) had titres of $\frac{1}{40}$ to EHV-1 on Day 1 but did not show rising titres on subsequent testing. Only one pony (PH 60) showed seroconversion to EHV-1 with a rising titre of $\frac{1}{40}$ - $\frac{1}{160}$ from Day 1 to Day 11, suggesting that it had an active virus infection.

(4) SEM findings:

The SEM appearance of the respiratory tract surfaces will be described for each group of ponies

(Table 7.1, page 182) and for the 2 adult ponies together as a group, as within the groups of animals the SEM findings were generally similar.

1. Group 1 : The surface appearance of the nasal cavity and nasopharyngeal sites was, in most respects, similar to that described in the normal horse in Chapter 4. In one animal (PH 57) in the dorsal nasal concha, there was a large area (about $\frac{1}{3}$ of the total) of nonciliated microvillous cells while the remaining surface was well ciliated with intervening mucous cells protruding from among the cilia. Surface bacteria were observed in 2 animals (PH 55 and PH 56) without apparent damage to the underlying cells. In the nasal septum of PH 56, small groups of cocci were found adhering to the surface of the microvillous cells (Fig. 7.5). These bacteria appeared as single cells or diplococci and had distinctly rough or "fuzzy" surfaces from which fine strands projected connecting the bacteria to one another and to the surface of the microvillous cells (Fig. 7.6). In PH 55, in the basal fold of the ventral nasal concha, groups of rod-shaped bacteria with fine surface filaments appeared to adhere to the squamous cells (Fig. 7.6) particularly in the narrow hairless zone which lies between the hairy skin of the nasal vestibule and the nasal mucosa proper (as described in Chapter 4).

In the guttural pouch large amounts of surface mucus were present and most of the surface cells were well

ciliated. However, there were small scattered patches of microvillous cells (Fig. 7.7) and poorly ciliated cells were often present at the periphery of the patches.

Surface features of the epiglottis were similar to those of normal horses described in Chapter 4. In the ventral larynx and trachea the surface was mostly covered by well ciliated cells interspersed with mucus-secreting cells, the latter most numerous in the ventral trachea. However, many small patches of nonciliated microvillous cells were present in both larynx and trachea similar to those seen in the guttural pouch (see Fig. 7.7).

The surface appearance of bronchi, bronchioles and alveolar membrane resembled that described in normal horses in Chapter 6.

2. Group 2 : The appearance of surfaces in the nasal cavity and nasopharynx generally resembled that of normal horses. On the basal fold of the ventral nasal concha in 2 animals (PH 58 and PH 59) many rod-shaped bacteria, similar to those described above in Group 1, were present on the squamous cells. In the medial edge of the pharyngeal opening of the auditory tube of one animal (PH 59) there were several circumscribed raised areas (Fig. 7.8) covered by microvillous cells, which probably represented underlying lymphatic tissue, and were similar to lymphatic nodules found in the guttutal pouch of a normal horse described in Chapter 4 (see Figs. 4.29, 4.30).

In the guttural pouches, large amounts of surface mucus had trapped many inflammatory cells, most of which were degenerate (Fig. 7.9) and difficult to identify, but were probably neutrophils and lymphocytes. There was considerable loss of the normal well ciliated appearance and much of the surface was covered by microvillous cells and scattered ciliated cells with short sparse cilia (Fig. 7.10).

The nasal surface of the soft palate was deeply folded and coated with large amounts of mucus. The surface cells were similar to those of the normal nasopharynx described in Chapter 4. In one animal (PH 59) smooth, raised circumscribed areas were present which were similar to those described above in the medial auditory tube opening of the same animal. Only a small quantity of mucus was present on the oral surface of the soft palate. The squamous surface cells had distinct boundaries and were covered with microplicae or stubby microvilli. Many groups of cocci, either single cells or diplococci, were observed adhering to the squamous cells (Fig. 7.11). These bacteria had a smoother outline (Fig. 7.12) than those described on the nasal septum in Group 1 but they also had slender filaments extending from their surfaces to attach to the surface of the squamous cells.

The epiglottis appeared normal.

The ventral larynx and trachea had similar surface appearances to those described above in Group 1, i.e., ciliated cells and patches of microvillous cells. However, in Group 2 the lobar bronchi also had many small patches of microvillous cells (Fig. 7.13) and in the small bronchus of one pony (PH 59), there were similar nonciliated patches (Fig. 7.14). The surfaces of small bronchi of the remaining ponies in Group 2 were normal and the surface appearance of bronchioles and alveolar membrane in all the ponies was similar to that described in normal horses in Chapter 6.

3. Group 3 : The nasal cavity and nasopharyngeal surfaces were similar to those in normal horses except that many rod-shaped bacteria were observed adhering to the squamous cells in the basal fold of the ventral nasal concha (see Fig. 7.6) in both animals in this group (PH 62 and PH 63).

In the guttural pouch well ciliated cells with intervening mucus-secreting cells covered the surface in one pony (PH 63), while in the other pony (PH 62) the normal appearance was lost and the surface was mainly covered by microvillous cells among which were poorly ciliated cells (see Fig. 7.10).

The surfaces of the larynx and trachea were similar to those described above in Group 2.

The appearance of the bronchi, bronchioles and

alveolar membrane resembled that of normal horses, as described in Chapter 6.

4. Group 4 : The surfaces of the rostral nasal cavity appeared normal. There was widespread loss of ciliation in the caudal nasal cavity (Fig. 7.15), i.e., the dorsal nasal concha and conchofrontal sinus. These surfaces were covered by microvillous cells, cells with sparse, stunted cilia or even a single cilium or cilia which adhered to each other (Fig. 7.16). The guttural pouches had a similar appearance.

An obvious surface feature in the nasopharynx and pharyngeal openings of the auditory tubes were many smooth, raised circumscribed areas (Fig. 7.17) similar to those described in one pony (PH 59) in Group 2. Microvillous cells were the predominant type in the centre of these areas; elsewhere a mixture of ciliated and microvillous cells covered the surface.

The nasal and oral surfaces of the soft palate resembled those described above in Group 2, but fewer groups of cocci were observed adhering to the squamous cells of the oral surface.

The surface of the epiglottis appeared normal. In the ventral larynx and trachea most of the ciliated surface cells were replaced by a mixture of microvillous cells and poorly ciliated cells, these changes being most marked in the ventral larynx.

Well ciliated surface cells with many intervening mucous cells covered most of the surfaces of the lobar and small bronchi but a few small patches of microvillous cells were observed (see Figs. 7.13, 7.14). The bronchioles and alveolar membranes were normal in appearance.

5. Adult Ponies (PH 34, PH 42)

Large amounts of mucus were present in the nasal cavity and nasopharynx but the surface cells in all sites were similar to those of normal horses and are described in Chapter 4. In one animal (PH 34) there were a few small groups of cocci adhering to the microvillous cells in the nasal septum. These bacteria had a "fuzzy" surface appearance similar to those described in one animal in Group 1 (PH 56).

The guttural pouches also had large quantities of surface mucus which had trapped cell debris and neutrophils. The normal well ciliated surface was interrupted by patches of microvillous cells and poorly ciliated cells (see Fig. 7.10). Surfaces in the ventral larynx and trachea were similar to that of the guttural pouches. These changes in the surface appearance were more marked in pony No. PH 34.

The surfaces of the epiglottis, bronchi, bronchioles and alveolar membranes were normal.

(5) Histological findings:

1. Group 1 (PH 55, PH 56, PH 57)

Excluding the stratified squamous epithelium of the nasal vestibule, the epithelium of the nasal cavity was of a stratified cuboidal type rostrally and pseudo-stratified columnar ciliated caudally and both these types of epithelium were present on nasopharyngeal surfaces. Submucosal glands were present and were most numerous in the rostral nasal cavity and nasopharynx; they were of a serous nature rostrally and became sero-mucous in type caudally. With the exception of the extreme rostral portion of the nasal cavity, surface mucous cells were present at all sites in the nasal cavity and nasopharynx. The mucosubstance in both surface mucous cells and glands was predominantly acidic or mixed in its staining reaction. These histological features were similar to those of normal horses and are described comprehensively in Chapter 4. However, a noticeable difference in the nasal cavity and nasopharynx of animals affected with Strangles was a cellular infiltration of the subepithelial connective tissue; lymphocytes predominated but plasma cells and neutrophils were also present (Fig. 7.18). Many cells were found to be migrating through the epithelium. Aggregations of lymphocytes with follicle formation were observed, particularly in the nasopharynx (Fig. 7.19).

Pseudostratified columnar ciliated epithelium

covered the guttural pouches but the generally well ciliated surface was interrupted by small gaps where the cilia were absent. Many lymphocytes and neutrophils were seen passing through the epithelium. There was marked lymphoid hyperplasia in the lamina propria and cellular infiltration (Fig. 7.20) including neutrophils, plasma cells and macrophages. In one animal (PH 55) microabscesses had developed and lymphoid follicles had formed germinal centres.

Apart from a mild cellular infiltration (lymphocytes, neutrophils and plasma cells) of the lamina propria of the larynx, trachea and lobar bronchi, the histological features of the lower respiratory passages and lungs were similar to those of normal horses as described in Chapters 4 and 6.

2. Group 2 (PH 58, PH 59, PH 60)

The histological features of the upper respiratory tract sites were similar to those described above in the animals in Group 1. In the guttural pouch of the animal (PH 58) there was oedema of the subepithelial connective tissue with development of microabscesses.

The histological appearance of the nasal surface of the soft palate resembled that of the nasopharynx. The oral surface was covered by a thick stratified squamous epithelium (up to 8 nuclei deep) and many acidic staining mucous glands were present in the subepithelial connective

tissue. Follicular lymphoid hyperplasia, with many germinal centres, was an obvious feature (Fig. 7.21). Many groups of Gram positive cocci were seen on the epithelial surface (Fig. 7.22), which confirmed the observations with SEM.

The lower respiratory passages and the lung were normal.

3. Group 3 (PH 62, PH 63)

The histology of the nasal cavity, larynx and lower respiratory tract was similar to that of normal horses as described in Chapters 4 and 6. While the epithelium and glands of the nasopharyngeal sites and the guttural pouches were normal there was a distinct cellular infiltration in the lamina propria, mainly of lymphocytes with fewer numbers of plasma cells and neutrophils. Follicular lymphoid hyperplasia accompanied by the formation of germinal centres was a noticeable feature, particularly in the nasopharynx (Fig. 7.23).

4. Group 4 (PH 65, PH 66)

The histological features of the upper respiratory tract were similar to those described in horses in Group 3. The histological features of the soft palate were similar to those described in Group 2, except that no bacteria were observed on the oral surface.

The respiratory epithelium of the ventral larynx, trachea and major bronchi had a patchy loss of

surface cilia and mild cellular infiltration of lymphocytes, plasma cells and neutrophils was present in the lamina propria. The lungs were normal.

5. Adult Ponies (PH 34, PH 42)

The nasal cavity was similar to that of normal horses described in Chapter 4. Subepithelial lymphoid hyperplasia, including germinal centres, was present in the nasopharynx. There was patchy loss of cilia from the surface of the guttural pouches and an increased number of lymphocytes, plasma cells and neutrophils in the lamina propria. The histological features of the larynx and lower respiratory tract were normal.

(6) TEM findings:

The TEM appearance of small bronchi, bronchioles and alveolar membranes was similar to that of normal horses as described in Chapter 6.

DISCUSSION

A review of the literature has clearly shown that Streptococcus equi infection, or Strangles, is a common and important disease of horses, recognised for centuries and yet there have been surprisingly few reports of the necropsy findings or, indeed, the histopathological lesions of the disease; even more surprisingly there appear to be no descriptions of the ultrastructural features of respiratory tract surfaces of horses affected

with Strangles.

The present ultrastructural and histological study was undertaken to investigate the effects of Streptococcus equi infection in the respiratory tracts of horses and to characterise the sequential changes in the surface morphology during the early and late course of the disease and in the recovery period.

The dramatic enlargement of the mandibular and retropharyngeal groups of lymph nodes, with abscess formation and accompanying tissue reaction, found at necropsy in ponies in the acute stage of Strangles (Groups 1 and 2 and the adult ponies) were similar to changes described by Knight et al (1975) in a brief report of necropsy findings in experimentally infected ponies killed at 21 days post infection. Purulent exudate in the guttural pouches was also noted by these workers but they made no mention of any changes in the nasopharynx. In the present study, excess mucous and lymphoid hyperplasia in the nasopharynx were usually found in acute Strangles. Another feature was the presence of purulent exudate at the nostrils, although the surfaces in the nasal cavity were remarkably clean and there was no evidence of ulceration of the nasal mucosa reported as a characteristic feature of Strangles by Jubb et al (1985). Ponies in Groups 2 and 4, which had recovered from Strangles and appeared clinically normal, still had slight enlargement

of the retropharyngeal lymph nodes and one animal in each group had plugs of inspissated pus in the guttural pouches. The latter finding would certainly support the suggestion that Strangles could predispose to chronic guttural pouch disease (Knight et al, 1975; Clabough, 1987; Wilson, 1988).

The inflammatory changes observed at necropsy in the present study were almost entirely confined to the upper respiratory tract and adjacent lymph nodes. The lower respiratory passages appeared normal in all but 2 ponies and in these animals a small amount of mucopurulent exudate was present in the trachea. Streptococcus equi infection does not commonly cause clinical pneumonia (Wilson, 1988) and there was no evidence of spread of infection to the lungs in any of the ponies.

All the ponies in the present study had positive nasal swab cultures of Streptococcus equi and, except for one animal, all those killed in the acute stage of the disease had at least one positive swab culture of Streptococcus equi from the upper respiratory tract. The organism was not cultured from the lower respiratory passages or the lungs except in one other animal, which had a positive swab culture from tracheal exudate. In contrast, Streptococcus zooepidemicus was cultured from material taken at necropsy from the clinically recovered animals in Groups 3 and 4. Streptococcus zooepidemicus

is regarded as the most common secondary invader of the upper and lower respiratory tracts of horses (McAllister, 1982), and has been isolated from many equine tissues, both normal and abnormal, including mandibular and retropharyngeal lymph nodes, tonsil and nasopharynx (Woolcock, 1975; Wilson and Salt, 1978; Gillespie and Timoney, 1981; McAllister, 1982). Although none of the clinically recovered ponies in the present study appeared to be carriers of Streptococcus equi, it has been reported recently that a carrier state does indeed exist and that clinically normal horses can shed the organism for considerable periods of time (up to 11 months in one case) after recovery from Strangles (George et al, 1983; Timoney, 1988[b]; Sweeney et al, 1989).

Infection with respiratory viruses was evident in only one pony which had a rising titre to EHV-1, but this did not appear to cause any significant differences in the clinical signs or in the gross pathological or microscopical lesions.

In the acute stage of Strangles, histological lesions were mostly confined to subepithelial tissues. Except for patchy loss of surface cilia in the guttural pouches, the epithelium of both the upper and lower respiratory tracts appeared intact and the surface features were similar to those of normal horses (Mair et al, 1987; see Chapters 4 and 6). Infiltration of

subepithelial connective tissue by lymphocytes, plasma cells and neutrophils was the most noticeable feature of the upper respiratory tract mucosa and, although present, was much less marked in the trachea and bronchi. Lymphocytes predominated and formed follicles, particularly in the nasopharynx and guttural pouches, where many germinal centres developed. In the guttural pouches small abscesses formed with surrounding tissue oedema. This latter observation was in contrast to that of Knight et al (1975) who stated in a brief histological report that there was little sign of inflammation in the guttural pouches in cases of acute Strangles.

The animals in Groups 3 and 4 had marked follicular lymphoid hyperplasia with germinal centres in the nasopharynx and, while the epithelial surfaces were normal in both animals in Group 3, both animals in Group 4 had patchy loss of cilia in the larynx, trachea and bronchi. Although the ponies in Groups 3 and 4 appeared clinically normal and showed few gross abnormalities at necropsy, the persistence of histological lesions indicated that the time taken for complete recovery from Strangles was considerable and that the respiratory tract mucosa had not regained normality even 3 months after initial onset of clinical signs. Subepithelial lymphoid hyperplasia and progressive development of germinal centres, particularly noticeable in nasopharyngeal tissues,

in the course of the disease and in the recovered animals, could indicate a local mucosal immune system response to Streptococcus equi antigens. Indeed significant levels of antibody to the M protein of Streptococcus equi have been found in nasopharyngeal mucus of horses recently recovered from Strangles and, although many of these animals had not yet developed significant serum levels of antibodies, they were remarkedly resistant to further challenge with Streptococcus equi. This suggested that locally produced antibodies could be important in protection, perhaps by preventing adhesion and penetration of the organism (Galan and Timoney, 1985).

The histological appearance of the nasal and oral surfaces of the soft palate were similar to those described by Nickel et al (1979) and Leeson et al (1985). In Group 2 animals only, cocci were observed on the oral surface and proved to be Gram positive when stained by the Gram-Engbaek technique.

SEM studies revealed more extensive changes in the surface epithelium than were observed by the light microscope but the marked lymphoid hyperplasia, which was such an obvious histological feature, was not so noticeable with SEM and only the more protuberant nodules were represented by smooth, raised circumscribed areas in nasopharyngeal surfaces similar to lymphoid nodules observed in the guttural pouch of a normal horse (see

Chapter 4).

In the acute stage of Strangles surface changes were most marked in the guttural pouches where there was considerable loss of surface cilia and increased amounts of mucus which had trapped many degenerate cells and the latter correlated with the mucopurulent exudate noted at necropsy. Except in one animal which had loss of cilia in the dorsal nasal concha, nasal cavity surfaces appeared similar to those of normal horses (see Chapters 4 and 5).

Although Strangles is regarded as an upper respiratory disease, there were some changes in the lower respiratory passages where small groups of nonciliated microvillous cells were present in the usually well ciliated surfaces of the trachea in all the acutely ill ponies and these changes extended into the bronchi in 3 animals. However, no abnormalities were detected in the bronchioles or alveolar membranes in any animal.

Of the recovered animals, those in Group 3 still had evidence of loss of cilia from surfaces in the guttural pouches, larynx and trachea. However, the extensive loss of normal ciliated surfaces, not only in the guttural pouches and lower respiratory passages but also in the caudal nasal cavity, was an unexpected finding in the ponies in Group 4, killed at 90 days after the initial case first presented.

Ciliated cells are extremely sensitive to infection and irritants in the respiratory tract and local loss of cilia is the common response, usually followed by regeneration provided the injury is not repetitive or lethal (Sturgess, 1989). It is generally accepted that pathogenic strains of beta haemolytic streptococci release a considerable number of soluble products including toxins and enzymes which are probably involved in local pathogenicity (Taussig, 1984), and Bazeley (1943) demonstrated that young cultures of Streptococcus equi produced strong haemolysin and exotoxins so it seems reasonable to suppose that at least some of these products could have a deleterious effect on ciliated surfaces. Extensive damage to respiratory tract mucosa, with loss of surface cilia followed by almost complete return to normal in 28 days, has been demonstrated in the dog by Majid (1986) in a SEM and histological study of experimental Bordetella bronchiseptica infection. A similar sequence of events has been described in the respiratory tract of ferrets infected with Influenza A virus, where patchy loss of cilia persisted up to 20 days post infection (Chevance et al, 1978). In the present study the effect of Streptococcus equi infection on the ciliated respiratory tract surfaces of ponies killed in the acute stage of the disease, would appear to be similar, though less severe, than that of Bordetella

bronchiseptica in the dog and Influenza A virus infection in the ferret (Majid, 1986; Chevance et al, 1978). However, whether or not the loss of surface cilia observed in the clinically recovered ponies could still be attributed to the effect of Streptococcus equi infection after 40 and 90 days is difficult to explain as this organism was not cultured at necropsy. Although Streptococcus zooepidemicus has been regarded as a secondary invader of the respiratory tract in horses and of less pathogenic importance than Streptococcus equi (McAllister, 1982), the fact that the former organism was cultured from necropsy samples of ponies in Groups 3 and 4 could be significant and could indicate that it had contributed to the surface changes noted in these animals.

The SEM appearance of surfaces in the soft palate correlated well with the histological appearance. While a few Gram positive cocci were observed by the light microscope on the oral surface in Group 2 animals, many cocci were visualised by SEM in this site, adhering to the squamous surface cells in animals in Groups 2 and 4, with the greatest concentration of bacteria in the former. The isolation of Streptococcus zooepidemicus from the soft palate of one pony in Group 4 suggests that the bacteria observed, both with the light microscope and SEM, were indeed streptococci, at least in this animal.

Adherence of bacteria to nonciliated surfaces

in the nasal cavity was also observed by SEM, though not by light microscopy. Two ponies in the acute stage of the disease had groups of cocci adhering to the microvillous cells in the nasal septum. Both animals had positive nasal swab cultures for Streptococcus equi taken at necropsy and it is tempting to suggest that these were the bacteria observed with SEM. Ponies in all groups had rod-shaped bacteria adhering to squamous cells in the basal fold of the ventral nasal concha and these bacteria possibly represented the plump gram negative rods cultured from a nostril swab from one pony, but were not further investigated as they were not considered to be significant.

The streptococci have a component of the cell wall, known as the M protein, present as hairlike filaments which appear as a surface "fuzziness" when viewed with TEM and it has been demonstrated that the presence of M protein is a prerequisite for virulence in Streptococcus pyogenes and that the surface "fuzz" functioned in allowing attachment of the organism to cells of the tongue and buccal mucosa of rats, mice and humans (Ellen and Gibbons, 1972). Frost et al (1977), in a SEM study, demonstrated bacterial adherence in bovine mammary gland duct cells, in vitro, and suggested that virulence was related to adherence in Streptococcus agalactiae. Srivastava and Barnum (1983) found that Streptococcus equi adhered firmly to nasal, tongue and buccal cells of ponies,

in vitro, and suggested that the M protein has a possible role in adherence. In the present study there was circumstantial evidence to suggest that the cocci observed adhering to the nonciliated cells in the nasal septum and oral surface of the soft palate were streptococci and it is possible that the fine filaments which projected from their surface and sometimes gave a surface "fuzziness" could represent the M protein.

It was noted, in the present study, that many more bacteria were adherent to the soft palate than to the nasal septum, suggesting that the former was a preferred site and this is supported by the observations of Timoney (1988[b]) who concluded that the epithelial surfaces of the soft palate and tonsillar area were the most likely sites of localization, penetration and subsequent maintenance of Streptococcus equi and that adhesion in other sites, such as the nasal mucosa, was not important in the development of infection. It has been clearly demonstrated in pigs that the palatine tonsil was the portal of entry for beta haemolytic streptococci which rapidly invaded the mandibular and retropharyngeal lymph nodes causing a "strangles-like" illness in that species (Gosser and Olsen, 1973; Williams et al, 1973) and this supports the observations of Timoney (1988[b]) and those of the present study.

In this systematic study of the entire

respiratory tracts of ponies naturally infected with Streptococcus equi, the gross pathological and microbiological features of Strangles have been detailed and the surface features of the mucosa have been characterised by SEM, light microscopy and TEM, during the course of the disease and in the recovery period. The ultrastructural features of the respiratory tract of animals affected with Strangles have not been described before and SEM revealed a greater extent of damage to the respiratory tract epithelium than had been previously suggested, both in acute Strangles and in apparently recovered animals. The loss of cilia from respiratory tract surfaces and the activity in local lymphoid tissue, up to 3 months after the initial infection, would suggest that the effects of the disease are prolonged for a greater length of time than had been hitherto suspected. SEM also proved more useful than light microscopy in detection of surface bacteria and demonstrated that the oral surface of the soft palate was a common site for adherence of streptococci. Thus some new observations of the effects of Streptococcus equi infection have been made and those of other workers confirmed and the usefulness of SEM, used in conjunction with light microscopy, has been demonstrated in the study of an important respiratory disease of horses.

TABLE 7.1

TABLE OF AFFECTED PONIES IN THE STRANGLES OUTBREAK WHICH WERE USED IN THE SEM, HISTOLOGICAL AND TEM STUDY

Group	Animals	Status of Illness	Day Killed*
		Early stage of disease	
1	PH 55 PH 56 PH 57	Febrile for 4 days Febrile for 3 days Febrile for 2 days	4
		Disease developing	
2	PH 58 PH 59 PH 60	Febrile for 8 days Febrile for 8 days Febrile for 9 days	11
		Fully developed disease now recovered	
3	PH 62 PH 63	Febrile for 14 days Febrile for 14 days	40
		Fully developed disease now recovered	
4	PH 65 PH 66	Febrile for 13 days Febrile for 17 days	90

* Day 1 = day of initial case of Strangles in the outbreak. All ponies developed the disease within 5 days of Day 1.

TABLE 7.2

NECROPSY FINDINGS IN PONIES AFFECTED WITH STRANGLES*

Animals	Lymph Node Groups		Nasopharynx	Guttural Pouches	Trachea, Bronchi	Nasal/Ocular Discharge
	Mandibular	Retropharyngeal				
Group 1						
PH 55	Enlarged 2+ small abscesses 1-2 mm	Enlarged 3+ small abscesses 1-5 mm	Excess mucus	Surface granular	Normal	Serous 1+
PH 56	Enlarged 2+	Enlarged 3+ small abscesses 0.5-1 cm	Excess mucus Follicular lymphoid hyperplasia 1+	Surface granular	Normal	Serous 1+
PH 57	Enlarged 2+	Enlarged 3+ small abscesses 0.5-1 cm	Excess mucus Follicular lymphoid hyperplasia 1+	Surface granular Purulent exudate in left side	Normal	Serous 1+
Group 2						
PH 58	Enlarged 2+	Enlarged 4+ Abscesses>1 cm	Excess mucus	Copious purulent exudate	Normal	Mucopurulent 1+
PH 59	Enlarged 4+ Abscesses>1 cm Oedema	Enlarged 4+ Abscesses>1 cm Oedema	Excess mucus	Copious purulent exudate	Normal	Mucopurulent 1+
PH 60	Enlarged 2+	Enlarged 4+ Abscesses>1 cm	Excess mucus	Small amount purulent exudate	Normal	Mucopurulent 2+

TABLE 7.2 (Cont'd)

Animals	Lymph Node Groups Mandibular	Retropharyngeal	Nasopharynx	Guttural Pouches	Trachea, Bronchi	Nasal/Ocular Discharge
Group 3						
PH 62	Normal	Enlarged 1+	Follicular lymphoid hyperplasia 2+	Mucosa thickened opaque	Normal	None
PH 63	Normal	Enlarged 1+	Normal	Plugs of inspissated pus 0.5 x 1 cm	Normal	None
Group 4						
PH 65	Normal	Enlarged 1+	Normal	Normal	Normal	None
PH 66	Normal	Enlarged 1+	Normal	Plugs of inspissated pus in right side 1 x 0.5 cm	Muco-purulent exudate in trachea	None
PH 34	Enlarged 5+ Abscesses>1 cm Oedema	Enlarged 4+ Abscesses>1 cm Oedema	Follicular lymphoid hyperplasia 1+	Mucosa thickened surface granular	Muco-purulent exudate in trachea	Mucopurulent 3+
PH 42	Enlarged 5+ Abscesses>1 cm ruptured Oedema	Enlarged 4+ Abscesses>1 cm	Follicular lymphoid hyperplasia 1+	Purulent exudate in right side	Normal	Mucopurulent 2+

* Lesions and nasal/ocular discharge graded 1+ to 5+ according to severity.

TABLE 7.3

ISOLATION OF STREPTOCOCCUS EQUI AND STREPTOCOCCUS ZOOEPIDEMICUS FROM SWABS AND TISSUE SAMPLES TAKEN POST-MORTEM

	SWABS				TISSUES			
Animals	Nasal Cavity	Nasopharynx	Guttural Pouches	Trachea & Bronchus	Mandibular Lymph Node	Retro-pharyngeal Lymph Node	Lung	Other
PH 55	+ ve Strep. equi	- ve	- ve	- ve	- ve	- ve	- ve	
PH 56	+ ve Strep. equi	+ ve Strep. equi	- ve	- ve	- ve	+ ve Strep. equi	- ve	
PH 57	- ve	+ ve Strep. equi	+ ve Strep. equi	- ve	- ve	- ve	- ve	
PH 58	- ve	- ve	- ve	- ve	- ve	- ve	- ve	
PH 59	+ ve Strep. equi	- ve	- ve	- ve	- ve	- ve	- ve	
PH 60	- ve	- ve	+ ve Strep. equi	- ve	- ve	- ve	- ve	
PH 62	- ve	+ ve Strep. zooepidemicus	- ve	- ve	- ve	+ ve Strep. zooepidemicus	- ve	
PH 63	+ ve Strep. zooepidemicus	+ ve Strep. zooepidemicus	+ ve Strep. zooepidemicus	- ve	- ve	not done	- ve	

TABLE 7.3 (Cont'd)

Animals	SWABS				TISSUES			
	Nasal Cavity	Nasopharynx	Guttural Pouches	Trachea & Bronchus	Mandibular Lymph Node	Retro-pharyngeal Lymph Node	Lung	Other
PH 65	+ ve Strep. zooepidemicus	+ ve Strep. zooepidemicus	+ ve Strep. zooepidemicus	- ve	- ve	- ve	- ve	Soft Palate - ve
PH 66	+ ve Strep. zooepidemicus	+ ve Strep. zooepidemicus	+ ve Strep. zooepidemicus	- ve	- ve	- ve	- ve	Soft Palate + ve Strep. zoo-epidemicus
PH 34	+ ve Strep. equi	+ ve Strep. equi	+ ve Strep. equi	Trachea + ve Strep equi	+ ve Strep. equi	+ ve Strep equi	- ve	Nostril + ve Strep. equi
PH 42	+ ve Strep. equi	+ ve Strep. equi	+ ve Strep. equi	- ve	+ ve Strep. equi	+ ve Strep. equi	- ve	

CHAPTER 8

AN ULTRASTRUCTURAL AND HISTOLOGICAL STUDY OF HORSES WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) has long been recognised as a major lung disease of horses and is probably the most common cause of chronic coughing and premature loss of use of horses in Britain and Western Europe (Gerber, 1973; Littlejohn, 1979; Wilkie, 1982; Thomson and McPherson, 1988).

The disease is characterised clinically by chronic coughing, exercise intolerance leading to poor work performance and increased respiratory effort with abnormal lung sounds. There appears to be no sex or breed predisposition and animals as young as 6 months of age may be affected. The majority of cases, however, have been reported in horses over 5 years old and the prevalence of the disease increases with age (Littlejohn, 1979; McPherson and Thomson, 1983; Mirbahar and Eyre, 1986). The principal pathological lesion is chronic bronchiolitis which may be accompanied by alveolar over-inflation and emphysema (Alexander, 1959; Thurlbeck and Lowell, 1964; Foley and Lowell, 1966; Gerber, 1973; Nicholls, 1978; Breeze, 1979; Winder and von Fellenberg, 1986). COPD is essentially a disease of domestication and is world wide in distribution; however, it is uncommon in warm climates where horses can spend most of the time at grass (Littlejohn, 1979; Mirbahar and Eyre, 1986; Derksen, 1987; Dixon and McGorum, 1990[a]).

There is now good evidence that the disease is associated with dusty atmospheres and moulds in hay, other foodstuffs and bedding materials (Cook and Rossdale, 1963; Gillespie and Tyler, 1969; Eyre, 1972; Cook, 1976). There is also substantial evidence that COPD, at least in Britain, takes the form of a pulmonary hypersensitivity to organic dust antigens (McPherson et al, 1979). These latter workers demonstrated that the predominant aetiological antigens are Micropolyspora faeni (a thermophilic actinomycete) and Aspergillus fumigatus (a fungus), although grass pollens and occasional unidentified agents may also be involved. Recently, however, sporadic cases of COPD in Britain have been recorded in horses at grass in the summer and, in these cases, pulmonary allergy to grass pollens or oil seed rape pollen has been suggested as the underlying cause (Hannant, 1988; Dixon and McGorum, 1990[a,b]; Hackett, 1990). A similar pasture-associated COPD occurs in horses in the south-eastern United States in the warm, humid summer months (Beadle, 1986). Whether or not other infectious agents have an important role in the development of COPD is not known but it has been suggested that both influenza virus and streptococcal infections can predispose to its development (Gerber, 1973; McPherson and Lawson, 1974). More recently, in Canada, high levels of antibodies against influenza A

Equi I were demonstrated in the tracheal mucus of affected horses and it was suggested that this was consistent with repeated or continuous antigenic stimulation (Thorsen et al, 1983).

The understanding of the disease has been complicated by the fact that over the years COPD has also been known by many names such as Broken wind, Heaves and chronic alveolar or pulmonary emphysema (Littlejohn, 1979; McPherson and Thomson, 1983; Derksen, 1987) and the disease has a long history going back centuries (Smith, 1919). Aristotle, in 333BC, was probably the first to describe its characteristic abdominal expiratory effort or "heave" (Smith, 1919). The symptoms of Broken wind were linked to pulmonary emphysema by Floyer (1698) and the loss of lung elasticity associated with emphysema was noted by Gibson (1751). Later, Stommer (1887) noted similarities between emphysema in the horse and in man. Faulty husbandry and feeding of poor quality hay were thought to be the cause of Broken wind by Percival (1853) and Fitzwygram (1903) regarded poor quality hay to be an important aetiological factor. Later in the present century interest in emphysema in man led several workers to compare the human condition with emphysema in Broken wind or Heaves in horses (Alexander, 1959; McLaughlin and Edwards, 1966; Gillespie and Tyler, 1967[b], 1969; Nowell et al, 1971).

However, it has been noted by some workers that emphysema is not a consistent feature of the disease (Thurlbeck and Lowell, 1964; Lowell, 1964; Foley and Lowell, 1966). A respiratory allergy in horses with Broken wind was first demonstrated by Eyre (1972) and an allergic aetiology has since been confirmed (McPherson et al, 1979; Halliwell et al, 1979). As there seems to be no complete agreement on what is understood by the terms Broken wind, Heaves or alveolar emphysema in the veterinary literature, Sasse (1971) introduced the phrase "chronic obstructive pulmonary disease" to describe this long recognised condition and the term became accepted by most workers. In this study this will be the adopted phrase.

Although numerous reports and reviews of COPD have described the aetiology, clinical signs and pulmonary pathology (Gerber, 1973; Muyelle and Oyaert, 1973; Nicholls, 1978; McPherson et al, 1978, 1979; Littlejohn, 1979; Breeze, 1979; McPherson and Thomson, 1983; Thomson and McPherson, 1983; Mirbahar and Eyre, 1986; Clarke, 1987; Robinson, 1989; Dixon and McGorum, 1990[a]) there have been few reports of the ultrastructural features of the disease. As these have been confined to selected lower respiratory passages and the alveolar membrane (Gillespie and Tyler, 1966; Nowell et al, 1971; Nicholls, 1978; Drommer et al, 1986; Kaup et al, 1986)

it seemed appropriate to carry out a systematic ultrastructural and histological study of the entire respiratory tract in order to establish a more comprehensive picture of the mucosal changes present in horses affected with COPD.

MATERIALS AND METHODS

(1) Animals:

Ten horses were used in this study and were obtained through the clinical departments of Glasgow University Veterinary School and the Royal (Dick) School of Veterinary Studies, Edinburgh. Each horse had a prolonged clinical history of COPD and was destroyed for humane reasons. The general details of the horses are listed in Table 8.1, page 226).

The diagnosis of COPD was based on the following clinical signs:

1. Coughing, of more than 3 months duration.
2. Increased respiratory effort, often with a noticeable double expiratory effort or "heave".
3. Exercise intolerance indicated by reduced capacity for work and exercise often accompanied by respiratory distress and increased coughing.
4. Increased breathing sounds on auscultation (harsh sounds, crackles, squeaks).

5. Reduced partial pressure of arterial oxygen (PaO_2) $\leq$ 82 mm Hg.

Other notable clinical signs included occasional productive coughing, increased respiratory rate at rest (see Table 8.2, page 227), serous or mucoid nasal discharge, gradual weight loss and mucopurulent exudate in the trachea observed by endoscopy.

Tracheal aspirates were taken for routine bacteriological examination and were examined for the presence of cells. The clinical observations and the results of the examination of the tracheal aspirates are summarised in Table 8.2, page 227.

Routine examination of faeces samples for the presence of lungworm (Dictyocaulus arnfieldi) larvae was carried out in 6 horses (PH 44, PH 51, PH 52, PH 53, PH 67 and PH 68) and a negative result was obtained in each case.

(2) Methods of Investigation:

The method of destruction and the post-mortem procedures carried out are described in Chapter 3. In addition, swab samples for routine bacteriological examination were taken from the nasal cavity, nasopharynx, guttural pouch, trachea and lobar bronchi and pieces of lung tissue.

Samples for histology, SEM and TEM were taken from the entire respiratory tract and the sample sites

and procedures were the same as those used in the study of normal horses, described in Chapter 4 (Fig. 4.1 and Fig. 4.2) and Chapter 6 (Fig. 6.1). Additional samples were taken for SEM from the following sites (Fig. 8.1) :

i. Right cranial segmental bronchus

ii. Right caudal segmental bronchus

iii. Right caudal small bronchus

iv. Lung slices from 2 different locations in the right caudal lobe.

Samples iii and iv were taken after perfusion of the right caudal lobe of the lung via the caudal segmental bronchus with Karnovsky's fixative.

Additional samples for histology were taken from the following sites (Fig. 8.1) :

i. Left cranial segmental bronchus

ii. Left caudal segmental bronchus

iii. Left caudal small bronchus

iv. Lung slices from 2 different locations in the left caudal lobe.

Samples iii and iv were taken after perfusion of the left caudal lobe of the lung via the caudal segmental bronchus with 10% neutral buffered formalin.

All tissues for SEM and TEM were immersed in Karnovsky's fixative and tissues for histology were fixed in 10% neutral buffered formalin. Subsequent methods of processing, staining and examination were carried out as

described in Chapter 3.

RESULTS

(1) Post-mortem findings:

The gross pathological lesions were confined to the lower respiratory tract. Apart from the presence of discharge at the nostrils in 4 animals, (PH 44, PH 54, PH 67, PH 68), the nasal passages, nasopharynx, guttural pouches and larynx appeared normal, except for one animal (PH 54) which had severe atrophy of the left dorsal cricoarytenoid muscle but no gross abnormalities within the larynx. In all but 2 horses (PH 8 and PH 41), mucopurulent material was present in the trachea and in copious amounts in 3 horses (PH 44, PH 51, PH 52). Excess mucus or mucopus was present in the bronchi of all animals. Except for one horse (PH 54), the lungs had puffy areas of overinflation, particularly obvious in the dorsal and ventral borders of both cranial and caudal lobes. In 5 horses (PH 41, PH 51, PH 52, PH 67 and PH 69) the lungs were voluminous, pale and pitted on digital pressure and one of these animals (PH 41) had noticeable costal impressions on the pleural surfaces (Fig. 8.2). In other respects the pleural surfaces appeared normal in all but 2 horses; in PH 41 there were a few fibrous tags on the ventral borders of the lungs (Fig. 8.3) and in PH 67 a small area of

adhesion was found between the left caudal lobe and the costal pleura. The bronchial lymph nodes appeared normal. No significant lesions were observed in other organs or tissues.

(2) Bacteriological findings:

A variety of bacteria and a few fungi were cultured from swabs and tissues taken after death and are listed in Table 8.3,(page 229). While there was no further identification of many of the organisms cultured, specific bacteria were identified in cultures from 3 horses: Streptococcus zooepidemicus from the nasopharynx of PH 67, Pasteurella pneumotropica from the nasopharynx of PH 69 and Bordetella bronchiseptica from the guttural pouch of PH 68. Although these organisms are potential respiratory pathogens there was no evidence of inflammation in the nasopharynx of PH 67 and PH 69 and patchy loss of surface cilia was the only abnormality in the guttural pouch of PH 68. Thus it would seem that none of these bacteria were playing a significant role in the disease at the time of death.

(3) SEM findings:

The SEM appearance of the respiratory tract is described for all the horses as the changes observed differed only in degree in different individuals.

1. Nasal cavity and nasopharynx:

Mucosal surfaces in the nasal cavity and

nasopharynx were generally similar to those of normal horses as described in Chapter 4. Loss of surface cilia in patches was observed in the guttural pouch of 4 horses (PH 51, PH 67, PH 68 and PH 69).

2. Larynx:

The epiglottis appeared normal with surface features as described in Chapter 4. The ventral larynx was well ciliated in 6 horses (PH 8, PH 41, PH 54, PH 67, PH 68 and PH 69) but in the remaining 4 animals there were patches of microvillous cells (Fig. 8.4).

3. Trachea, Lobar and Segmental bronchi:

In 2 horses (PH 8 and PH 69) the surfaces were all well ciliated and resembled the trachea and bronchi of normal horses described in Chapter 6. Patches of poorly ciliated cells or microvillous cells were observed in the remaining horses; in the trachea of PH 44, PH 52, PH 53, PH 54, PH 67 and PH 68, in the lobar bronchi of PH 44, PH 51 and PH 52 and in the segmental bronchi of PH 41, PH 44 and PH 53 (Fig. 8.5).

4. Small bronchi:

In 3 horses (PH 8, PH 54 and PH 68) the small bronchi appeared normal with ciliated cells interspersed with mucus-secreting cells giving a "moth-eaten" appearance which is described fully in Chapter 6. All the other horses had patches of microvillous cells or poorly ciliated cells (see Fig. 8.5). These patches

were extensive in 3 horses (PH 41, PH 51 and PH 53). Surface mucus was a usual feature in all the airways.

5. Lung:

A noticeable feature was the focal nature of lesions in the lungs in spite of the fact that in 5 horses (PH 41, PH 51, PH 52, PH 67 and PH 69) the necropsy appearance of the lungs appeared to indicate a more diffuse involvement. Although the changes were widespread, there were also many completely normal small respiratory passages, alveolar ducts and alveoli similar to those described in Chapter 6.

(a) Bronchioles: Surface mucus and inflammatory cells were present in affected bronchioles (Fig. 8.6). Mucus in the terminal bronchioles often spilled over into the alveolar ducts (Fig. 8.7). In places the mucus blanket trapped degenerate cells (Fig. 8.8) which were difficult to identify with certainty, but most were probably neutrophils (Fig. 8.9). Ciliated cells and nonciliated Clara cells populated the surface of the bronchioles. Some of the former had few cilia, often concentrated at the cell periphery (Fig. 8.10). While some Clara cells appeared normal, others were flat (Fig. 8.10) or had a "crumpled" appearance (Fig. 8.11) and were less protruberant than normal. Macrophages were also observed on some bronchiolar surfaces (Fig. 8.12) and at the junction of terminal bronchioles and alveolar

ducts (Fig. 8.13). No distinct mucus-secreting cells were observed in any bronchiole with SEM.

(b) Alveolar membrane: Under low power, focal areas of overinflation, with loss of normal alveolar structure (emphysema), were observed in all but one horse (PH 54) (Fig. 8.14). Obvious subpleural emphysematous areas (Fig. 8.15) were present in 2 horses (PH 41 and PH 51). In affected areas interalveolar pores were numerous (more than 20 per exposed surface (Fig. 8.16) in many alveoli), varied greatly in size (Fig. 8.17) and some appeared multilocular with each space separated from the other by fine strands of alveolar membrane. Mucous exudate was present in many alveolar ducts and in some alveoli (Fig. 8.18). The type I pneumocytes appeared normal. There was, however, an increased number of type II pneumocytes, particularly noticeable at the junction of the alveolar ducts and terminal bronchioles (Fig. 8.19). Some type II pneumocytes had large numbers of central pores (Fig. 8.20) and some appeared to be degenerating (Fig. 8.21) and (were possibly) sloughing off from the alveolar membrane surface (Fig. 8.22). Alveolar macrophages were numerous, especially in alveolar ducts and at their junction with terminal bronchioles (see Fig. 8.19 and Fig. 8.21). While the changes described in bronchioles and alveolar membranes were present in some degree in all the horses, they were

most marked in 6 animals (PH 41, PH 44, PH 51, PH 52, PH 67 and PH 69). No differences were noted between the cranial and caudal lobes of the lung.

(4) Histological findings:

In general the mucosal surfaces of the nasal cavity, nasopharynx and larynx were similar to those described in normal horses in Chapter 4. However, loss of surface cilia over small areas in the guttural pouch was observed in 4 horses (PH 41, PH 52, PH 68 and PH 69) and in the ventral larynx of another (PH 53). In the lower respiratory tract the mucosa of the larger passages was in general similar to that described in normal horses in Chapter 6 but focal loss of surface cilia was noted in the trachea of 3 horses (PH 44, PH 53 and PH 68), in the cranial lobar bronchus of one horse (PH 67) and in the segmental bronchi of one horse (PH 51) without any noticeable change in the number or type of mucous cells.

The small bronchi appeared normal in 3 horses (PH 53, PH 54 and PH 68), with low respiratory epithelium and scanty mucosal glands. In all the other horses there was a mild peribronchiolar cellular infiltration consisting mainly of lymphocytes but also a few plasma cells and neutrophils. The epithelium appeared normal but mucus and neutrophils were present in the lumen of a few small bronchi (Fig. 8.23).

The histopathology was mainly confined to the bronchioles and lung parenchyma and was focal in nature. All the horses had histological changes in some but not all bronchioles. Many affected bronchioles, both large and small, had epithelial hyperplasia (2-3 cells deep) and some surface cells contained mixed or acidic mucosubstances in their apical portions (Figs. 8.24, 8.25). The lumen of many bronchioles was occluded by mucus and neutrophils (Figs. 8.26, 8.27). There was marked peribronchiolar cellular infiltration of lymphocytes and plasma cells (Fig. 8.24), the former predominating and occasionally forming follicles. One animal (PH 68) also had a mild peribronchiolar eosinophil infiltration. Peribronchiolar fibrosis was a feature in some bronchioles (Fig. 8.24) and there were occasional macrophages containing haemosiderin in the peribronchiolar tissue.

In the lung parenchyma there were focal areas of alveolar overinflation, usually associated with affected bronchioles. Emphysema was present in 6 animals (PH 41, PH 44, PH 51, PH 52, PH 67 and PH 69) and was often most obvious in the immediate subpleural areas (Fig. 8.28). Small focal areas of alveolar epithelial hyperplasia and mild septal fibrosis were observed adjacent to affected bronchioles and small bronchi and some alveoli contained mucous exudate and macrophages. The histological changes were present in

both the cranial and the caudal lobes of the lung and were most severe in 6 horses (PH 41, PH 44, PH 51, PH 53, PH 67 and PH 69) and less marked in the remaining animals.

(5) TEM findings:

Changes in the bronchioles and alveolar membrane were most severe in 7 horses (PH 8, PH 41, PH 42, PH 44, PH 52, PH 67 and PH 69) and less marked in the remaining animals. In the lung samples, small bronchi, bronchioles and alveolar membranes were all visualised with TEM. The changes observed involved some but not all of these tissues and generally corresponded with the focal nature of the lesions observed histologically and with SEM. Normal airways and alveoli were present and were similar to those described fully in Chapter 6. Similar changes were observed in all the horses and differed only in severity in individual animals.

(a) Small bronchi: The epithelium generally resembled that of normal horses described in Chapter 6, with ciliated cells, mucous cells and occasional basal cells; however, in some of the former, cilia were sparse. Occasional lymphocytes were found migrating between the epithelial cells.

(b) Bronchioles: The epithelium was either simple or hyperplastic (2-3 cells deep), with ciliated cells and Clara cells reaching the lumen (Fig. 8.29). The basal lamina was thin and often difficult to distinguish.

Numerous cytoplasmic interdigitations were present between adjacent cells (Fig. 8.30). While some ciliated cells appeared normal, a variety of changes were observed in others. Some had few cilia and large cytoplasmic vacuoles were present which contained membranous remnants and fine granular mucous-like material (Fig. 8.31). Other features were lipid-like droplets, myelin configurations and cytoplasmic glycogen (Figs. 8.32, 8.33, 8.34). A few Clara cells contained normal granules but, in some, they were smaller or few in number and less electron-dense than usual (Figs. 8.29, 8.30, 8.36), while some cells were agranular (Fig. 8.35) and contained large amounts of smooth endoplasmic reticulum (Fig. 8.36). Many Clara cells appeared flattened and no longer protruded into the bronchiolar lumen (Fig. 8.30). Cytoplasmic glycogen was also a feature of some cells (Fig. 8.34). Cells containing mucous granules (Fig. 8.37), the latter often floccular in appearance (Fig. 8.38), were present in some bronchioles. These airways had few, if any, recognisable Clara cells. Many mononuclear cells, most of which were lymphocytes, were present below the basal lamina or migrating between the epithelial cells.

(c) Alveolar membrane: There was a noticeable increase in the number of type II pneumocytes in some alveoli (Fig. 8.39). Many of these cells contained enlarged lamellar vacuoles which often appeared partially or

completely empty (Figs. 8.40, 8.41). Some type II cells appeared electron dense and degenerate. Type I pneumocytes appeared normal. Alveolar macrophages were numerous and appeared active with numerous phagolysosomes and extensive, slender cytoplasmic processes. Macrophages were often in the vicinity of type II pneumocytes and some were observed within alveolar pores (Fig. 8.42). A further feature of the alveolar membrane was an increase in the amount of collagen (Fig. 8.40) and number of cells, some of which were fibroblasts, in the interalveolar septum.

DISCUSSION

COPD is probably the most common cause of chronic coughing and impaired work performance in horses and frequently results in premature loss of individual animals (Gerber, 1973; McPherson and Thomson, 1983; Thomson and McPherson, 1988). While most of the previous work has concentrated on the clinical manifestations and aetiological factors, necropsy and histopathological findings have also been documented (Alexander, 1959; Thurlbeck and Lowell, 1964; Nicholls, 1978; Winder and von Fellenberg, 1986). However, ultrastructural features of the disease have appeared in only a few reports, all of which deal with the lung and the lower respiratory passages (Gillespie and Tyler, 1967[b];

Nowell et al, 1971; Drommer et al, 1986; Kaup et al, 1986); there is no comprehensive ultrastructural and histological survey of the respiratory tract as a whole. The present study was undertaken to examine, for the first time, systematically, with SEM, light microscopy and TEM, the surfaces of the upper and lower respiratory tracts of horses affected with this disease.

Changes in the gross appearance of the lungs was an obvious necropsy finding in most horses in the present study. The lungs appeared generally voluminous, due to trapped air, in 5 animals and focal puffy, pale areas of overinflation were obvious, especially in the cranial lobes and ventral borders of the caudal lobes in all of the horses except in one horse where overinflation was minimal. There was a mucopurulent exudate in the trachea of all but 2 animals and excess mucus or mucopus was present in many of the smaller airways. The surfaces of the upper respiratory passages appeared normal. These gross pathological features are similar to those described by Nicholls (1978) in a pathological study of 25 horses with chronic pulmonary disease and in an earlier brief report of chronic alveolar emphysema in the horse (Alexander, 1959).

Although a variety of organisms were cultured from swabs and lung tissue taken after death, none were regarded as significant in the course of the disease.

Although it has been suggested that infectious agents can play a role in the aetiology of COPD (Littlejohn, 1979), in a more recent study of tracheobronchial washings from affected horses, it was concluded that the role of pathogenic organisms in the disease is limited (Nuytten et al, 1983). In an investigation of elastase-producing microorganisms in horse lungs, Grunig et al (1986) found that 17 out of 21 horses with chronic pulmonary disease had pulmonary microorganisms, with considerable numbers in 7 horses. They could, however, find no correlation between numbers of microorganisms and pulmonary lesions.

In the present study, the histological appearance of the mucosa of the upper respiratory tract and the larger lower respiratory passages was generally similar to that described in normal horses (Mair et al, 1987; see Chapters 4 and 6). However, there was focal loss of surface cilia from a number of sites; in the guttural pouches, the larynx, the trachea and segmental bronchi. Mild inflammatory changes were noted in the small bronchi of all but 3 horses. Chronic bronchitis and bronchiolitis have been described as features of COPD (Alexander, 1959; Gerber, 1973) although Nicholls (1978) found no evidence of generalised bronchitis. The findings in the present study, however, seem to indicate that mild bronchitis, affecting small bronchi,

is a feature in some cases.

In the present study, focal bronchiolitis was the most consistent histological feature. Intraluminal plugs of mucus and inflammatory cells and hyperplastic epithelium, with some surface cells containing mucosubstances, were features of affected bronchioles. Various degrees of peribronchiolar thickening were observed associated with mononuclear cell infiltration and fibrosis, the latter only in the more severely affected airways. Alveolar changes were also focal; overinflation was the most common finding, with emphysema, as defined by the Ciba Symposium in 1958 (Fletcher, 1959), present in the more severe cases. Small islands of peribronchial and peribronchiolar alveolar epithelial hyperplasia were also present.

Descriptions of the light microscopic appearance of COPD vary considerably; Alexander (1959), in a brief account of the histopathology of chronic alveolar emphysema in horses, observed that distended and ruptured alveoli, chronic bronchitis and bronchiolitis were the most consistent features. Bronchiolitis, with mucopurulent exudate, peribronchiolar fibrosis and associated alveolar fibrosis and epithelial hyperplasia, were described in 7 horses with "heaves" by Thurlbeck and Lowell (1964) but widespread emphysema was not a feature in these cases. While similar changes were

observed in 2 out of 4 affected horses by Foley and Lowell (1966), these workers described one horse which had evidence of both bronchiolitis and emphysema; another animal had emphysema only but this was considered to be unusual. On the other hand, emphysema was considered to be the major histopathological lesion in horses with "heaves" by McLaughlin and Edwards (1966) but, in a comparative study with emphysema in man, they only examined the lungs of 2 animals.

In her comprehensive study of 25 horses with COPD, Nicholls (1978) found that chronic bronchiolitis was the most consistent histopathological change and she listed the main bronchiolar changes as epithelial hyperplasia (2-3 cells deep), epithelial metaplasia with the appearance of mucous cells, peribronchiolar cellular infiltration of lymphocytes and plasma cells, peribronchiolar fibrosis and mucous exudate with neutrophils in the lumen. The latter worker also described over-inflation as the main alveolar change and, although emphysema was observed, it was not considered to be an important part of the pathology of the disease and focal alveolar epithelial hyperplasia was observed in only 5 out of the 25 horses. Breeze (1979), in agreement with the work of Nicholls (1978), summarised the histopathological features of COPD but emphasised that all the lesions were not necessarily present in every case, nor

were they present to the same degree. In more recent studies of horses with COPD in Switzerland, similar histological changes were described and varied in severity in different individuals and it appeared that the degree of changes corresponded to the severity of the clinical signs (Winder and von Fellenberg, 1988).

Breeze (1979) also considered that most horses with COPD did not have significant emphysema if significant meant that this lesion was the cause of all, or most of the functional abnormalities of the lungs. He supported this by stating that many horses with COPD improved clinically and functionally after appropriate therapy and change in their environment and that this would be improbable if significant emphysema was present.

In the present study the histological changes observed in the lungs were generally similar to those recorded by other workers (Thurlbeck and Lowell, 1964; Foley and Lowell, 1966; Nicholls, 1978; Breeze, 1979; Winder and von Fellenberg, 1986, 1987, 1988).

The peribronchiolar cellular infiltration observed in the present study and by other workers (Foley and Lowell, 1966; Nicholls, 1978; Breeze, 1979, Winder and von Fellenberg, 1986, 1987, 1988), consisted mainly of lymphocytes and plasma cells. The presence of these cells suggests an immune response. It has been

demonstrated, in immunofluorescence and immuno-histochemical studies, that increased numbers of cells containing IgA and IgG are present in the submucosa and lamina propria of the bronchioles of horses with COPD, compared with normal horses and this was attributed to persistent mucosal antigenic stimulation (Winder and von Fellenberg, 1986, 1988). These workers also suggested that IgG immune complexes might activate the complement system with the subsequent attraction of neutrophils, large numbers of which are a prominent feature within many bronchioles of horses affected with COPD.

A variable degree of pulmonary eosinophilia has been noted in some cases of COPD (Thurlbeck and Lowell, 1964; Gerber, 1973; Nicholls, 1978, Breeze, 1979). An association with parasitic infection was ruled out by Nicholls (1978) in her cases but the possibility that an allergic reaction (asthma or type I hypersensitivity reaction) was responsible for the presence of eosinophils was suggested. Gerber (1973) also suggested that an allergic reaction can be responsible for the presence of these cells but that their absence did not exclude an allergic aetiology for COPD. While it is known that eosinophils are important inflammatory cells in human asthma (Barnes, 1989), in contrast, the neutrophil is now recognised as

one of the most important cells in COPD in horses and, indeed, the presence of large numbers of these cells in tracheobronchial aspirates is regarded as diagnostic of the disease (Nuytten et al, 1983; Whitwell and Greet, 1984; Derksen et al, 1985; Mair, 1987; Thompson, 1989).

Pulmonary eosinophilia was not a feature of horses in the present study except for one animal which had a mild infiltration in peribronchiolar connective tissue. Neutrophils, however, were present in large numbers in tracheal aspirates of 6 out of 8 horses and of the remaining 2 animals, one had a few macrophages and the other had many eosinophils (Table 8.2, page 227). It has been reported that the presence of eosinophils in tracheal lavage samples could be indicative of lungworm infection (Whitwell and Greet, 1984; Mair, 1987), however, none of the horses in the present study had any evidence of lungworm. Nuytten et al (1983) found eosinophils in tracheobronchial aspirates in a small number of horses with COPD (4 animals in a group of 135) and in another study (Derksen et al, 1985) these cells were also present in 3 out of 6 horses with COPD. None of these authors could explain the presence of eosinophils and the reason why these cells are present in lavage fluids of some horses with COPD remains obscure (Derksen et al, 1989).

SEM studies of the upper respiratory tract

confirmed the observations with the light microscope. Surfaces in the nasal cavity and nasopharynx were generally similar to those of normal horses (see Chapter 4), although 4 horses had patchy loss of cilia in the guttural pouches. With SEM patches of poorly ciliated cells or microvillous cells were noted with increasing frequency distally from the ventral larynx to the trachea and larger bronchi and were more extensive than the changes observed histologically. All but 2 horses had some evidence of damage to these ciliated surfaces and the larynx, trachea and larger bronchi were all involved in 3 horses. Similar changes were observed in small bronchi in all but 3 horses. Surface changes observed with SEM did not always correlate with the histological findings but this was not surprising given the apparent patchy distribution of the lesions, the very large surface area of the passages involved and the relatively small size of the samples. However, from both SEM and light microscopic observations, it seems reasonable to conclude that focal loss of surface cilia, in the lower respiratory passages, was a distinct feature in the horses in the present study.

Ciliated cells are very sensitive to irritants and infections in the respiratory tract and are replaced by undifferentiated microvillous cells which normally undergo ciliogenesis and differentiate into mature

ciliated cells although repeated injury can interfere with this (Sturgess, 1989). The lower respiratory passages of horses with COPD commonly contain mucopurulent exudate, which probably contains degradation products of inflammatory cells which could have a deleterious effect on the ciliated cells. The loss of cilia or damage to cilia in the lower respiratory passages could be partly responsible for the impaired mucociliary transport rate observed in horses with COPD (Turgut and Sasse, 1989). In a SEM and TEM study of 13 horses with COPD, loss of ciliated cells and replacement by microvillous cells has also been observed in the trachea and bronchi by Drommer et al (1986). A further TEM observation by these workers was epithelial hyperplasia, especially in "middle-sized" bronchi of severe cases. In the present study, bronchial epithelial hyperplasia was not a demonstrable histological feature, nor was it found by TEM. The loss of surface cilia in small bronchial surfaces observed with SEM was not reflected in the TEM appearance but this could be due to the small number of these passages available for examination by the latter method.

The focal nature of lesions in the lungs observed with the light microscope was also a feature of the SEM studies. Many small bronchi, bronchioles and alveoli appeared similar to those described in normal

horses (Nowell and Tyler, 1971; Tyler et al, 1971; see Chapter 6). The most obvious SEM feature of affected bronchioles was the presence of surface mucus, which often contained degenerate inflammatory cells sometimes spilling out of the terminal bronchioles into the alveolar ducts. This reflected the plugs of muco-purulent material noted with light microscopy within bronchioles. There appeared to be no loss of ciliated cells although the cilia were sparse on some cells. While some Clara cells appeared normal, others were flat or "crumpled". Surprisingly there appeared to be no evidence of typical mucus-secreting cells in any of the bronchioles. The light microscopic appearance of affected bronchioles with many surface cells containing mucosubstances did not seem to be reflected in the SEM appearance. A similar observation was made by Nicholls (1978). In an earlier SEM study of chronic pulmonary emphysema in the horse Nowell et al (1971) reported that the bronchioles appeared normal. It is difficult to explain why no obvious mucus-secreting cells were observed with SEM but it might indicate that the cells containing mucosubstances in affected bronchioles were not morphologically identical, at least on the surface, to normal mucous cells elsewhere in the respiratory passages.

TEM studies revealed profound changes in

many bronchioles. Epithelial hyperplasia was observed in some of the passages. While many ciliated cells appeared normal, others possessed few cilia and a considerable number contained cytoplasmic vacuoles, mucus-like and lipid-like droplets and myelin configurations which probably represented degenerative changes in the cells. Glycogen deposits were also present in some cells. Nicholls (1978) found normal ciliated cells in bronchioles of horses with COPD but examined only a small number of samples with TEM. Drommer et al (1986), in their SEM and TEM study, described ciliated cells with few cilia and the presence of ciliary defects, attributed to precipitous cell proliferation, in the larger respiratory passages. They also mentioned degenerative changes in bronchiolar ciliated cells but did not specify what these changes were nor did they illustrate them. However, horses with bronchiolitis, experimentally induced by administration of 3-methylindole, had some degenerative changes in the bronchiolar ciliated cells similar to those observed in the present study (Turk et al, 1983).

In affected bronchioles there were few Clara cells with a normal complement of granules. Some cells had a few irregular granules and others were agranular. In the latter, the smooth endoplasmic reticulum, which is a normal feature of horse Clara cells (Plopper et al,

1980[b]), became particularly prominent. The apical portions of some cells were less protruberant than normal and others were distinctly flattened and this corresponded to the SEM appearance of crumpled or flattened Clara cells.

It is now generally recognised that the nonciliated columnar cells of respiratory epithelium are the usual progenitor cells (McDowell *et al*, 1984; Evans *et al*, 1986) and that Clara cells respond to injury by degranulating and dividing to produce agranular cells which then differentiate either into new Clara cells or ciliated cells (Evans *et al*, 1978). In the present study the Clara cells in affected bronchioles, which lacked granules, could represent similar cells to those described by Evans *et al* (1978). Damage to Clara cells, with loss of granules, was also observed with TEM by Turk *et al* (1983) in their attempt to produce an experimental model of COPD in horses.

In some bronchioles there were few, if any, recognisable Clara cells and in these airways cells containing mucous granules, which often had a floccular appearance, were present among the ciliated cells. That these airways were indeed bronchioles and not small bronchi was confirmed by re-examining the original 1 µm toluodine blue stained sections used for choosing the final sections cut for TEM (see Chapter 3). Mucus-containing "goblet cells" replacing the Clara cells

have been described in bronchioles of horses affected with COPD in other TEM studies (Nicholls, 1978; Drommer et al, 1986). In the present study with TEM, the mucus-containing bronchiolar cells did not always exactly resemble the mucous cells of normal equine respiratory epithelium observed with TEM (see Chapter 6), as the granules sometimes appeared floccular and often seemed to be confined to a rather shallow area in the apical cytoplasm and the cell surfaces appeared relatively flat. However, these granule-containing cells would appear to correspond to the cells containing mucosubstances observed histologically in affected bronchioles. Nicholls (1978) reported that the bronchiolar mucous cells in horses with COPD contained mainly acidic mucosubstances and many of these cells in the present study contained similar mucosubstances. However, some cells showed a mixed staining reaction and the presence of glycogen in both ciliated cells and Clara cells could account for the positive staining with the PAS staining method.

From the changes observed in the present combined study of light microscopy, SEM and TEM, there seems to be no doubt that damage to bronchiolar epithelial cells is a feature of COPD in horses and that mucus-containing cells develop in the bronchiolar epithelium. These findings are generally in accord

with the work of others (Nicholls, 1978; Drommer et al, 1986). It would seem, therefore, that both ciliated cells and Clara cells are involved in the disease process and that the latter respond in their regenerative role (Evans et al, 1978) and in some instances form mucous cells. This would account for mucus in the bronchiolar lumen observed histologically and with SEM and ciliated cell damage could seriously interfere with mucociliary clearance, thus contributing to bronchiolar obstruction. Drommer et al (1986) considered that the Clara cells were the target cells and were especially involved initially and transformed into mucus-secreting "goblet cells" and that degenerative changes in ciliated cells occurred later in severe cases. In the present study, as both ciliated cells and Clara cells showed evidence of damage, it was impossible to determine whether one cell was affected before the other.

The focal alveolar overinflation and emphysema observed histologically was also obvious with SEM, which gave a dramatic three-dimensional picture of alveolar changes, including an increase in the number and size of interalveolar pores many of which had a "lacy" appearance. Similar changes in the lung parenchyma have been recorded in other SEM studies of horses with COPD (Tyler et al, 1971; Nowell et al, 1971;

Nicholls, 1978; Kaup et al, 1986). There is an increase in the number of alveolar pores in conditions in which hyperventilation occurs (Desplechain et al, 1983) and this could be an explanation for the increased number of pores in horses with COPD. It has been reported that the role of alveolar pores in collateral ventilation appears to be of little importance in normal lungs but may be important in lung disease (Desplechain et al, 1983; Gillett et al, 1989) and it has been suggested that the focal emphysema in COPD is possibly the result of air trapped and collateral ventilation, distal to obstructed bronchioles (Kaup et al, 1986).

In the present study, the type I pneumocytes appeared normal with SEM but there was an obvious increase in the number of type II pneumocytes in many alveoli and alveolar ducts. In the latter, clusters of these cells were often present at the junction with terminal bronchioles. Many type II cells appeared exhausted or degenerate and had increased numbers of surface pores. TEM confirmed the observations with SEM; the type I pneumocytes had a normal appearance but many type II pneumocytes contained enlarged lamellar bodies and others appeared electron dense and degenerate.

Although the type I pneumocytes appeared normal with SEM and TEM, the presence of more than usual

numbers of type II pneumocytes suggests that there must have been some alveolar damage and loss of type I cells. It is now generally accepted that the type II pneumocytes proliferate in order to establish a continuous epithelium in alveoli after damage to type I pneumocytes which are the more susceptible cells (Mason and Williams, 1977; Burri, 1985). In a SEM and TEM study of COPD, Kaup et al (1986) also noted an increase in the number of type II pneumocytes, which they suggested had replaced lost type I pneumocytes. They illustrated, without comment, the remaining type I cells with SEM and they appeared normal so it must be assumed that Kaup and his co-workers considered them also to be so.

The SEM and TEM features of type II pneumocytes in the present study suggested increased production of surfactant. Kaup et al (1986) found similar changes in type II cells in their study and suggested that increased surfactant production was indicated by the large number of alveolar macrophages, containing phagocytosed surfactant material, which were present in the vicinity of the type II cells. It has been demonstrated experimentally in laboratory animals that hyperventilation increases surfactant secretion but the mechanism for this remains unknown (Voelker and Mason, 1989). It has also been demonstrated that sublethal experimental injury to alveoli causes hyperplasia of

type II pneumocytes and increased secretion of surfactant (Kikkawa and Smith, 1983). Horses with COPD frequently hyperventilate, possibly stimulating increased surfactant production from type II pneumocytes. There is no doubt that there are increased numbers of these cells in some alveoli of horses with COPD and circumstantial evidence to suggest that they probably produce more surfactant than usual. Presumably there must be focal damage to the alveolar membrane which stimulates the proliferation of the type II cells and, as this proliferation seems to occur in alveoli and alveolar ducts close to affected bronchioles, it seems reasonable to suppose that the factors which cause bronchiolar damage and reactivity such as antigens and immune complexes (McPherson et al, 1979; Winder and von Fellenberg, 1988), could also cause focal damage to the alveolar membrane, but this requires further investigation.

A further TEM observation was thickening of the alveolar membrane with excess collagen and number of cells, some of which were fibroblasts and this mirrored the histological appearance of focal alveolar septal fibrosis. Alveolar fibrosis is regarded as a reactive process in response to a number of aetiological factors in man, including hypersensitivity to organic dusts, such as those found in mouldy hay, which cause the disease known as Farmer's Lung (Cotran et al, 1989).

Similarities between "heaves" in horses and Farmer's Lung in man were noted by Thurlbeck and Lowell (1964) but they concluded that the two conditions were not identical as the disease of horses was essentially a bronchiolitis, while that of man was an alveolitis. The focal alveolar fibrosis in COPD is regarded as an extension of peribronchiolar fibrosis by Kaup et al, (1986).

As mentioned earlier in the discussion, Kaup et al (1986) noted an increase in the number of alveolar macrophages in COPD and this was also a feature in the present study. These cells were visualised with the light microscope, SEM and TEM and were prevalent in alveoli and alveolar ducts close to affected bronchioles and sometimes also within the latter passages. The dramatic SEM appearance of the macrophages with their numerous, long, slender pseudopodia reaching out over the surfaces gave an impression of intense activity which was confirmed by TEM, with the presence of numerous cytoplasmic phagolysosomes. Traditionally regarded as phagocytes, alveolar macrophages are now known to secrete a variety of biologically active substances which, for example, can stimulate fibroblasts to secrete collagen and other cells to initiate inflammatory responses. In addition, it has been shown that they can enhance or suppress

immune reactions (Herscowitz, 1985; Bowden, 1987). Thus alveolar macrophages have the potential not only to defend the lung from harmful agents, by phagocytosis and modulation of inflammatory responses, but also to cause harm by secretion of proteases or stimulation of fibroblasts (Bowden, 1987). So it would appear that alveolar macrophages are involved in regulation of the delicate balance between health and disease in the lung (Herscowitz, 1985) but their precise role in COPD in horses has yet to be defined.

In the present study with TEM, there did not appear to be an increased number of mast cells in peribronchiolar tissue or in alveolar septa, although they have been noted in the latter site associated with alveolar fibrosis (Kaup et al, 1986). In a histological study to determine the distribution of mast cells in the lungs of normal horses and horses with COPD, Nicholls (1978) found that 7 out of 10 horses with COPD had increased numbers of mast cells while the remaining 3 horses had fewer than normal. There seemed to be no satisfactory explanation for the differences in animals with COPD but it was suggested that mast cell numbers might vary with the time of year (Nicholls, 1978) and Breeze (1979) suggested that there might be more pulmonary mast cells in cases of COPD in the summer months. Special fixation and staining methods for the

detection of mast cells histologically was not carried out in the present study but this could possibly be useful in future investigations.

In this study the entire respiratory tracts of horses with COPD were examined systematically. The gross pathological and microbiological features were noted and the surface features of the mucosa, as visualised by light microscopy and SEM, were described in detail. In addition, TEM was used to further elucidate features of the small respiratory passages and the alveolar membrane. Although COPD is primarily regarded as a disease of the lungs, it was interesting to note patchy loss of cilia from the larger airways, including the larynx with the probable consequence of impaired mucociliary transport. SEM proved to be a useful tool in detection of abnormalities in the ciliated epithelium of the airways but at bronchiolar level did not seem to reflect as clearly the changes in the Clara cells which were an obvious TEM feature. The presence of mucosubstances in bronchiolar epithelial cells observed histologically was confirmed with TEM but the surface morphology of these cells did not appear to be the same as that of mucus-secreting cells in normal equine respiratory epithelium as visualised by SEM.

It is generally agreed that chronic

bronchiolitis with alveolar overinflation and sometimes emphysema are the main pathological features of COPD in horses (McPherson and Thomson, 1983; Winder and von Fellenberg, 1988) and that, at least in Britain, COPD is regarded as a pulmonary hypersensitivity to moulds and sometimes pollens, in the environment (McPherson and Thomson, 1983). Over the past 30 years there have been many reports on the various aspects of the disease from Alexander in 1959 to Thomson in 1989, with later workers concentrating on the lung at cellular level (Drommer et al, 1986; Kaup et al, 1986; Winder and von Fellenberg, 1988). However, the sequence of changes in the bronchioles and alveoli and the role of particular cells, such as the Clara cells, type II pneumocytes, alveolar macrophages and the various inflammatory cells, in the development of the disease or in its course, is not entirely clear at the present time.

The situation of pulmonary disease in man was admirably summed up by Ayers and Jeffery (1988) who stated that "the lung remains relatively unexplored with regard to the factors which control proliferation and differentiation of its many distinct cell types both in health and disease" and this statement would certainly seem to apply to the horse also. In COPD many questions remain unanswered and further investigation of the various cell types in the lung will be required before

the problems of this important respiratory disease of horses can be solved.

TABLE 8.1

HORSES IN THE COPD STUDY

No.	Age in Years	Sex	Type	Duration of Illness
PH 8	20	G	Hunter	Over 2 years
PH 41	11	G	Thoroughbred	5 months
PH 44	16	G	Showjumper	2 years
PH 51	25	F	Thoroughbred	3 years
PH 52	22	G	Thoroughbred X	4 years
PH 53	12	G	Hunter	5 months
PH 54	16	F	Irish Draught	5 years
PH 67	15	F	Pony	5 years
PH 68	20	F	Pony	3 years
PH 69	18	G	Riding Horse	4 months

G = Gelding

F = Female

TABLE 8.2

CLINICAL OBSERVATIONS IN HORSES IN THE COPD STUDY

Observations	PH 8	PH 41	PH 44	PH 51	PH 52	PH 53	PH 54	PH 67	PH 68	PH 69
Coughing	+	+	+	+	+	+	+	+	+	+
Productive Cough	+	-	+	+	-	-	-	-	-	-
Increased respiratory effort	+	+	+	+	+	+	+	+	+	+
Exercise intolerance	+	+	+	+	+	+	+	+	+	+
Increased breathing sounds	+	+	+	+	+	+	+	+	+	+
Pa O_2 in mm Hg*	ND	59-82	77.2	72-75	90	ND	77.5-81	62.9-72	84.9	80.2
Respiratory rate** at rest/min.	18	20-22	32-40	22-36	30-36	16-20	12	16-24	12-16	20-26
Nasal discharge	-	-	+	-	-	-	+	+	+	-
Weight loss	+	+	+	+	+	-	-	-	+	-
Tracheal exudate observed by endoscopy	ND	ND	+	+	+	-	-	-	+	+

TABLE 8.2 (Cont'd)

CLINICAL OBSERVATIONS IN HORSES IN THE COPD STUDY

Observations	PH 8	PH 41	PH 44	PH 51	PH 52	PH 53	PH 54	PH 67	PH 68	PH 69
Bacteria in tracheal aspirate	ND	ND	None	Pseudo-monas spp	Non-haemo-lytic strepto-cocci	Pseudo-monas spp	None	None	None	Alpha Haemo-lytic Strepto-cocci
Cells in tracheal aspirate	ND	ND	Neutro-phils	Neutro-phils	Eosino-phils	Neutro-phils	Neutro-phils	Neutro-phils	A few Macro-phages	Neutro-phils

* Normal PaO_2 = 90 $\pm$ 8 mm Hg (McPherson et al, 1978)

** Normal resting respiratory rate = 8-14/min.

ND = Not done

\+ Observation present

\- Observation absent

TABLE 8.3

ORGANISMS CULTURED FROM SWABS AND LUNG TISSUE TAKEN AT NECROPSY

Horse	Nasal Cavity	Nasopharynx	Guttural Pouch	Trachea	Cranial Lobar Bronchus	Caudal Lobar Bronchus	Lung Tissue
PH 8	-	-	-	Bacillus Species	Unidentified Streptococcus	Unidentified Streptococcus	-
PH 41	-	-	Mucoraceous Fungi	-	-	-	-
PH 44	Gram negative cocci Actino-Bacillus-like Organism	Actino-Bacillus-like Organism	-	-	-	-	-
PH 51	-	Alpha-haemolytic Strepto-cocci Gram negative cocci	-	Gram negative cocci	-	-	-
PH 52	-	-	-	Nonhaemo-lytic Strepto-cocci	-	-	-

TABLE 8.3 (Cont'd)

Horse	Nasal Cavity	Nasopharynx	Guttural Pouch	Trachea	Cranial Lobar Bronchus	Caudal Lobar Bronchus	Lung Tissue
PH 53	-	-	-	Unidentified Streptococci and Staphylococci	Unidentified Streptococci and Staphylococci	Unidentified Streptococci and Staphylococci	-
PH 54	Alphahaemolytic Streptococci	Alphahaemolytic Streptococci	Alphahaemolytic Streptococci	-	-	-	-
PH 67	Pseudomonas Species	Streptococcus zooepidemicus	Gram negative rods	Bacillus Species	-	-	-
PH 68	-	Unidentified Streptococci	Bordetella Bronchiseptica	-	Unidentified Streptococci	-	-
PH 69	-	Pasteurella Pneumotropica	Unidentified Fungus	Unidentified Fungus	Unidentified Fungus	-	-

- = None Cultured

ADDENDUM

Since this work was completed 2 papers have appeared in the literature describing ultrastructural features of the lower airways and lungs of horses affected with COPD.

1. Kaup, F.J., Drommer, W. and Deegan, E. (1990). Ultrastructural findings in horses with chromic obstructive pulmonary disease (COPD) I : alterations of the larger conducting airways. Equine Veterinary Journal, 22, 343-348.

2. Kaup, F.J., Drommer, W., Damsch, S. and Deegan, E. (1990). Ultrastructural findings in horses with chronic obstructive pulmonary disease (COPD) II : pathomorphological changes of the terminal airways and the alveolar region.

Parallel studies to those described in Chapter 8 were carried out by these workers. Their results were generally similar to those described in this thesis, although their studies were confined to the lower respiratory tract.

CHAPTER 9

SUMMARY AND CONCLUSIONS

There were two main aims in the present study. The first was to characterise the normal surface features of the equine respiratory tract with SEM, light microscopy and, where appropriate, TEM. It was apparent from the literature that, although numerous studies of respiratory tract morphology have been carried out in many mammalian species, most workers have concentrated on a section of the tract or even on particular cells. It emerged that much more interest has been shown in laboratory animals such as small rodents and monkeys than in the domestic animals; the larger domestic species such as cattle and particularly the horse have received the least attention. In the horse, the histological features of both upper and lower respiratory tracts have been briefly recorded but the few ultrastructural reports were confined to selected parts of the lower respiratory passages and the lungs.

The second objective was aimed at establishing a picture of respiratory tract surfaces in horses affected with respiratory disease and to assess the nature and extent of damage caused by the various disease processes. Two very different respiratory conditions were chosen for this study; Streptococcus equi infection or Strangles, which primarily affects the upper respiratory tract and COPD which is a disease of the lungs. Although disease of the respiratory system is a serious problem in horses

and Strangles and COPD are two of the most common types, there have been very few comprehensive reports of the histological or ultrastructural features of either of these conditions, or indeed of any other equine respiratory disease.

The main objectives of the work, i.e., characterisation of surface features of the respiratory tract in normal horses and in horses with respiratory disease was achieved. This is the first time that such detailed ultrastructural and histological studies from the nasal vestibule to the alveolar membrane has been carried out in horses.

A number of diverse and complex anatomical structures combine to form the respiratory tract of mammals. In any morphological study, particularly ultrastructural studies, these create a number of technical difficulties. In the larger species of domestic animals the length of the tract and its surface area are particularly extensive and there are some features in the upper respiratory tract of horses which are peculiar to Equidae. Thus it was thought worthwhile to underline the subsequent ultrastructural and histological studies by firstly giving an outline of the gross anatomical features of the equine respiratory tract in Chapter 2.

In Chapter 4, a SEM and histological study of

the upper respiratory tract was undertaken and, in addition, because there was scant information available on the distribution and nature of mucus-secreting cells in the epithelium and mucosal glands, a histochemical analysis was also carried out using AB-PAS stain. In this part of the study, 21 normal horses of various types and sex were used, with ages ranging from 2 days to over 20 years. Two female donkeys were also included in the study. Samples for SEM, histological and histochemical studies were taken from multiple sites from the basal fold of the ventral nasal concha to the ventral larynx.

Patterns of ciliation in the upper respiratory tract mucosa were generally similar in all the animals, regardless of type or age and SEM proved to be more useful than light microscopy for defining the nature of epithelial surfaces by virtue of the relatively large areas available for examination. However, the light microscopic and SEM features were generally similar. Excluding the hairy, stratified squamous epithelium of the nasal vestibule, the most rostral area of the nasal cavity of the horse was found to be clothed with a nonciliated stratified cuboidal type of epithelium with surface mucous cells. The latter contained acidic and mixed muco-substances and were often associated with the numerous duct openings of underlying serous-secreting mucosal glands. SEM revealed a "cobblestone" surface appearance

with polygonal cells covered with microvillous processes. Some of these cells were mucous cells and the latter were observed in various stages of their secretory cycle. While the junction between the nasal vestibule and the nasal mucosa proper was abrupt, the change in the epithelium from the nonciliated rostral mucosa to the well ciliated caudal surfaces was gradual. Ciliated surface cells initially appeared singly, then in groups and became more numerous until the epithelium was almost completely ciliated. This gradual change from non-ciliated rostral to well ciliated caudal surfaces, in the nasal cavity, has been described in a number of mammals and the partially ciliated area, which can be extensive, has been termed the transitional zone. This zone appeared to represent a considerable proportion of the nasal mucosa in the horse and extended at least to the level of the first cheek tooth.

Nasopharyngeal surfaces were clothed with patches of ciliated cells and microvillous cells while the guttural pouches were usually well ciliated. Stratified squamous epithelium merged with a stratified cuboidal type on the epiglottis and in the latter a few ciliated cells were usually present. The surface of the ventral larynx was covered with well ciliated epithelium.

Surface mucous cells and mucosal glands were plentiful in the upper respiratory tract. The glands

were serous in type rostrally and sero-mucous caudally. The contained mucosubstances were usually acidic or mixed in the surface cells and glands, although a few neutral staining cells were present; the latter were most numerous in the glands.

In this initial study, the number of sample sites in the nasal cavity was limited to five and it was felt that, given the overall length of the nasal cavity, this did not give a complete picture of the true extent of the transitional zone. With this in mind, Chapter 5 was devoted to a more thorough systematic SEM and histological study of the nasal cavity of 4 horses. Eight levels were chosen, rostral to caudal, which corresponded to precise anatomical landmarks and at each level samples were taken from the dorsal nasal concha, ventral nasal concha and nasal septum (24 samples per horse). In addition, in order to characterise more fully the nature of the sparsely ciliated rostral epithelium, samples for TEM were taken from the 4 rostral levels (12 samples per horse), from 2 of the horses. As a result of this study, the patterns of ciliation in the nasal cavity were more clearly mapped out. Only the most rostral level was completely nonciliated and caudally cilia first appeared on the dorsal nasal concha, then the ventral nasal concha and finally on the nasal septum. In the latter site the number of ciliated cells was

generally less than in the former; indeed even at the most caudal level where the nasal conchae were well ciliated, there were still patches of microvillous cells on the surface of the nasal septum. Using measurements made in the head of an average 14.2 h.h. adult animal, it was calculated that the transitional zone, which extended to the level of the second cheek tooth (a distance of 10.5 cm), represented approximately 35% of the total length of the nasal cavity of the horse.

Olfactory epithelium in the horse is mostly confined to the most caudo-dorsal area of the nasal cavity and was not present in any of the sample sites used in Chapters 4 and 5. To complete the upper respiratory tract study, samples were taken from the ethmoidal conchae of 2 horses for SEM and histology and the results indicated that the olfactory epithelium of horses resembles that of other mammals.

In Chapters 4 and 5 the detailed ultrastructural features of equine upper respiratory tract surfaces were established for the first time. Using multiple sample sites it was possible to create a comprehensive map of the areas of nonciliated and ciliated epithelium. SEM proved to be the most useful method for detailing epithelial surface characteristics while TEM confirmed the presence of stratified cuboidal type of epithelium in the rostral nasal cavity.

In Chapter 6, an ultrastructural, histological and histochemical study of the lower respiratory tract was carried out. For SEM, histology and histochemistry samples were taken from sites in the dorsal and ventral trachea, cranial and caudal lobar bronchi. After airway perfusion, via the right cranial lobar bronchus for electron microscopy and the left cranial lobar bronchus for light microscopy, small bronchial samples and lung slices were taken.

Light microscopy revealed that, in common with many other mammals, the trachea and bronchi of the horse were clothed with pseudostratified columnar ciliated epithelium, i.e., respiratory epithelium. Mucus-secreting surface cells were numerous and contained mixed and acidic mucosubstances. Mucosal sero-mucous glands were not numerous in the trachea and large bronchi and were scanty in small bronchi. A luxuriant carpet of cilia was revealed with SEM in the trachea and large bronchi, with mucous cells interspersed among the ciliated cells. The former became more numerous and obvious in the small bronchi giving a "moth-eaten" appearance to the surface.

In the lung slices, small bronchi, bronchioles, alveolar ducts and alveoli were all visualised. Respiratory bronchioles were not present. The bronchiolar epithelium of the horse appeared to be similar to that

of other mammals, i.e., a simple cuboidal type with ciliated cells and nonciliated bronchiolar epithelial (Clara) cells. As with the examination of the upper respiratory tract, SEM gave a much clearer picture than that offered by light microscopy alone and revealed that the ciliated cells became progressively sparse as the bronchioles decreased in size while the Clara cells increased in number and became the predominant cell in the terminal bronchioles. However, both cell types were present at the junction between these passages and the alveolar ducts and the junction itself was abrupt with bronchiolar epithelial cells lying adjacent to the cells of the alveolar membrane. The distribution of epithelial cell types in bronchioles was confirmed by TEM, as was the nature of the equine Clara cells, the latter containing numerous electron-dense apical granules and large amounts of smooth endoplasmic reticulum.

In the lung parenchyma interalveolar pores were present in all the animals and the number appeared to increase with age. In younger horses 0-6 pores per exposed portion of alveolus was usual while in animals over 6 years old most alveoli had an increased number of pores with as many as 20 per alveolus in old animals. Histologically, details of cells of the alveolar membrane was not clear but SEM clearly distinguished the surface features of type I and type II pneumocytes.

The former, which covered most of the alveolar surface, had a few small microvillous processes on their uneven surfaces and cell boundaries were clearly visible. The distinctive type II pneumocytes occurred singly or in small groups of 2 or 3 cells, protruded into the alveolar space and possessed a peripheral fringe of microvilli surrounding a smooth central area which often contained small depressions or pores. A third cell type was the alveolar macrophage which was readily observed in all the animals. These cells had uneven or ruffled surfaces and their most characteristic feature was the presence of long, slender cytoplasmic processes which extended from the cell borders. Macrophages were often observed either emerging from, or descending into, alveolar pores.

Mucosal lymphoid tissue was recognised histologically and ultrastructurally in the complete study of the normal respiratory tract. Its morphology and distribution resembled that described in previous studies by Mair et al (1987, 1988).

Brush cells and pulmonary neuroendocrine cells described in some other mammals were not identified in the respiratory tract mucosa of the horse.

The combined studies of Chapters 4, 5 and 6 now provide more information on the entire respiratory tract of normal horses than has hitherto been available and gives a sound basis for future studies in horses with

respiratory disease.

Following on from the studies of the normal equine respiratory tract, an investigation of Streptococcus equi infection or Strangles was conducted in Chapter 7. Spontaneous clinical disease in a group of 19 young ponies under one year old obviated the need to infect healthy animals with Streptococcus equi experimentally. The infection was allowed to spread naturally within the group and the ponies were monitored clinically and bacteriologically for a period of 3 months after the initial case. Ten ponies, divided into 4 groups each representing a stage in the course of the disease from day 4 to day 90 of the outbreak, were used in a systematic histological, SEM and TEM study of the entire respiratory tract. A further 2 adult ponies, in the acute stage of Strangles, were also included in the study. The sample sites were the same as those used in Chapters 4 and 6, with additional samples in selected animals for SEM and histology from the soft palate.

All the ponies in the study had positive swab cultures of Streptococcus equi in the early stages of the illness, however, in later stages and in the recovery period Streptococcus zooepidemicus was cultured. Only one pony had evidence of concurrent virus infection on serological examination, which suggests that this was not an important factor in the development or course of

Strangles.

At necropsy, all the ponies had some degree of upper respiratory tract disease, ranging from acute inflammation in the nasopharyngeal region with associated lymphadenitis and abscess formation in the early stages, to mild enlargement of retropharyngeal lymph nodes and the presence of inspissated pus in the guttural pouches in clinically recovered animals.

During the acute stage of the disease the main histological feature was marked subepithelial lymphoid hyperplasia in the nasopharynx with formation of follicles and migration of lymphocytes and neutrophils through the epithelium. In clinically recovered animals, follicular lymphoid hyperplasia with germinal centres was a notable feature of the nasopharynx and this suggested a local mucosal immune system response.

SEM proved to be more useful than light microscopy in detecting damage to the epithelial surfaces. Patchy loss of surface cilia was observed in the guttural pouches, larynx, trachea and bronchi. A point of interest was that loss of cilia was most marked in the clinically recovered group of animals killed at day 90 of the outbreak. This loss of surface cilia, not only from upper respiratory tract surfaces but also from lower respiratory passages suggests that the effects of Streptococcus equi infection are more widespread and

more prolonged than had been previously described.

The subepithelial lymphoid hyperplasia, which was such an obvious histological feature, was less noticeable with SEM and detected in only a few animals by the presence of raised, circumscribed areas in nasopharyngeal surfaces.

In the acute stage of the disease, groups of Gram positive cocci were observed on the oral surface of the soft palate. Groups of cocci were also visualised by SEM adhering to nonciliated surface cells in the soft palate and in the nasal cavity of acutely ill ponies and, in the former site, in clinically recovered animals. The greatest number of bacteria were found on the oral surface of the soft palate suggesting that this was a preferred site for adherence and could create a reservoir of infection.

None of the ponies showed any evidence of disease in the lungs histologically or with SEM and TEM.

In this study of Streptococcus equi in horses, the surface features of the respiratory tract have been clearly defined for the first time. Although Strangles is considered to be a disease of the upper respiratory tract, SEM studies showed damage to both upper and lower respiratory tract epithelium. Adherence of bacteria to nonciliated surfaces in the nasal cavity and soft palate was also demonstrated with SEM. The opportunity to

study affected ponies over a long period proved to be useful as it demonstrated that effects of Streptococcus equi infection are prolonged and can persist for at least 3 months. The combination of studies with SEM and light microscopy confirmed previous reports and added to the overall knowledge of the disease.

Further studies of spontaneous respiratory disease were carried out in the final chapter of investigative work (Chapter 8), with the examination of 10 horses affected with chronic obstructive pulmonary disease (COPD). The primary aim was to conduct a systematic histological and ultrastructural study to determine the extent of surface changes in the respiratory tract mucosa, not only in the lung but also in the larger, lower respiratory passages and in the upper respiratory tract. The sample sites for SEM, TEM and light microscopy used in Chapters 4 and 6 were repeated in the horses with COPD with additional samples taken from cranial and caudal segmental bronchi and the caudal lobes of the lung.

The severity of the clinical signs was usually reflected in the gross pathological, light microscopic and SEM findings. In general, upper respiratory tract surfaces were normal with the exception of patchy loss of surface cilia from the guttural pouches of 6 horses and the ventral larynx of 4 horses. Nonciliated patches

became increasingly frequent in lower respiratory tract surfaces from the trachea to the small bronchi. This loss of cilia probably contributed to impaired mucociliary transport which was indicated by the presence of mucus or mucopurulent exudate in the trachea and bronchi of most horses at necropsy and also observed histologically and with SEM in the small bronchi and bronchioles. The most obvious lesions, however, were confined to the lungs and this was apparent at necropsy and on histological and ultrastructural examinations.

Bronchiolitis was the main histopathological lesion and many, but not all, bronchioles were affected. Mononuclear cell infiltration of the peribronchiolar tissue was a prominent feature with lymphocytes predominating and occasionally forming follicles. Changes in the epithelium included hyperplasia and the presence of cells containing acidic and mixed mucosubstances. Many bronchioles contained mucus and neutrophils. Focal alveolar overinflation was also a feature and emphysema was present in 6 horses.

The changes in alveolar structure were particularly dramatic when viewed with SEM, which also revealed an increase in the number and size of alveolar pores. At the bronchiolar level changes in the epithelium were focal. Many bronchioles contained mucous exudate and degenerate cells, some ciliated cells

had sparse cilia and Clara cells often appeared collapsed and flattened, however, no obvious mucus-secreting cells were observed.

With TEM there were profound changes in the structure of both ciliated cells and Clara cells. In the former, loss of cilia, the presence of vacuoles, lipid-like material, myelin configurations and glycogen were common, while partial or complete loss of granules was the most obvious change in the latter. In some bronchioles nonciliated cells contained what appeared to be mucous granules, although the latter often had a floccular appearance. It was interesting that apparent mucus-secreting cells in bronchioles were observed with light microscopy and TEM but were not seen with SEM. It is difficult to explain this discrepancy but it could indicate that the mucus-secreting cells which develop in bronchioles in COPD are not morphologically the same as the mucous cells present normally in the respiratory tract epithelium. The nature and origin of these mucous cells awaits a more detailed investigation.

Changes in the cell population of the alveolar membrane include an increased number of type II pneumocytes and alveolar macrophages in some alveoli. The former often had large central pores or craters or appeared degenerate, suggesting increased surfactant secretion to the point of exhaustion. TEM confirmed

the appearance of type II pneumocytes and the increased number of aveolar macrophages, which often contained phagocytosed surfactant material. Ultrastructurally the type I pneumocytes appeared morphologically normal, but the presence of many more than usual type II pneumocytes suggests that there must have been some damage to the former, as the latter are regarded as progenitor cells and capable of repairing the alveolar epithelial surface. Focal alveolar septal fibrosis noted with TEM also suggest damage to the alveolar membrane.

In combining histological and ultrastructural methods of investigation, in the entire respiratory tract, the studies in Chapter 8 have confirmed some of the results of previous work and have detailed more fully the changes at bronchiolar and alveolar level. However, many questions remain unanswered and more detailed studies of the cell types which are involved in the disease process will have to be undertaken before there is a full understanding of COPD in horses.

In conclusion, the present work has demonstrated the usefulness of SEM, not only in the study of normal equine respiratory tract surfaces, but also in the investigation of respiratory disease in horses. In addition it has shown the value of combining SEM and TEM for the complete characterisation of certain cell types.

This is the most comprehensive study ever undertaken of respiratory tract surfaces in the horse (and probably in any domestic mammal). Furthermore, it has clearly demonstrated that only by carrying out systematic studies of the entire respiratory tract can a complete picture be gained of the varied surface morphology of the mucosa in normal horses and in horses with respiratory disease.

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