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COMPARATIVE PULMONARY STRUCTURE AND FUNCTION IN TWO LARGE MAMMALS, THE HORSE AND THE COW

By

© GORDON JAMES GALLIVAN, B.SC., M.SC.

A Thesis

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ABSTRACT

Pulmonary structure and function were compared in healthy adult horses and cows to examine the hypothesis that differences in pulmonary function between two species of similar size were related to differences in pulmonary structure. The functional residual capacity and tidal volume were higher in the horses than in the cows while the respiratory rate was higher in the cows. The pattern of air flow differed between the two species, and flow rates were higher in the cows. The dynamic compliance was higher in the horses while the work of breathing was higher in the cows. Separation of the lower pulmonary system (trachea to pleural cavity) from the total pulmonary system (nares to pleural cavity) indicated that most of the resistance and work of breathing were in the upper airways (nares to trachea), and there were no differences in the mechanical indices of pulmonary function in the lower pulmonary system between the two species.

To more adequately characterize the lungs, horses and cows were anaesthetized and pressure-volume manoeuvres were performed to determine the lung capacities and quasistatic compliance. The lung capacities were significantly greater in the horses, but the lung compliance did not differ significantly between the two species.

Quantitative and qualitative observations revealed few significant differences between the structure of the lung parenchyma in

horses and cows. The obvious structural differences were in the size and shape of the lungs, the branching pattern of the airways, and the amount and distribution of the interlobular septa.

During the measurements of pulmonary mechanics in the standing animals there were several anomalous observations and examination of the pressure recordings revealed phase lags between the total and lower pulmonary systems. It would appear that it is invalid to assume that inertia is a negligible factor in pulmonary function in large mammals such as the horse and the cow, due to the large abdomen in these species. The size and shape of the abdomen has probably been a significant factor in the evolution of lung structure in horses and cows, and abdominal movements are important in determining the breathing patterns in these two species.

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INTRODUCTION

The primary function of the mammalian respiratory system is the exchange of metabolic gases between the body and the environment. It performs this function in mammals ranging in size from the 2.5 gm shrew to the 150 metric ton blue whale. In spite of this seemingly vast array of subjects from which to choose, much of our knowledge of respiratory function comes from studies conducted on man as he is a co-operative, if not always willing, subject. However, man is a unique animal and one must question whether concepts developed to explain his respiratory function can apply to other mammals of diverse size exposed to a wide range of environmental conditions.

To gain a broader understanding of the form and function of the respiratory system, several workers (Agostoni 1972, Amoroso et al. 1963, Crosfill and Widdicombe 1961, Drorbaugh 1960, Leith 1976, Taylor and Weibel 1981, Tenney and Remmers 1963, Spells 1969/70, Stahl 1967, Weibel 1973) have compared various indices of pulmonary function in several species. Often allometric equations have been used to describe the relationships between the various indices and body weight, or between indices of lung structure and function. These equations have shown an excellent correlation ($r > 0.9$) between the various indices of pulmonary function and body weight (Stahl 1967), however, there are some limitations to the interpretation of these relationships. For many indices of pulmonary function, such as lung compliance, diffusing capacity and work of breathing, there were no data for species larger than

man, and the allometric equations should not be extrapolated to include large mammals. Even in those cases in which the data spanned a wide range of body weights, often most of the data was clustered within a relatively small weight range and the slope depended on one or two outlying values. Also, the variability about the calculated regressions is high with mean standard errors of estimate ranging from 20 to 50% (Stahl 1967). For animals of the same weight, the value of a given index can differ by a factor of 3 or 4. This suggests that while allometric relationships may provide insight into the relationship between body size and lung function, other factors must also influence lung function.

The veterinary literature has long recognized that there are differences in the gross anatomical structure of the lungs of domestic mammals (Sisson 1914). McLaughlin et al. (1961a) extended these observations and on the basis of lobular pattern, bronchovascular relationships and terminal airways categorized the lungs of domestic mammals and man as Types I, II or III. The basic differences between these types are summarized in Table I. McLaughlin et al. (1961a, 1961b) suggested that the differences in lung structure were related to differences in function, and that the structural differences should be considered when making interspecific comparisons. This would appear to be a logical conclusion as pathophysiologists have long recognized that changes in lung function occur when pathological processes alter the structure of the lung. More recently, Weibel (1973) and Taylor and Weibel (1981) have postulated that structure and function are related in the normal lung, and that differences in the microscopic

Table I. Subgross lung types found in seven mammalian species and man (after McLaughlin et al. 1961a, 1961b).

Subgross Type	I	II	III
Species	cow, sheep, pig	monkey, dog, cat	horse, man
Lobulation	extremely well developed	absent	imperfect
Pleura	thick	thin	thick
General broncho-Vascular Relationships	blood vessels close to airways from hilum to periphery	Pul. vein has an independent course from periphery to hilum	Pul. vein near bronchi at periphery, apart at hilum
Termination of Bronchial Artery	distal airways	distal airways	distal airways and alveoli
Terminal Bronchioles	present, predominant distal airway	absent	present
Repiratory Bronchioles	infrequently observed, extremely poorly developed	present, very well developed	present, poorly developed
Intrapulmonary Shunts	present	not demonstrated	present

structure of the lungs of mammals are related to the O_2 requirements at rest or in exercise. Lung structure may also influence other indices of pulmonary function, such as collateral resistance (Robinson and Sorenson 1978, Woolcock and Macklem 1971), but there have been no comprehensive studies examining the influence of lung structure on the mechanical properties of the lungs and on the breathing pattern of animals. The present study, therefore, is an examination of the relationship between lung structure and pulmonary function to determine if the differences in lung structure between species could account for some of the variability about the allometric equations.

Multiple regression analysis provides one approach for the comparison of the relationship between the structure and function of the different types of lungs in different species. However, complete data on pulmonary structure and function are probably only available for the rat, dog and man. It is both time-consuming and expensive to collect sufficient data from enough species to justify the use of multiple regression analysis to test the hypothesis that lung structure is related to pulmonary function (Taylor and Weibel 1981). An alternative approach is to compare pulmonary function in animals of similar size, but which have differences in lung structure. This approach was the basis for the present study of pulmonary structure and function in the horse and the cow.

Several species, man, rat, cat, dog, sheep, pig, horse and cow, were considered for the comparison of pulmonary structure and function in the present study. Mature horses and cows were chosen as they are of similar size and posture, and are larger than the species normally

used in pulmonary function studies. It is well recognized that there are differences in the gross structure of the lungs of horses and cows (Sisson 1914, McLaughlin et al. 1961a, 1961b), and is generally accepted that there are differences in the breathing patterns of the two species (Smith 1921, Kelly 1974). As such, these species provided an interesting opportunity to examine the hypothesis that differences in lung structure are related to differences in pulmonary function in mammals of similar size. Also, they provided an opportunity to further information on the influence of body size on pulmonary function.

LITERATURE REVIEW

In the present study many aspects of pulmonary structure and function were examined. As there is an extensive literature on almost all aspects of pulmonary structure and function, a detailed review of all aspects was not feasible. Therefore, this review is restricted to the literature describing the pulmonary anatomy and function in horses and cows.

Anatomy:

Gross:

Horses and cows breathe through the nose during normal quiet breathing. In the horse the upper respiratory tract, from the nares to the trachea, accounts for approximately 80% of the pulmonary resistance (Viel 1980, Willoughby and McDonell 1979), and as such is an important component of the respiratory mechanics. Therefore, this section will include a description of both the upper and lower respiratory tracts. Unless otherwise indicated the anatomical descriptions are based on the reviews of Hare (1975a, 1975b).

During quiet breathing the nares of horses and cows are shaped like inverted commas and are oriented obliquely, so that they are closer ventrally than dorsally. In the horse, the nares are an

important site of airflow obstruction (Cook 1966), and during normal breathing the resistance of the nares accounts for approximately 77% of the resistance of the upper respiratory tract during inspiration and 68% during expiration (Robinson et al. 1975).

The nares lead to the nasal cavity which is divided into the dorsal, medial and ventral meati by the dorsal and ventral conchae. The ventral meatus is the primary pathway for airflow in the horse (Cook 1966) and airflow probably follows a similar route in the cow (Pass et al. 1971). The nasal conchae are larger in the horse than in the cow, and in the horse an opening in the ventral concha communicates with the maxillary sinus. In both species the conchal folds and lining of the nasal septum contain venous sinuses. In the horse the sinuses are cavernous, and during exercise are emptied of blood to enlarge the ventral and common meati (Cook 1966).

From the meati air passes to the pharynx. In the horse the pharynx is relatively narrow and Cook (1966) has postulated that this is another site of airflow obstruction. Measurements of the pressure drop across the pharynx during inspiration (Robinson et al. 1975) do not support this hypothesis. There is no information on the pressure change across the pharynx during expiration. As with the other structures of the upper respiratory tract, there are no measurements of the dimensions of the pharynx in horses and cows.

The larynx of horses and cows is located at the posterior border of the mandible. The distance from the nares to the larynx is greater in the horse than in the cow (Lodge 1969). Cook (1966) also postulated that the larynx is another site of airflow obstruction in

the horse. During normal breathing the resistance of the larynx accounts for approximately 16% of the resistance in the upper respiratory tract during both inspiration and expiration (Robinson et al. 1975).

The trachea of cows is 65 cm in length, and is ellipsoid, with the greatest diameter in the sagittal plane. The trachea of the horse is 70 to 80 cm in length, and at the origin forms a circle 5.5 cm in diameter. At the midpoint it is flattened dorsoventrally forming an ellipse 5 cm high and 7 cm wide.

The lungs of horses and cows are approximately 45 cm in height, but the equine lung is longer than the bovine lung. The equine lung weighs approximately 1.02% of body weight, whereas the bovine lung weights approximately 0.73% of body weight (Altman et al. 1958). In both species the right lung is larger than the left lung. In the horse the ratio is 55:45 (McDonell 1974) and in the cow the right lung is 1.5 to 2 times larger than the left. The relative position of the lungs in both speices is shown in Figure 1. The apex, or most anterior tip of the lung is slightly anterior to the cranial edge of the first rib, and the dorsal border angles upward at an angle of 15 to 20 degrees. In the horse the lung extends caudally to the 16th intercostal space. The lateral aspect of the basal or diaphragmatic border is convex, passing from the vertebral end of the 16th intercostal space downward across the 11th rib slightly ventral to its middle, and along the 8th and 9th costal cartilages to terminate at the costochondral junction of the 6th rib. In the cow the lateral aspect of the basal border extends in a straight line from the vertebral end of the 11th rib to

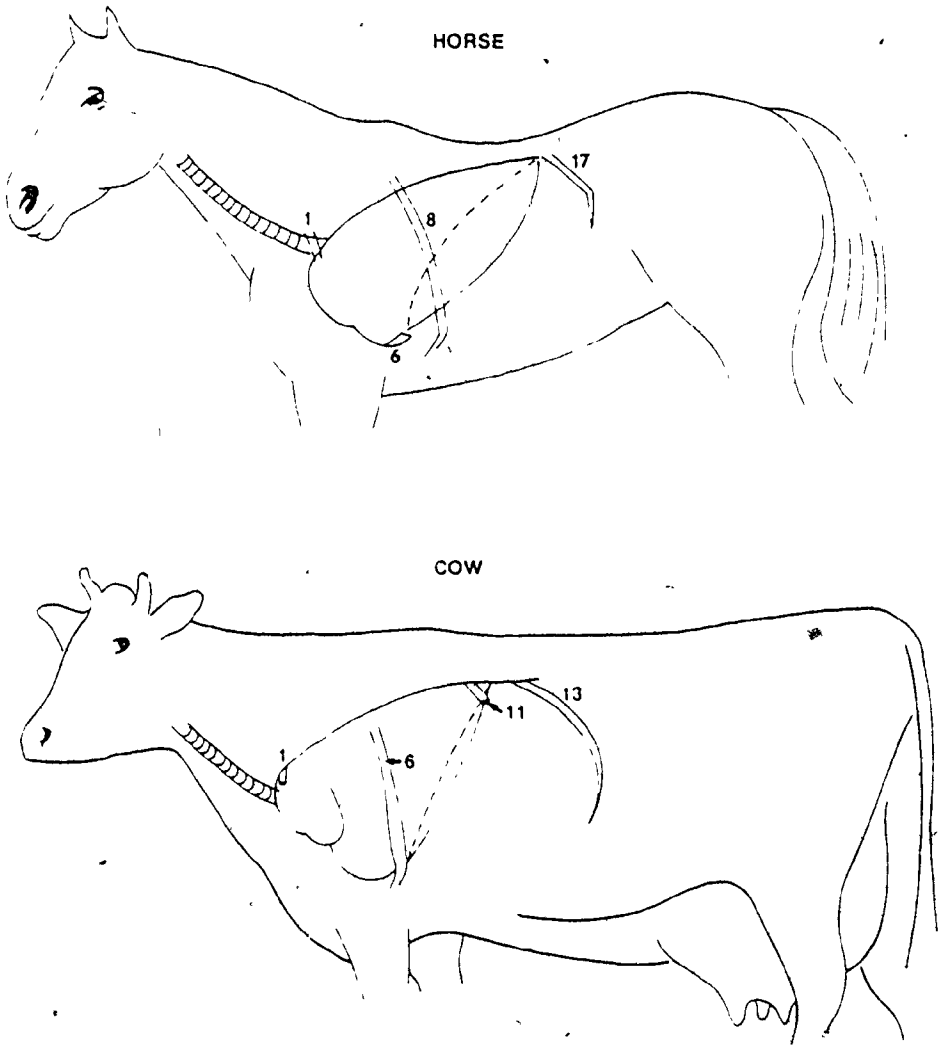


Figure 1. Anatomical sketch of a horse and cow showing the position of the lungs in the body. The numbers indicate the appropriate ribs, and the dotted line indicates the cranial aspect of the diaphragm. (after Getty 1964)

the costochondral junction of the 6th rib. In the horse the diaphragm is conical in shape with the cranial aspect extending well forward of the lateral edges of the basal border. In the cow the diaphragm is relatively flat (Figure 1).

The equine lung is not obviously divided into lobes and each lung appears to form a single unit. In contrast the lobes of the bovine lung are separated by deep fissures. Anatomically, the left lung of the horse is divided into cranial and caudal lobes, and the right lung is divided into the cranial, caudal and accessory lobes. In the cow the left lung is divided into the cranial, middle and caudal lobes while the right lung is divided into the cranial, middle, caudal and accessory lobes. In the cow the cranial lobe of the left lung is much smaller than the cranial lobe of the right lung and this accounts for much of the size difference between the two lungs.

In both species the hilum is at approximately mid-lung height. It is located at the level of the 5th intercostal space in both the horse and the cow. Despite the differences in the lobes of the lungs, the branching patterns of the primary bronchi and pulmonary vasculature in the horse and cow are similar to those in other species (Suzuki and Ohkubo 1977). An important exception is that the right apical lobe of the bovine lung communicates with the trachea via an accessory bronchus which arises cephalad to the carina.

McLaughlin et al. (1961a) described the bovine lung as obviously lobulated with complete interlobular septa. The pulmonary vasculature is closely associated with the bronchial tree, the bronchial artery terminates at the distal bronchioles, the terminal

bronchiole is the distal airway, and respiratory bronchioles are infrequent and poorly developed. In their scheme, the bovine lung was classified as Type I. In contrast lobulation of the equine lung is incomplete, with well defined but haphazardly arranged interlobular septa. The pulmonary artery closely parallels the bronchial tree while the pulmonary vein is close to the bronchi at the periphery, and diverges towards the hilum. The bronchial artery has two branches paralleling each bronchus and the bronchial artery extends to the alveoli. The horse has both terminal and respiratory bronchioles. This pattern of lobulation, broncho-vascular relationships and distal airways was classified as Type III.

Histology:

As in other species, the upper respiratory tract, trachea and bronchi of horses and cows are lined with pseudostratified columnar epithelium. Pass et al. (1971) described the regional variations in the thickness of the mucosa and in the frequency of goblet cells in the nasal epithelium of calves. Jericho and Magwood (1977) described the changes in the frequency of goblet cells, vacuolation and polymorphonuclear leucocytes in the airway epithelium of calves exposed to different environmental conditions. From their results it is difficult to appreciate any significant differences in the frequency of cell types from the nose to the bronchioli. Allan (1978) reported that brush cells are present in the bovine airway but are not found distal to the bronchi. The only study of the airway epithelium of the horse is that of Viel (1980) who reported that the relative

frequency of goblet cells increases from the upper trachea to the bronchi.

Studies using the electron microscope (EM) (Epling 1964, Gillespie and Tyler 1967, Rybicka et al. 1974a) have shown that the cell types and basic structure of the alveolar septa of equine and bovine lungs are similar to those reported for other species (Meyrick and Reid 1970, Weibel 1973). The alveolar septum is composed of Type I and Type II pneumocytes, endothelial cells, fibroblasts, collagen and elastin. There are also macrophages, and occasionally mast cells, plasma cells, lymphocytes and polymorphonuclear leucocytes are seen. Epling (1964) stated that the bovine lung contains few capillaries and little elastin, but provided no quantitative evidence. He also reported that there are few intra-alveolar macrophages. This latter observation has been confirmed by Rybicka et al. (1974a) and in a subsequent publication (Rybicka et al. 1974b), this group reported that most of the macrophages are intravascular. Rybicka et al. (1974a) also reported collagen and elastin in the alveolar septum of the bovine lung. Gillespie and Tyler (1967) quantitated the distribution of cell types in the alveolar septum of the horse. They reported that the distribution of cell types is similar to that in other species, and that there is only a small amount of collagen.

In their paper describing the use of the scanning electron microscope in the study of lung morphology, Nowell and Tyler (1971) described the terminal airways and alveolar septum of equine lungs, and demonstrated alveolar macrophages, and Type I and Type II pneumocytes. Mariassy et al. (1975) described the bovine lung, reporting

that it is divided into distinct lobules, alveolar macrophages are rare and that the pores of Kohn are small and rare. This is in contrast to the equine lung in which pores of Kohn are common and large (Gillespie and Tyler 1967, Nowell and Tyler 1971). In contrast to the findings of McLaughlin et al. (1961a), Mariassy et al. (1975) reported abundant respiratory bronchioles in the bovine lung, but noted that they are short and poorly developed.

There is little information on the morphometry of equine and bovine lungs. From the data of Tenney and Remmers (1963), at an inflation pressure of 20 cm H_2O, the alveolar diameter of the cow is about 97 µm and the alveolar surface area is approximately 316 m^2. More recently, Gehr et al. (1977) reported that the alveolar surface density was 755 cm^2 of alveolar surface per cm^3 of lung parenchyma in the equine lung and 635 cm^2/cm^3 in the bovine lung. The respective diffusing capacities were 6.93 and 2.5 ml O_2/min/torr/kg. A more complete description of the morphometry of the lungs of two horses is provided by Gehr and Erni (1980). They reported that 86.2% of the lung was gas exchange parenchyma, and alveolar volume was 52.7 mg/kg body weight, and the capillary volume and tissue volume were equal at 5.5 ml/kg. The alveolar surface area was 2457 m^2 and the capillary surface area was 1663 m^2. Gehr and Erni (1980) observed significant differences in capillary volume, tissue volume, capillary surface density and diffusing capacity between the cranial and caudal lobes. However, the differences between the cranial and caudal lobes may be artefacts as the lungs were fixed in situ while the horses were positioned in dorsal recumbency (supine). Radiographic evidence (McDonell

1974, McDonell et al. 1979) shows that in horses in dorsal recumbency there is significant compression of the caudal lobes by the abdominal viscera.

Pulmonary Function:

Gas Exchange and Metabolism:

The resting metabolic rates of horses and cows are similar over a wide range of body weights, and the basal metabolic rates of these species are similar to those predicted for mature mammals of equivalent size (Brody 1945). While several other authors have reported metabolic rates of horses and cows, often it is not possible to compare their results to those of Brody (1945). Some authors (Grover et al. 1963, Hales and Findlay 1968a, Orr et al. 1975, Will et al. 1978) did not report the weight of their animals or the measurements were made on fasting animals, whereas the animals used by Brody (1945) were not always post-absorptive. The fasting metabolic rates of standing horses (Mauderley 1974, Thomas and Fregin 1981) are less than the metabolic rates reported by Brody (1945), while the fasting metabolic rates of cattle (Bisgard et al. 1973, Kiorpes et al. 1978, Reeves et al. 1962) are equal to or greater than Brody's values. In a more recent study of the comparative metabolic rates of ponies and steers (Taylor et al. 1978), the resting metabolic rate was the same in both species, but the maximum O_2 uptake ($\dot{V}_{O_2}$ max) of the ponies was three times higher than that of the steers.

Many papers contain data on the minute ventilation ($\dot{V}_E$), tidal

volume (V_T) and respiratory rate (f_R) of horses and cows. Figure 2 is a summary of this data. It would appear that V_T is similar in the two species, but that f_R and $\dot{V}_E$ are higher in the cow.

Arterial blood gases are routinely sampled in many studies, therefore there is a considerable body of information on the arterial blood gases of horses and cows. However, one must exercise caution in interpreting this data as many authors do not specify the health of their animals, and experimental and environmental conditions vary. This is particularly important for cows as they hyperventilate in response to mild thermal stress (Findlay 1957, Hales and Findlay 1968a, McMurtry et al. 1975), and much of the published work was conducted at high altitude (Grover et al. 1963, Hecht et al. 1962, Kuida et al. 1961, McMurtry et al. 1975, Will et al. 1978).

The arterial pH (pH_a) of normal horses at or near sea level ranges from 7.38 (Hilledge and Lees 1975, Willoughby and McDonell 1979) to 7.46 (Gillespie et al. 1964), while the pH_a of normal cows at sea level ranges from 7.31 (Bisgard et al. 1973) to 7.49 (Musewe et al. 1979). The arterial CO_2 pressure (P_{aCO_2}) of horses ranges from 35.3 (Meister 1976) to 47.0 torr (Hilledge et al. 1975) and the arterial O_2 pressure (P_{aO_2}) ranges from 83.6 (Willoughby and McDonell 1979) to 103 torr (Gillespie et al. 1964). For cows, P_{aCO_2} ranges from 38.7 (Bisgard et al. 1973) to 48.8 torr (Hales and Findlay 1968a) and P_{aO_2} ranges from 73.3 (Kiorpes et al. 1978) to 93.6 torr (Donawick and Baue 1968).

The alveolar ventilation ($\dot{V}_A$) of ponies is 64 to 65 ml/kg/min, and the dead space to tidal volume (V_D/V_T) ratio is 0.77 (Orr et al.

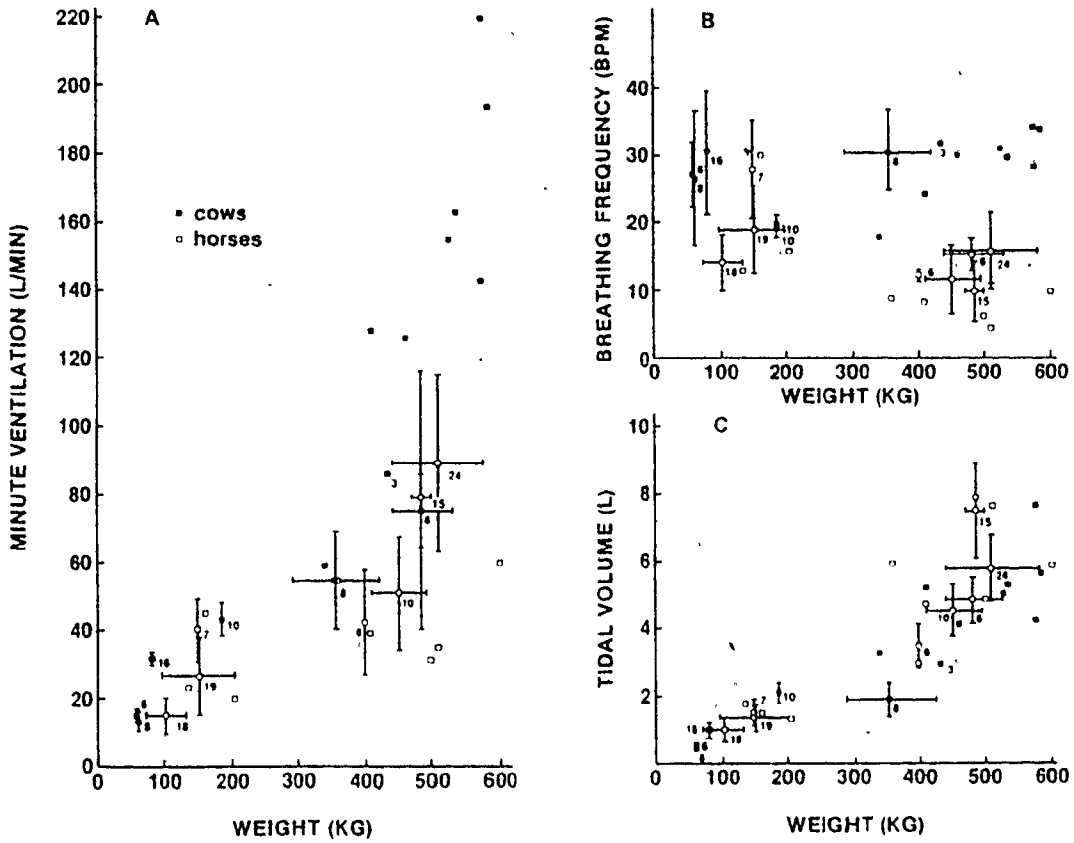


Figure 2. A summary of the values for minute ventilation ($\dot{V}_E$) (A), respiratory rate (f_R) (B) and tidal volume (V_T) (C) reported for horses and cows. The vertical and horizontal lines represent 1 standard deviation, and the numbers are the sample sizes. The points without lines or numbers represent individual animals. The values for $\dot{V}_E$, f_R and V_T were reported by Amoroso et al. (1963), Bisgard et al. (1973), Bisgard et al. (1979), Garner et al. (1971), Gillespie et al. (1966), Hales and Findlay (1968a), Kiorpes et al. (1978), Mauderley (1974), Muir et al. (1975), Musewe et al. (1979), Orr et al. (1975), Patterson et al. (1965), Purchase (1965), Sasse (1971), Viel (1980), Willoughly and McDonell (1979).

1975, Bisgard et al. 1979). However, the measurements of V_D/V_T are questionable as the f_R was 28 whereas the normal value is 12 (Kelly 1974). The V_D/V_T ratios of 0.62 and 0.67 reported by Forster et al. (1976) and Hall et al. (1968) are more likely to be correct. The $\dot{V}_A$ of calves is even higher than that of ponies ranging from 75 ml/kg/min (Hales and Findlay 1968a) to 114 ml/kg/min (Kiorpes et al. 1978). The V_D/V_T ratio of calves ranges from 0.57 (Kiorpes et al. 1978) to 0.66 (Hales and Findlay 1968a). The difference between the $\dot{V}_A$ of ponies and calves is most likely due to the age of the animals. The ponies were mature whereas the calves were immature. Immature animals have a high $\dot{V}_{O_2}$ and $\dot{V}_E$ relative to mature animals of a similar weight (Kleiber 1975).

The values reported for the alveolar-arterial O_2 pressure differences ($P_{(A-a)O_2}$) in normal horses varies widely. Gillespie and coworkers (Hall et al. 1968, Gillespie and Tyler 1969) reported $P_{(A-a)O_2}$ values of 18 and 26 torr respectively, and Garner et al. (1971) reported a $P_{(A-a)O_2}$ of 15.7 torr. Other workers (Mauderley 1974, Viel 1980) have reported that $P_{(A-a)O_2}$ ranges from 7.5 to 11 torr. The reported $P_{(A-a)O_2}$ of calves ranges from 11.7 (Donawick and Baue 1968) to 23.0 torr (Kiorpes et al. 1978).

There are no calculated values of the O_2 diffusing capacity of equine or bovine lungs but the CO diffusing capacity (D_{LCO}) has been reported for the equine lung. In the normal adult light horse D_{LCO} is 366 ml/torr/min (Gillespie and Tyler 1968) and in ponies ranges from 35 to 50 ml/torr/min (Mauderley 1974). Further comparison of these values is difficult as Gillespie and Tyler (1968) did not report the weight of their horses.

Lung Volumes:

Horses and cows will not inspire and expire maximally on command, therefore other than V_T, the functional residual capacity (FRC) is the only lung volume which can be measured in the unanaesthetized standing animal. The FRC of normal light horses is approximately 48 ml/kg body weight (Willoughby and McDonell 1979, Viel 1980). Mauderley (1974) reported that the FRC of ponies ranges from 35 to 76 ml/kg, but this range seems rather large. The FRC of cows is 52 ml/kg (Patterson et al. 1965), and the reported FRC of calves ranges from 40 (Peters et al. 1973) to 58 ml/kg (Kiorpes et al. 1978). The similarity of the FRC in horses and cows is interesting as the weight of the lung differs between the two species.

Measurements of the total lung capacity (TLC) and residual volume (RV) of horses and cows can only be made on anaesthetized animals. However, this procedure is not without drawbacks. Sedating a horse drops the FRC from 48 ml/kg (Willoughby and McDonell 1979, Viel 1980) to between 35 (McDonell and Hall 1974) and 41 ml/kg (Leith and Gillespie 1971). Complete anaesthesia and positioning in sternal recumbency (prone) does not appear to cause a further drop in FRC as the reported FRC of anaesthetized horses in sternal recumbency is approximately 36 ml/kg (Sorenson and Robinson 1980). In contrast, positioning the horse in lateral recumbency (lateral decubitus) or dorsal recumbency (supine) results in a significant reduction in FRC (McDonell and Hall 1974, Sorenson and Robinson 1980) due to the compression of the caudal lobes of the dependent lung by the abdominal viscera (McDonell 1974, McDonell et al. 1979).

Total lung capacity has been defined as the lung volume at a transpulmonary pressute (P_{TP}) of 30 cm H 0 (Leith 1976). In sedated horses (Leith and Gillespie 1971), or in anaesthetized horses in sternal recumbency (Sorenson and Robinson 1980), TLC is 93 to 95 ml/kg. Residual volume has been defined as the lung volume at P_{TP} of -20 cm H_2O, and is 19 to 20 ml/kg in the sedated or anaesthetized horse (Leith and Gillespie 1971, Sorenson and Robinson 1980). The closing volume (CV) of the horse is 15.7 ml/kg and the anatomical dead space (V_{Dan}) was 4.4 ml/kg (Sorenson and Robinson 1980). As with FRC, TLC and RV are significantly reduced when the horse is positioned in lateral and dorsal recumbency, but CV and V_D are not (Sorenson and Robinson 1980).

The TLC of an excised bovine lung was 25 ml/kg (Tenney and Remmers 1963). The other lung volumes have not been reported for cows.

PUlmonary Mechanics:

Amoroso et al. (1963) described the patterns of airflow during inspiration and expiration in horses and cows. In both species expiration is longer than inspiration. Airflow is uniform during inspiration, but the horse has a double peak during expiration, while in the cow flow peaks at the beginning of expiration and then declines. Amoroso et al. (1963) reported that the peak flow rates are similar in the horse and the cow, ranging from 105 to 313 L/min during inspiration and from 140 to 271 L/min during expiration. Purchase (1965) reported that the peak flow rate of cows is from 290 to 460 L/min during inspiration and from 390 to 540 L/min during expiration. The mean inspiratory flow

rates of horses are 190 to 217 L/min and the mean expiratory flow rates are 152 to 200 L/min (Gillespie et al. 1966, Muylle and Oyaert 1973, Sasse 1971), within the range of the peak flow rates reported by Amoroso et al. (1963). No values are available for the mean flow rates of cows.

The intrathoracic pressure of horses and cows has been measured using both oesophageal balloons and intrapleural catheters. The two methods give similar results (Amoroso et al. 1963, Derkson and Robinson 1980, Dewes et al. 1974, Gillespie et al. 1966, Muylle and Oyaert 1973). During normal quiet breathing the maximum change in transpulmonary pressure ($\Delta P_{TP}max$) is approximately 6.5 cm H_2O in both horses and cows (Amoroso et al. 1963). Other reported values of $\Delta P_{TP}max$ in horses range from 3.6 (Gillespie and Tyler 1969) to 10.3 cm H_2O (Sasse 1971). Most recently, Derkson and Robinson (1980) have reported that the $\Delta P_{TP}max$ in horses varies with lung height, and is greatest about the middle of the lung. Musewe et al. (1979) reported that the $\Delta P_{TP}max$ in cows is 7 cm H_2O, similar to the value reported by Amoroso et al. (1963).

Dynamic compliance (C_{dyn}) has been measured in standing horses and cows. In horses the reported C_{dyn} ranges from 0.8 (Dewes et al. 1974) to 6.13 L/cm H_2O (Gillespie and Tyler 1969), with most of the values between 2.0 and 2.5 L/cm H_2O (Muylle and Oyaert 1973, Sasse 1971, Willoughby and McDonell 1979, Viel 1980). The reported C_{dyn} of cows is 0.53 L/cm H_2O (Musewe et al. 1979).

The static or quasistatic compliance of equine and bovine lungs (C_L) can only be measured on anaesthetized animals or excised lungs. Leith and Gillespie (1971) reported that the C_L of sedated horses is

3.4 L/cm H_2O while Sorenson and Robinson (1980) reported that in anaesthetized horses C_L is 1.35 L/cm H_2O. The static compliance of bovine lungs has not been reported, but Patterson et al. (1965) presented the deflation pressure-volume curve of an excised bovine lung at ambient temperature (22°C).

The pulmonary resistance (R_L) of horses varies throughout inspiration and expiration, and is highly dependent upon the breathing pattern (Gillespie et al. 1966). Gillespie et al. (1966) reported that the peak R_L was 0.41 cm H_2O/L/sec during inspiration and 0.55 cm H_2O/L/sec during expiration, while Dewes et al. (1974) reported that the mean R_L was 0.62 and 0.73 cm H_2O/L/sec during inspiration and expiration respectively. Robinson et al. (1975) and Viel (1980) adopted the convention of reporting R_L at 25, 50 and 75% of V_T during both inspiration and expiration. Both authors (Robinson et al. 1975, Viel 1980) reported similar values, and R_L did not differ significantly at 25, 50 or 75% of V_T during inspiration or expiration. Robinson et al. (1975) reported that 70 to 90% of R_L is in the lower pulmonary system (from the trachea to the pleural cavity), but Willoughby and McDonell (1979) state that 80% R_L is in the upper tract (from the nares to the trachea). The results of Viel (1980) show that the R_L of the lower pulmonary system is less during inspiration than during expiration, and accounts for a maximum of 35% of the total R_L in normal horses.

There are no reported values for R_L in cows, but Kiorpes et al. (1978) reported that in calves R_L is 3.1 cm H_2O/L/sec. These values are not comparable to those of the horses as the calves weighed only 60 kg, whereas the horses weighed approximately 450 kg.

There are three reports of respiratory resistance in anaesthetized horses. Leith and Gillespie (1971) reported that the resistance of the total respiratory system was 0.40 cm H_2O/L/sec with 50% due to the chest wall and 50% due to the lung. The resistance of the total respiratory system has also been reported by Purchase (1966) and Mapleson and Weaver (1969). The values reported by Purchase (1966) range from 0.61 to 2.68 cm H_2O/L/sec. Mapleson and Weaver (1969) reported their results using the equation: $P = k_1\dot{V} + k_2\dot{V}^2$, where P = pressure, $\dot{V}$ = flow rate, and k_1 and k_2 are constants. The derived values for k_1 and k_2 were 0.54 cm H_2O/L/sec and 0.05 cm H_2O/L/sec respectively. However, the results of Purchase (1966) and Mapleson and Weaver (1969) are questionable as some of their horses were not healthy and the experimental protocol was not consistent.

The work of breathing (W_b) has been reported for both the horse and the cow, but the methods differed significantly, thus the values are not comparable. For the horse, W_b was reported as the non-elastic work of breathing, measured by the integration of the area with the pressure-volume loop (Muylle and Oyaert 1973, Sasse 1971, Viel 1980, Willoughby and McDonell 1979), and the reported values range from 2.39 (Viel 1980) to 5.43 kg.cm/L (Sasse 1971). In the cow, the total metabolic cost of breathing was measured by the change in metabolic rate between successive levels of hyperventilation (Hales and Findlay 1968b). In CO_2-induced hyperventilation, the O_2 cost was linear and was 3 to 4 ml O_2/L, while the O_2 cost was nonlinear in response to thermally-induced hyperventilation. The non-linear response during

thermally-induced hyperventilation was due to an increase in dead space, and when ventilation was expressed as $\dot{V}_A$ rather than $\dot{V}_E$, the O_2 cost was 13.4 ml O_2/L. If it is assumed that the $\dot{V}_A/\dot{V}_E$ ratio did not change during CO_2-induced hyperventilation, and the $\dot{V}_A/\dot{V}_E$ ratio was 1/3, then the cost of $\dot{V}_A$ during the CO_2-induced hyperventilation was 9 to 12 ml O_2/L, which is reasonably close to the value obtained during thermally-induced hyperventilation.

STATEMENT OF PURPOSE

Based on a review of the literature, horses and cows have the same O_2 requirements, yet have markedly different lung structure and breathing patterns. Despite their apparent availability and economic importance there have been few thorough studies of pulmonary structure or function in these two species. It is difficult to compare the two species as many indices of pulmonary structure and function have not been reported for one or both species. Even in those cases where information is available, comparisons are often difficult as there were differences in the ages of the animals used, the methods varied between laboratories, or some of the animals may not have been healthy. The present study, therefore, was an attempt to measure the pulmonary function of mature healthy horses and cows using similar techniques, and to provide a qualitative and quantitative description of the structure of the lungs of these species. It addressed the basic hypothesis that the apparent differences in pulmonary function between horses and cows are due to structural differences in the lungs, particularly the lung parenchyma.

MATERIALS AND METHODS

Experimental Animals:

This study was conducted with six horses and seven cows (Table II). All of the animals were free of clinically-detectable respiratory disease as determined by a thorough clinical examination and pulmonary function tests. These findings were confirmed by post-mortem examination of four of the horses and five of the cows. The horses were older and smaller than the cows but the differences were not statistically significant.

Pulmonary Function Tests:

The pulmonary function tests were divided into two groups; those made to characterize normal quiet breathing in the standing, awake animal, and those made to characterize the mechanical properties of the lungs. The latter measurements were made on anaesthetized animals as horses and cows will not voluntarily inspire to TLC, or control the rate of inspiration and expiration.

Breathing in the Standing Animal:

The measurements to characterize normal quiet breathing were made while the animals were standing in a 1 x 2 m stock. Further restraint of the horses was limited to manual restraint of the head,

Table II. Description of the animals used in this study.

Animal	Age (years)	Weight (kg)	Sex	Breed	Clinical Problems	Post-mortem findings
Horses:						
Belle	10	426	F	Qtr	none	not euthanized
Speedy	3	375	F	Std	none	NAF
Archy	5	425	G	Std	none	NAF
Hoot	4	436	G	Std	ruptured tendons	a few larval nematodes in the lungs
1117	2	338	G	Std	bone cyst	NAF
Rocky	6	410	G	Std	none	not euthanized
Cows:						
65	2	342	F	Holst	infertile	not euthanized
221	4	499	F	Holst	none	not euthanized
864	4	589	F	Holst	none	NAF
86525	5	775	F	Holst	none	NAF
905	3	538	F	Holst	none	NAF
940	3	519	F	Holst	infertile	NAF
113	2	355	F	Holst	infertile	NAF

F - female; G - gelding; NAF - no abnormal findings; Qtr - quarterhorse; Std - standardbred; Holst - Holstein.

while the cows were tied in a head-gate stanchion similar to those in which they were tied in the barns. If further restraint was necessary the cows were cross-tied, so as to restrict, but not eliminate, head movement.

Gas Exchange:

The measurements to characterize gas exchange were: O_2 consumption ($\dot{V}_{O_2}$), CO_2 production ($\dot{V}_{CO_2}$), respiratory exchange ratio (R), tidal volume (V_T), respiratory rate (f_R), minute ventilation ($\dot{V}_E$), arterial O_2 pressure (P_{aO_2}), arterial CO_2 pressure (P_{aCO_2}), arterial pH (pH_a), arterial bicarbonate concentration ($[HCO_2^-]_a$), arterial base excess ($B.E._a$), physiological dead space (V_{Dphys}), dead space to tidal volume ratio (V_D/V_T), alveolar ventilation ($\dot{V}_A$) and alveolar O_2 pressure (P_{AO_2}). The equations used to calculate these measurements are given in Appendix I.

Figure 3 is a drawing of the apparatus used to measure gas exchange and determine the functional residual capacity (FRC). A mask, made of fibreglass and neoprene, was placed over the muzzle of the animal. An airtight seal between the face and the mask was achieved by compressing the hair on the face with petroleum jelly and covering the end of the mask with a neoprene band. The animal then inspired and expired through a non-rebreathing Lloyd-type valve which was connected to the mask by a short piece of flexible tubing. The valve was constructed from two dryer vents, plexiglass and 5 cm ID PVC tubing. The vents were obtained at a local hardware store and had openings 9 cm in diameter. Minimal pressure was required to open the flap and the

pressure drop across the valve was less than 1 cm H_2O at a flow rate of 500 L/min. To prevent leakage the flap was seated in a silicone ring. The ring was made by putting silicone sealant around the opening and then closing the flap until the sealant had partially set, and formed a perfect impression of the flap. The dead space of the mask, valve and tubing was approximately 1.5 L.

Heavy commercial vacuum cleaner tubing connected the inspiratory and expiratory ports of the valve to two PVC Y-pieces, each fitted with two one-way valves (Chemline Plastics, Toronto, Ont.). One of the valves on the inspiratory side of the Lloyd-type valve opened to room air and the other opened to a meteorological balloon which was used as an O_2 reservoir during the FRC determinations. One of the valves on the expiratory side also opened to room air, while the other opened to a 600 L spirometer (Warren E. Collins, Inc., Braintree, Mass). The original tubing in the spirometer had been replaced with 5 cm ID PVC tubing. This did not alter the linearity of the response but greatly reduced the resistance at high flow rates. The inner diameter of the vacuum cleaner tubing, Y-pieces and one-way valves was also 5 cm.

During the measurements of gas exchange the animals inspired room air and the expired air was collected in the spirometer. Samples of expired air were taken from the spirometer in oiled 50 cm^3 glass syringes and the O_2 and CO_2 concentrations were determined using a Scholander 0.5 cm^3 analyzer (Scholander 1947). A 600 L pneumotach (Fleisch #4, Instrumentation Associates, New York, N.Y.) was inserted between the breathing valve and the mask to monitor inspiration and expiration. The pressure changes across the pneumotach were measured

with a differential transducer (PM5E, Statham Inc., Hato Rey, Puerto Rico) and were recorded using a photographic recorder (VR-6, Electronics for Medicine, White Plains, N.Y.).

The arterial blood samples were taken while the expired air was being collected in the spirometer. The blood samples were collected anaerobically in chilled, heparinized glass syringes and placed in ice-water immediately. The P_{O_2}, P_{CO_2} and pH were determined within 60 minutes with a blood micro-system (BMS 3 Mk2, Radiometer, Copenhagen). The temperature of the water bath of the blood gas analyzer was 38.3°C, the mean rectal temperature of dairy cows (Andersson 1977). The mean rectal temperature of horses is 37.7°C (Andersson 1977) and the appropriate temperature corrections were made using the equations of Severinghaus (1966).

To minimize the formation of haematomas, 3.8 cm 20 gauge needles were used to take the blood samples. The samples from the horses were taken from the right carotid artery slightly above the thoracic inlet, and the samples from the cows were taken from the coccygeal artery just below the base of the tail. It would have been desirable to have taken blood from the carotid or brachial arteries in the cows but this was not practical. These arteries are deep, requiring 10 cm needles, and both are mobile and difficult to locate. Also, when the cows were in the stocks the head-gate blocked the approach to either vessel. Thus, the coccygeal artery was considered to be the most realistic source of arterial blood. Arterial placement of the needle was confirmed by the observation of pulsatile blood flow.

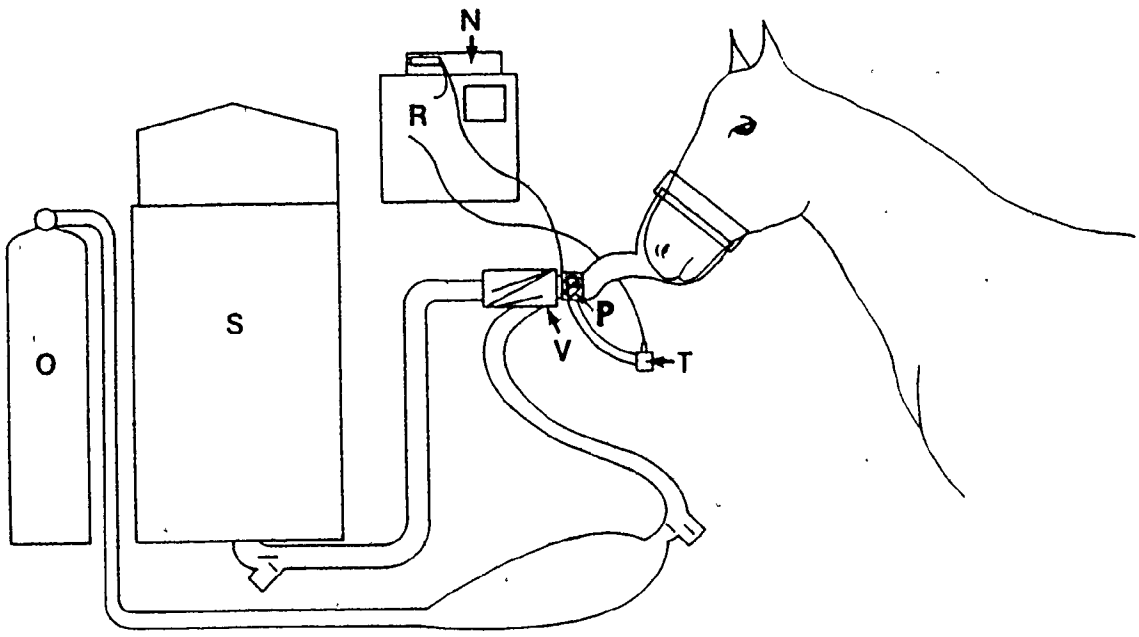


Figure 3. Drawing of the apparatus used to measure gas exchange and the FRC. V - one-way valve, T - pressure transducer, P - pneumotach, R - recorder, N - nitrogen analyzer, O - O_2 supply, S - spirometer.

FRC Determinations:

The FRC was determined using the open circuit N_2 washout technique (Comroe et al. 1962). The apparatus was basically the same as that used to measure gas exchange. At the beginning of the washout the one-way valves were switched so that the animal inspired 100% O_2 and the expired air was collected in the spirometer. The expired N_2 concentration was measured using a fast response N_2 analyzer (Nitroverter VR-3500, Vertek, Burlington, Vt.) and recorded as a function of the expired volume using the loop mode of the VR-6 recorder. The volume was obtained by the electronic integration of the flow signal from the pneumotach. The washout was continued until the end-tidal N_2 concentration was less than 1%. The mean expired N_2 concentration was then measured by emptying the spirometer past the sampling head of the N_2 analyzer. The equations used for the calculation of FRC are given in Appendix II.

Pulmonary Mechanics:

The measurements made to describe the pulmonary mechanics during normal breathing were: V_T, duration of inspiration (t_I) and expiration (t_E), maximum change in transpulmonary pressure ($\Delta P_{TP}max$), non-elastic work of breathing (W_b), dynamic compliance (C_{dyn}), flow rate during inspiration ($\dot{V}_I'$) and expiration ($\dot{V}_E'$), and pulmonary resistance (R_L) at peak flow and at 25, 50 and 75% of V_T during both inspiration and expiration. These measurements were made to describe the total pulmonary system, from the nares to the pleural cavity, and the lower pulmonary system, from the trachea to the pleural cavity.

The inspiratory and expiratory flow rates were recorded using the pneumotach, transducer and recorder described previously. The V_T was obtained by the electronic integration of the flow signal. The arrangement of the apparatus differed from that used in the gas exchange measurements in that the Lloyd-type valve and tubing were removed and the pneumotach was attached directly to the mask. This minimized the weight of the apparatus attached to the head of the animal and reduced the dead space.

The separation of the lower pulmonary mechanics from the total pulmonary mechanics was based on the techniques described previously for use in humans (Blide et al. 1964, Ferris et al. 1964, Hyatt and Wilcox 1961) and horses (Robinson et al. 1975, Viel 1980). The ΔP_{TP} of the total and lower pulmonary systems was measured using differential pressure transducers to monitor the changes in pressure between the nares and the pleural cavity, and between the trachea and pleural cavity respectively. The pressure at the nares was measured through a port in the mask while the pressure in the pleural cavity was measured using an oesophageal balloon, and the pressure in the trachea was measured using a 14 gauge catheter. The port in the mask, oesophageal balloon, and tracheal catheter were connected to the transducers by polyethylene tubing (5 mm ID, 6.5 mm OD, Becton, Dickinson and Co., Rutherford, N.J.).

The oesophageal balloon was made by attaching a condom to the end of a 2.5 m length of polyethylene tubing (3 mm ID, 4.5 mm OD, Becton, Dicinson Co.). To place the balloon in the oesophagus of a cow it was passed via the nares to the pharynx or upper oesophagus. It was

then partially inflated which induced the animal to swallow. In the horses a nasogastric tube was inserted into the upper oesophagus and the balloon was advanced via the tube. After the balloon was clear of the tube it was inflated and swallowed as in the cow. In both species the balloon was positioned in the distal third of the intrathoracic oesophagus. The distance from the nares to the distal third of the intrathoracic oesophagus was obtained by measuring from the nares to the larynx, to the point of the shoulder, to a point approximately 15 cm above and behind the olecranon. After the balloon was positioned, it was evacuated and 1.5 ml of air was added.

The pressure in the trachea was measured by introducing a 14 gauge stainless steel catheter into the trachea midway between the larynx and the thoracic inlet. The site was given a routine surgical preparation and infiltrated with local anaesthetic. In the horses the catheter and catheter introducer were then pushed through the skin and inserted into the trachea between two tracheal rings. In the cows it was necessary to make a small stab wound in the skin as the catheter introducer would not penetrate the thicker skin of this species. After the catheter was in the trachea, its patency was checked and it was then connected to the transducer.

The methods used to calculate C_{dyn} and R_L were based on the work of Neergaard and Wirz (1927) and Mead and Whittenberger (1953), as applied to the horse by Gillespie et al. (1966) and Dewes et al. (1974). These calculation are based on the theory that the pressure changes during breathing are due to the compliance of the lung, the pulmonary resistance and inertia of the system, such that:

$$\Delta P = \Delta P_C + \Delta P_R + \Delta P_{In} \quad (1)$$

where: ΔP = total change in transpulmonary pressure

ΔP_C = change in pressure due to the compliance of the lung

ΔP_R = chance in pressure due to pulmonary resistance

ΔP_{In} = change in pressure due to inertia

According to Mead and Whittenberger (1953) ΔP_{In} is essentially zero and equation 1 reduces to:

$$\Delta P = \Delta P_C + \Delta P_R \quad (2)$$

The compliance of the lung was estimated by C_{dyn}, which was calculated by dividing V_T by the change in pressure between the points of zero flow at end-inspiration and expiration. The change in pressure due to resistance was calculated by subtracting ΔP_C at a given volume from ΔP, and R_L was then calculated by:

$$R_L = \Delta P_R / \dot{V} \quad (3)$$

The non-elastic work of breathing was obtained by planimetry of the area inside the pressure-volume loops (Comroe et al. 1962).

System Calibration:

During the calibrations, the measuring devices and the recording system were treated as a unit. For example, pressure transducers were calibrated by comparing the output of the recorder to the pressure

change measured on a water manometer.

The pneumotach was calibrated using both steady-state and variable flow. For the steady-state comparisons, the flow was generated by a vacuum cleaner controlled by a rheostat, and the output of the recorder was compared against a standardized flow tube (Amatek, Schutte and Koertig Co., Cornwall Heights, Pa.). The response to variable flow was checked by pushing known volumes from the spirometer at varying flow rates. With steady-state flow the response of the pneumotach was linear from 0 to 300 L/min, but it had a positive error of 1 to 2%. Above 300 L/min the error increased progressively and was 5 to 6% at 600 L/min. The errors during the measurements with variable flow were similar to those obtained at equivalent flow rates in the steady-state. As most of the flow rates observed in the present study were less than 300 L/min, corrections were not made for the error in the pneumotach.

The pressure response of the balloon was tested by putting it in a vacuum bottle, and comparing the pressures in the balloon and the bottle while the pressure in the bottle was varied using a large animal ventilator (Mark 9, Bird Corp., Palm Springs, Ca.). The volume in the balloon was varied between 1 and 10 ml. At all volumes the balloon responded perfectly to pressure changes of up to 40 cm H_2O at frequencies up to 90 cycles per minute. The transducers and tubing used for these calibrations were the same as those used to measure pressure during the mechanics measurements.

The pressures measured with an oesophageal balloon are dependent not only on the characteristics of the balloon, but also on the recoil

pressure of the oesophagus. For man and the dog, the recoil pressure is negligible when the balloon volume is less than 0.5 ml (Gibson and Pride 1976, Mead and Whittenberger 1953), but there was no information on the effect of balloon volume in large mammals. In the present study the effect of balloon volume was examined by measuring $\Delta P_{TP}max$, W_b, C_{dyn} and R_L, at four balloon volumes; 1, 2, 3 and 5 ml. This experiment was conducted with four cows using a Latin square design. There were no significant differences ($P < 0.05$) due to balloon volume.

Several workers (Derkson and Robinson 1980, Dewes et al. 1974, Gillespie et al. 1966, McDonell pers. comm.) have reported that in the horse the pressure changes recorded with an oesophageal balloon are similar to those recorded with an intrapleural catheter. However, similar information was not available for the cow. Therefore, in the present study the changes in oesophageal pressure were compared to the changes in intrapleural pressure in the cow. The intrapleural pressure was measured at the level of the 10th intercostal space, 10 cm above a line from the tubar coxa to the point of the shoulder. The pressure changes in the oesophagus were indentical to the changes in intrapleural pressure.

McDonell (pers. comm.) examined the within-day and between-day variability of pulmonary mechanics measurements in horses. He compared measurements made six times on the same day, and measurements made on six days separated by on week intervals. Statistical testing of these results using an analysis of variance showed significant variability ($P < 0.05$) between days, and between successive measurements on the same day. Prior to the present study, the day to day variability of

pulmonary mechanics measurements was examined in two cows. The measurements were made on six days separated by interval of one day to several weeks. There were significant differences ($P < 0.05$) between days for all of the variables measured. The experiment to examine the effect of balloon volume provided an opportunity to examine the variability between successive measurements on the same day in cows. There was significant variability between measurements, and this was not due to the balloon volume.

The above paragraph indicates that the pulmonary mechanics measurements were not reproducible, but this is somewhat misleading. The variability did not differ significantly between measurements, and the significant results obtained with the analysis of variance were due to the difference between the extreme means. The means for separate measurements usually fell within the confidence limits of the overall mean, and there were no significant trends in the replications. These latter observations would suggest that the measurements were reasonably reproducible.

Experimental Protocol:

The measurements to characterize breathing in the standing animals were repeated twice, on separate days, and the results were pooled to obtain the mean values for each individual. All of the measurements were made in the morning after the animals had been fasted for 12 to 16 hours. This procedure slightly reduced the abdominal contents and minimized the increase in metabolic rate due to the heat increment of feeding. Before any measurements were made the

horses were allowed 15 to 20 minutes to settle in the stocks, and the cows were allowed 25 to 30 minutes.

The first procedure each day was the determination of FRC. Two determinations were made, each requiring 5 to 6 minutes. They were separated by a 7 to 10 minute period during which the animal breathed room air. The gas exchange measurements were begun 15 minutes after the completion of the FRC determinations. Four gas samples were collected for the measurement of $\dot{V}_{O_2}$ and $\dot{V}_E$. The collection periods were 4 to 5 minutes in duration and were separated by 4 to 5 minute intervals. As the formation of haematomas virtually eliminated any possibility of obtaining repeated arterial samples, blood samples were taken only during the last two gas collections on the second day. These gas collections were 0.5 to 1.0 minutes in duration as the collection was limited to the time during which the blood samples were being taken.

After the four gas collections were completed the oesophageal balloon was introduced into the trachea. Five pressure-volume (P-V) loops were then recorded, then the flow rate, volume and pressure changes of at least 10 "normal" breaths were recorded on a scalar trace. During routine testing only the mask to oesophageal pressure was recorded. The measurements to describe the lower pulmonary system (trachea to pleural cavity) were recorded at a later date using four horses and four cows. During these later measurements the starvation procedure was the same as during the routine measurements, but gas exchange and the FRC were not measured.

Measurements of Total Lung Capacity and Quasistatic Compliance:

Horses and cows are not the most co-operative of subjects, therefore it is virtually impossible to make any measurements which require more than minimal co-operation on the part of the subject. To characterize the mechanical properties of the lungs it was necessary to anaesthetize the animals and use positive pressure ventilation. This procedure was performed with four of the horses and five of the cows used to characterize breathing in the standing animal.

Anaesthesia:

As during routine general anaesthesia in the Large Animal Hospital, the animals were fasted overnight. Fifteen to twenty minutes prior to induction the horses were premedicated with 0.05 mg/kg of acepromazine maleate (Atravet®, Ayerst Laboratories, Montreal, P.Q.) to reduce the drug requirements for anaesthesia and to shorten the recovery period. No premedication was used in the cows as it was not deemed necessary. In both species anaesthesia was induced with the intravenous administration of 100 mg/kg of guaifenesin (Glycerol Guiacolate, B.D.H. Chemicals, Toronto, Ont.) and 4.2 and 5.6 mg/kg of thiamylal sodium (Surital, Parke, Davis and Co., Brockville, Ont.). Anaesthesia was maintained throughout the 20 to 25 minute period required for the measurements by the subsequent administration of small doses (1.0 to 1.5 mg/kg) of thiamylal sodium as required.

After anaesthesia was induced the animals were intubated. A 25 mm cuffed endotracheal tube was used for the cows and 30 mm cuffed tube was used for the horses. The animals were then positioned in

sternal recumbency and connected to the bag-in-box ventilator described below. The valves on the ventilator were positioned so that the animals inhaled room air and exhaled into the room.

To prevent the animals from fighting the ventilation procedures, and to minimize chest wall resistance, they were paralyzed using a depolarizing muscle relaxant, succinylcholine hydrochloride (Anectine, Burroughs Wellcome Ltd., LaSalle, P.Q.). This was administered intravenously at an initial dose of 0.15 mg/kg in the cows and 0.25 mg/kg in the horses. A single dose was sufficient to produce approximately 20 minutes of complete chest wall and diaphragmatic paralysis in the cows, but the horses required repeated administration of the initial dose at 5 to 8 minute intervals. Paralysis of the chestwall and diaphragm was considered to be complete when there was complete abolition of spontaneous pressure changes in the thoracic cavity.

Equipment and Protocol:

Figure 4 is drawing of the system used to make the measurements of quasistatic compliance and TLC. The inflation and deflation of the animal was accomplished by increasing or decreasing the pressure in the bag-in-box system. The bag-in-box system consisted of a plywood box which contained two meteorological balloons. The box was 90 x 120 x 60 cm. The sides and bottom were made of 1.8 cm thick plywood and the top was 0.6 cm plexiglass reinforced with 5 x 5 cm wood strapping. One of the meteorological balloons was filled with 100% O_2 and served as an inflation reservoir, while the other balloon served as a reservoir for the expired air. The animal was connected to the box via the tubing

and valves used for the measurements of gas exchange. To inflate the lungs, air was pumped into the box with a vacuum cleaner controlled by a rheostat. This increased the pressure in the box and forced 100% O_2 into the lungs. The lungs were deflated by gradually reducing the flow into the box and allowing the pressure in the box to decline.

The inflation and deflation procedure was performed at least six times for each animal. In each case the maximum pressure in the box was approximately 80 cm H_2O. Inflation was continued until the rate of change in pressure at the end of the endotracheal tube was more rapid than the change in volume. The first three inflations were used to check the system and to standardize the volume history of the lungs. Only the FRC was determined from these inflations. The next three inflations were used for the mechanical analysis. The time required to complete a cycle of inflation and deflation cycle varied from 60 to 90 seconds, with the rheostat being adjusted so that the rate of change in pressure was constant.

After the P-V loops for the measurement of compliance had been completed, two or three more inflation and deflation procedures were carried out for the determination of residual volume (RV_{an}), vital capacity (VC_{an}), expiratory reserve (ER_{an}) and closing volume (CV_{an}). Again the lungs were inflated to TLC, but when the volume had returned to FRC, the vacuum cleaner was reversed and air was pumped out of the box to generate a negative pressure. The pressure was lowered until the volume no longer changed.

During the inflation and deflation procedures the pressure at the end of the endotracheal tube, oesophageal pressure, flow rate,

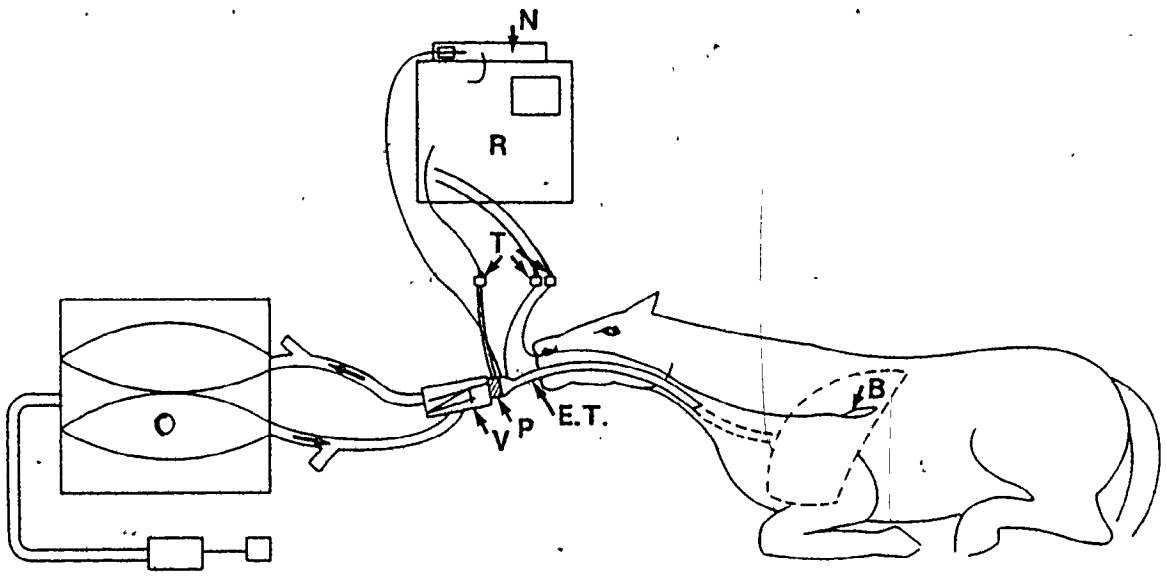


Figure 4. A drawing of the apparatus used to measure lung volume and quasistatic compliance. B - oesophageal balloon, E.T. - endotracheal tube, P - pneumotach, V - valve, R - recorder, N - nitrogen analyzer, T - transducers, O - O_2 reservoir. The arrows in the tubes indicate the direction of air flow. The dotted line shows the approximate position of the lungs and trachea.

volume and N_2 concentration were recorded using the equipment described in previous sections. Prior to anaesthesia, the oesophageal balloon had been introduced into the oesophagus and advanced to the stomach. After the animal was anaesthetized and positioned in sternal recumbency the balloon had been retracted into the distal third of the intrathoracic oesophagus. From the aforementioned measurements, the pressure-volume (P-V) curves of the lung and chest wall, FRC_{an} and inspiratory capacity (IC_{an}) were obtained. The absolute pressures across the lung and chest wall were not measured, thus the results are expressed only in terms of the change in pressure. The FRC_{an} was determined using a single-breath N_2 washout technique and IC_{an} was measured from the volume trace.

The lung compliance (C_L), chest wall compliance (C_W) and total compliance (C_T) were calculated from the deflation P-V curves. There is no standardized procedure for the calculation of compliance of large mammals so the compliance was calculated for one tidal volume above FRC, a measure equivalent to that used for humans (Pengelly 1977). Both the horses and cows had a significant drop in FRC when they were anaesthetized. As compliance changes as a function of lung volume (Comroe et al. 1962), adjustments were made for the drop in FRC and the adjusted compliance (C_L', C_W', C_T') was also calculated for one tidal volume above the normal FRC of the standing animal. The values for V_T and the FRC of the standing animals were available for each individual, as these animals had been used for the previous measurements to characterize breathing in the standing animal.

An alternative method to describe the P-V characteristics

of the lung is the use of exponential functions. One such function was described by Salazar and Knowles (1964), and Pengelly (1977) described a modified approach to its' use. This function which describes the P-V curve of the lung from FRC to TLC is:

$$V = V_{max}(1 - k_0 e^{k_1 P}) \quad (4)$$

where V = volume (L)

V_{max} = volume at infinite pressure (L)

P = pressure (cm H_2O)

and k_0 and k_1 are mathematically derived constants.

In their original publication Salazar and Knowles (1964) assumed that V_{max} was equal to TLC. However, Pengelly (1977) modified this assumption and calculated V_{max} by an iterative procedure. Different values of V_{max} were substituted so as to maximize the coefficient of determination, r^2, of the equation:

$$\ln(1 - V/V_{max}) = \ln k_0 - k_1 P \quad (5)$$

This procedure also provided the values for k_0 and k_1. The P-V characteristics of the lungs were then described in terms of the pressure required to inflate the lungs to one-half of IC (h), the pressure when the lung volume was zero (P_z), and the resting end-expiratory pressure (REEP). The factor, h, was determined by the equation:

$$h = \ln_2 / k_1 \quad (6)$$

while P_z was calculated by:

$$P_z = \ln k_0 / k_1 \quad (7)$$

and REEP was calculated by:

$$REEP = P_z - \ln(1 - FRC/V_{max})/k_1 \quad (8)$$

Lung Structure:

The second phase of this project was a study of the comparative structure of equine and bovine lungs. Four horses and four cows were used for this phase. All of these animals had been used for the preceding measurements of pulmonary function.

Lung Fixation:

The animals were euthanized with sodium pentobarbital (160 mg/kg; Euthanyl-Forte, MTC, Hamilton, Ont.) injected intravenously. Horses often exhibit an initial phase of barbiturate excitement during this type of euthanasia and thrashing during the excitement phase results in contusions, and ecchymotic and petechial hemorrhages in the lung. To eliminate this problem the horses were given xylazine (0.5 mg/kg; Rompun ®, Cutter Laboratories Inc., Mississauga, Ont.) intravenously 5 to 10 minutes prior to injecting the sodium pentobarbital.

After the animal was euthanized it was positioned in left lateral recumbency, an incision was made from the tip of the lower

jaw to the anal region, and the skin and right fore and hind legs were reflected back. The trachea was then dissected free and a 30 mm cuffed endotracheal tube was inserted. The cuff was inflated and the end of the tube was capped to hold the lungs at FRC when the thoracic cavity was opened. Previous experience had shown that it was virtually impossible to reinflate bovine lungs after they had collapsed to RV, hence it was necessary to maintain as much air in the lungs as possible. After the endotracheal tube had been capped, the abdomen was opened and the abdominal contents were allowed to fall outward so as to reduce the pressure on the thoracic cavity. An incision was then made along the diaphragm, and the rib cage was removed. The pluck (heart and lungs) was dissected free of the thoracic cavity and the lungs were quickly examined for any gross pathological changes. The pluck was then placed in a 45 x 60 x 60 cm tub which was partially filled with phosphate buffer. The cap was removed from the endotracheal tube and the tube was connected to a reservoir of 2.5% glutaraldehyde which was instilled into the lungs to a pressure of 50 cm. The formulae for the phosphate buffer and glutaraldehyde are given in Appendix III.

The time from euthanasia to immersion of the lungs in buffer was 45 to 60 minutes and inflation required another 15 to 20 minutes. After the lungs were inflated they were left for approximately 90 minutes to fix the alveolar structures, and then the pluck was removed from the buffer and immersed in 2.5% glutaraldehyde to fix the pleura. After 90 minutes the pluck was removed from the glutaraldehyde bath and the glutaraldehyde was drained from the lungs. The heart, great vessels and any excess fat or connective tissue were then

dissected from the lungs. To wash the glutaraldehyde out of the lungs, they were again immersed in phosphate buffer and reinflated with buffer. After approximately 90 minutes the lungs were removed from the buffer and the displaced volume was determined. The lungs were then sectioned for the morphometric measurements.

Sectioning and Tissue Sampling:

The left and right lungs were separated by cutting the primary bronchi at the level of the carina. In the cows it was also necessary to cut the accessory bronchus which arises anterior to the carina. After the lungs were removed, the length, volume and diameter of the trachea were measured. The lungs were then positioned so that the dorsal border formed an angle of 20 degrees with the horizontal plane. The lungs were cut into 5 cm thick horizontal sections. These sections were photographed, and then the tissue samples for light and electron microscopy were taken at random at three lung heights; 10, 20 and 30 cm from the top of the lung.

The tissue samples for light microscopy were 1.5 cm^3 blocks. Six blocks were taken at each lung height (three blocks from each lung). The blocks were stored overnight in phosphate buffer and the next day 2 mm thick slices were cut from the blocks and submitted to the Histopathology Laboratory of the Department of Pathology, University of Guelph. The slices were embedded in celloidin and paraffin, six micron sections were cut from the paraffin blocks, and stained with haematoxylin and eosin.

The tissue samples for electron microscopy were 1 mm^3 blocks. Ten blocks were taken from each lung height of each lung, giving a total of 60 blocks per animal. The blocks were processed through osmium tetroxide, stained with uranyl acetate, dehydrated with alcohol and embedded in plastic (Spurr, E.F. Fullham Inc., New York, N.Y.). These procedures are outlined in Appendix IV.

Morphometry:

Principles:

The morphometric measurements were made to quantitate some of the structural components of the lung, and the basic principles and procedures have been described by Weibel (1963, 1970/71). The relative proportions, or volume densities, of various components within a given lung volume are estimated from thin sections. The absolute volumes are then obtained by multiplying the volume densities by the volume of the lungs. The volume densities are obtained by point counting or line intersects. To calculate the relative portion, or volume density, of a given fraction, f, within a section, the section is covered by a grid and the number of points on "f" are then counted. The volume density of f ($V_V(f)$) is then obtained by the equation:

$$V_V(f) = P(f)/P(t) \qquad (9)$$

where:

P(f) = number of points on fraction, f

P(t) = total number of points

The relative surface area, or surface density, of fraction, f, is calculated by counting the number of intersects of the surface of f with a series of test lines. The surface density of f ($S_V(f)$) is then obtained by the equation:

$$S_V(f) = 2 \cdot I(f)/L(t) \qquad (10)$$

where:

I(f) = number of intersects with fraction, f

L(t) = total length of test lines

Procedures:

The 5 cm sections of the lung were photographed using 35 mm colour slide film, and the morphometric measurements were made by projecting the slides onto a grid for point counting. The projected image was approximately the same size as the original section. The grid was essentially a sheet of graph paper with lines 0.8 cm apart, and the points were formed by the intersection of the vertical and horizontal lines. Only those points falling on the lung were counted, and if the section was larger than the grid, the grid was moved until all of the section had been covered. The counts were made to determine the volume density of the parenchyma (bronchioles, alveolar ducts, alveoli, alveolar septum) and non-parenchyma, which included the airways and blood vessels greater than 1 mm in diamter, and interlobular septa.

The sections stained with haematoxylin and eosin were photo-

graphed at low power (25X) using 35 mm colour slide film. Ten fields were photographed at random from each lung at each lung height, yielding a total of 60 slides. From these 60 slides, 20 were selected at random for the determination of the volume densities of the alveoli, alveolar septa, alveolar ducts, terminal and respiratory bronchioles, interlobular septa, arterioles and venules to separate the gas exchange parenchyma (alveoli, alveolar septa and alveolar ducts) from the fine components (terminal and respiratory bronchioles, arterioles and venules, and interlobular septa) of the non-parenchyma.

The slides were projected onto a multipurpose grid described by Weibel et al. (1966). The magnification of the projected image was approximately 300X. The grid was composed of 84 lines, 2 cm in length, arranged in a lattice of equilateral triangles. There were 168 points on the grid and these were formed by the endpoints of the lines. Thus, for 20 slides a total of 3360 points were counted. This was sufficient to give a relative error of 5% if the volumetric fraction was 5% (Weibel 1963).

The final stage of the morphometry was the determination volume and surface densities of the various components of the gas exchange parenchyma (alveolar ducts, alveoli, and alveolar septa). As the relative error for the estimation of the volume of opaque bodies is dependent upon the section thickness (Weibel 1963), the sections for this level of counting were 0.5 μm in thickness. They were cut from the blocks prepared for electron microscopy, and were stained with 1% methylene blue/1% sodium borate. Sections were cut from three blocks at each lung height in each lung, yielding a total of 18 sections per

lung. They were photographed at high power (400X) using 35 mm colour slide film. Two fields were selected at random on each section, giving a total of 36 slides. Twenty of the 36 slides were chosen at random and projected onto the grid used for the counts of the sections stained with haematoxylin and eosin. The final magnification of these sections was 4700X. The volume densities of the air spaces ($V_V(A)$), capillaries ($V_V(c)$) and septal tissue ($V_V(s)$) were determined by point counting and the surface densities of the alveoli ($S_V(A)$) and capillaries ($S_V(c)$) were determined by the line intersects.

Electron Microscopy:

One block from each height in each lung was selected for examination with the transmission electron microscope. Sections 1000 to 1200 Å in thickness were cut from each block, mounted on Cu/Pd grids, and stained for two minutes with lead citrate. The sections were examined using a transmission electron microscope (Phillips 301, Phillips Electronics, Eindhoven, The Netherlands). No attempt was made to quantitate the structural components observed on the EM, as the quantitative studies of the light microscope sections only confirmed the obvious differences, and did not provide information on the distribution of the components within the lung.

Statistical Procedures:

The interspecific differences were tested statistically using a Students' t-test. The paired t-test, correlation coefficient,

analysis of variance and Latin square design were used to test other relationships. These are indicated in the text. Statistical significance was assumed at the level of the confidence interval ($P < 0.05$). The data are presented as the mean $\pm$ 1 standard deviation ($\bar{x} \pm s$).

RESULTS

Pulmonary Function Tests:

Gas Exchange and Metabolic Rate:

The variables measured to describe gas exchange in the standing animal are presented in Table III. In the cows, $\dot{V}_{O_2}$ and $\dot{V}_E$, expressed as ml/kg/min, were significantly correlated with body weight. When the unadjusted values for $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and $\dot{V}_E$ (ml/min) were regressed against body weight, the calculated exponents were closer to 0.75 than 1. Therefore $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and $\dot{V}_E$ were recalculated as a function of $W^{0.75}$, the metabolic body weight (Kleiber 1975). When $\dot{V}_{O_2}$ and $\dot{V}_E$ were expressed as ml/$kg^{0.75}$/min there was no longer any correlation with body weight.

Regardless of the units in which they were expressed, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and $\dot{V}_E$ were significantly higher in the cows than in the horses. The increased $\dot{V}_E$ in the cows was due to the relatively higher f_R. While V_T was 1.4 times larger in the horses than in the cows, f_R was twice as high in the cows.

The effect of blood sampling on $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, R, V_T, f_R and $\dot{V}_E$ was tested using a paired t-test. There were no significant differences for either species when the values from the six gas collections prior to the blood samples were compared with the values from the two gas collections during which blood was taken. Thus, it was assumed

Table III. Variables measured to describe gas exchange in the standing horses and cows. The values are presented as the mean ± 1 standard deviation, and those marked with an asterisk (*) are significantly different ($P < 0.05$) between the two species.

Variable	Cows	Horses	
n	7	6	
Weight (kg)	517 ± 147	402 ± 38	
$\dot{V}_{O_2}$ (ml/kg/min)	3.95 ± 0.35	3.26 ± 0.21	*
$\dot{V}_{O_2}$ ($ml/kg^{0.75}$/min)	18.41 ± 1.30	14.57 ± 0.75	*
$\dot{V}_{CO_2}$ (ml/kg/min)	3.56 ± 0.39	2.77 ± 0.32	*
$\dot{V}_{CO_2}$ ($ml/kg^{0.75}$/min)	17.07 ± 1.64	12.40 ± 1.31	*
R	0.90 ± 0.05	0.85 ± 0.05	
V_T (ml/kg)	10.39 ± 1.25	14.55 ± 1.52	*
f_R (bpm)	23.7 ± 3.3	11.7 ± 1.2	*
$\dot{V}_E$ (ml/kg/min)	244.6 ± 34.8	167.0 ± 9.1	*
$\dot{V}_E$ ($ml/kg^{0.75}$/min)	1152 ± 128	748 ± 32	*
P_{aO_2} (torr)	82.8 ± 5.5	85.7 ± 7.2	
P_{aCO_2} (torr)	39.6 ± 2.6	43.8 ± 0.8	*
pH_a	7.40 ± 0.03	7.36 ± 0.03	*
$[HCO_3^-]$ (meq/L)	24.4 ± 2.2	24.5 ± 1.9	
$B.E._a$ (meq/L)	-0.3 ± 2.5	-0.6 ± 1.9	
P_{AO_2} (torr)	101.5 ± 3.9	97.4 ± 4.3	
$P_{(A-a)O_2}$ (torr)	18.2 ± 4.3	11.7 ± 6.5	
V_D/V_T	0.64 ± 0.05	0.62 ± 0.04	
V_{Dphys} (ml/kg)	7.29 ± 1.26	10.06 ± 0.48	*
$\dot{V}_A$ (ml/kg/min)	86.8 ± 8.2	64.9 ± 7.3	*

that the blood gas values are representative of those during all of the gas collections.

There were no significant differences between P_{aO_2}, $B.E._a$ and $[HCO_3^-]$ in the two species, but P_{aCO_2} was higher, and pH_a was lower in the horses than in the cows. The V_D/V_T ratios were similar in both species, but V_{Dphys} was significantly higher in the horses and $\dot{V}_A$ was significantly higher in the cows. These latter differences parallelled the differences between V_T and $\dot{V}_E$ in the two species. Although P_{AO_2} and P_{A-aO_2} appeared to be higher in the cows, the difference between the two species was not statistically significant.

FRC Determinations and Pulmonary Mechanics:

The results obtained during the pulmonary mechanics measurements are presented in Tables IV and V and representative P-V loops and scalar traces of pressure, flow and volume are presented in Figures 5 and 6.

In the quiet, standing horses, FRC and C_{dyn} were significantly greater than in the cows. The difference in compliance remained even after compliance was expressed as the specific compliance (C_{dyn}/FRC), as the differences C_{dyn} were greater than the differences in FRC.

During normal breathing there was usually less breath-to-breath variability in the pattern of airflow in the cows than in the horses. The cows tended to have a uniform, or increasing flow rate throughout inspiration. During expiration the flow was initially very high and then declined exponentially. In the horses, flow was uniform during one or both of the phases of the breathing cycle in some breaths, but in other breaths there was a double peak in flow during inspiration

Table IV. Values for the pulmonary mechanics of standing horses and cows. The values are expressed as the mean ± 1 standard deviation, and those marked with an asterisk (*) are significantly different ($P < 0.05$) between the two species.

Variable	Cows	Horses
n	7	6
Weight (kg)	517 ± 147	402 ± 38
FRC (ml/kg)	39.37 ± 3.44	51.30 ± 6.53 *
ΔP_{TP}max (cm H_2O)	5.51 ± 0.95	3.78 ± 0.50 *
W_b (kg·cm/L)	3.29 ± 0.58	1.58 ± 0.51 *
C_{dyn} (L/cm H_2O)	1.25 ± 0.39	2.27 ± 0.40 *
C_{dyn}/FRC (per cm H_2O)	0.063 ± 0.013	0.109 ± 0.007 *
t_I (sec)	1.05 ± 0.36	2.08 ± 0.52 *
t_E (sec)	1.30 ± 0.33	2.53 ± 0.89 *
t_I/t_E	0.78 ± 0.08	0.85 ± 0.13

Table V. The flow rates and pulmonary resistances of standing horses and cows at peak flow and 25, 50 and 75% of V_T during inspiration and expiration (V_I and V_E respectively). The values are presented as the mean ± 1 standard deviation, and those marked with an asterisk (*) are significantly different ($P < 0.05$) between the two species.

Variable		Cows	Horses	
n		7	6	
Weight (kg)		517 ± 147	402 ± 38	
$\dot{V}_I$' (L/sec)	peak	5.56 ± 0.87	3.65 ± 0.70	*
	25	4.78 ± 0.71	2.83 ± 0.94	*
	50 % of V_I	5.13 ± 0.73	2.32 ± 0.44	*
	75	5.11 ± 0.86	2.98 ± 0.56	*
$\dot{V}_E$' (L/sec)	peak	6.20 ± 1.38	4.13 ± 0.98	*
	25	6.11 ± 1.33	3.13 ± 1.36	*
	50 % of V_E	4.67 ± 0.93	2.29 ± 0.61	*
	75	3.10 ± 0.55	2.54 ± 0.58	
$R_L I$ (cm H_2O/L/sec)	peak	0.43 ± 0.08	0.36 ± 0.08	*
	25	0.37 ± 0.09	0.27 ± 0.08	
	50 % of V_I	0.40 ± 0.09	0.23 ± 0.08	*
	75	0.43 ± 0.08	0.36 ± 0.07	
$R_L E$ (cm H_2O/L/sec)	peak	0.41 ± 0.08	0.37 ± 0.08	
	25	0.43 ± 0.08	0.40 ± 0.11	
	50 % of V_E	0.44 ± 0.09	0.36 ± 0.08	
	75	0.36 ± 0.08	0.32 ± 0.08	

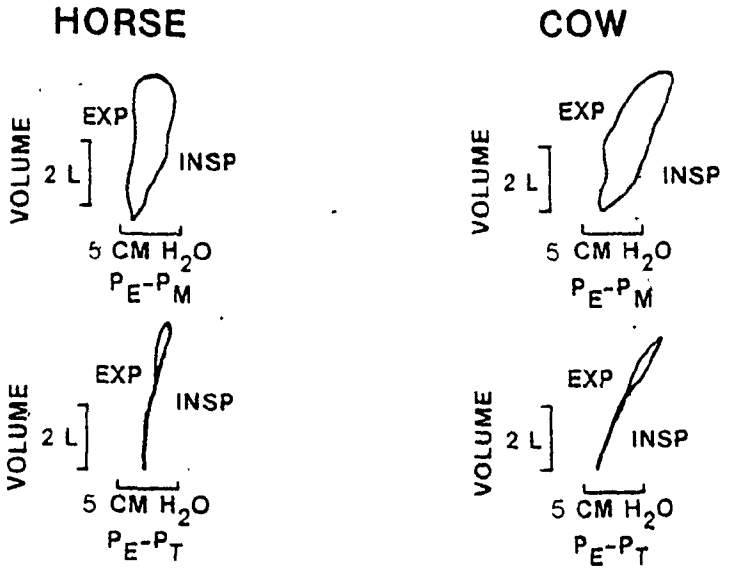


Figure 5. Representative pressure-volume (P-V) loops of normal breaths of a standing horse and cow. The upper loops are for the total pulmonary system, from the nares to the oesophagus, and the lower loops are for the lower pulmonary system, from the trachea to the oesophagus. There were no differences between the P-V loops of the total pulmonary system before or after the tracheal catheter was inserted. P_E - oesophageal pressure, P_M - mask pressure, P_T - tracheal pressure.

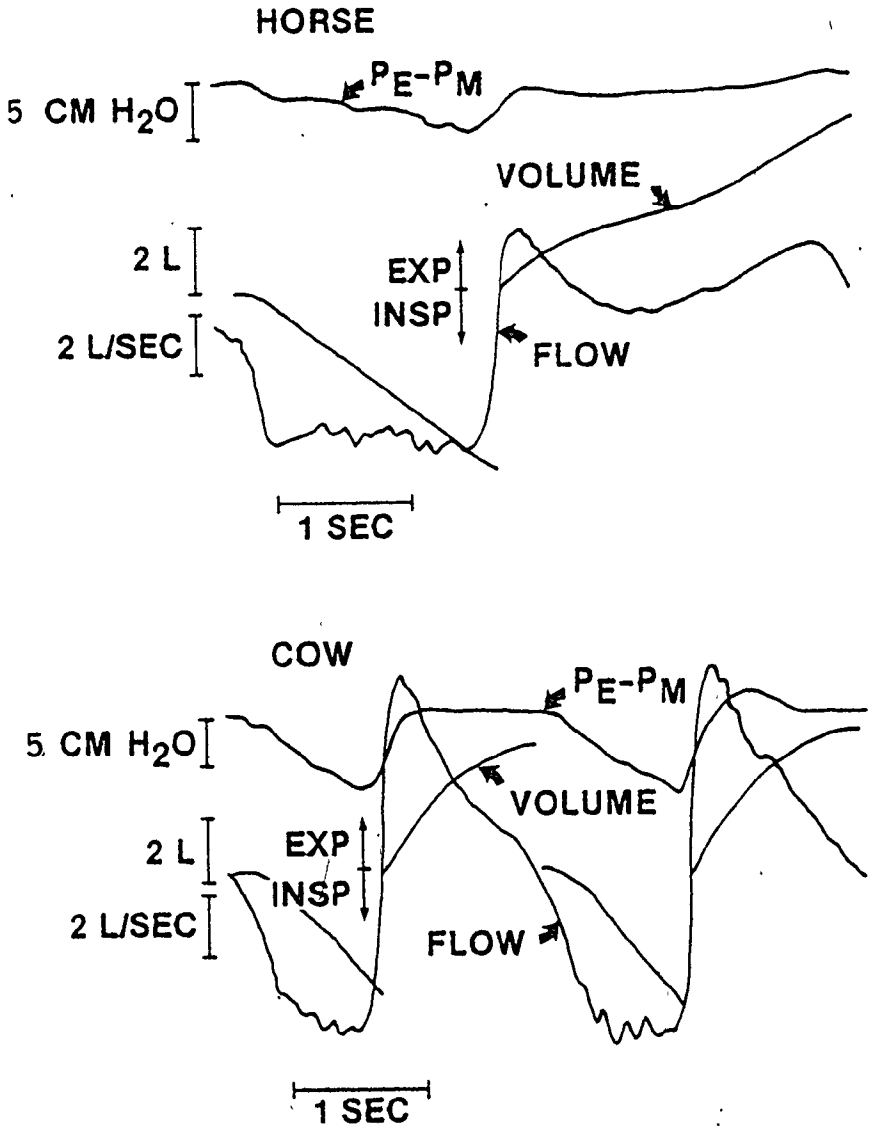


Figure 6. Representative scalar traces of flow rate, volume and transpulmonary pressure during normal breathing in a horse and a cow. P_E - oesophageal pressure, P_M - mask pressure.

and/or expiration. The duration of inspiration and expiration was significantly greater in the horses, but the t_I/t_E ratio did not differ significantly between the two species. Except for 75% of the expired volume, the flow rates during both inspiration and expiration were significantly higher in the cows than in the horses.

The ΔP_{TP}max and W_b were significantly higher in the cows, while FRC, C_{dyn} and the specific compliance (C_{dyn}/FRC) were significantly greater in the horses. The pulmonary resistance did not differ significantly between the various stages of the respiratory cycle in either species, and the only significant difference in R_L between the two species was at 50% of V_T during inspiration. There was no relationship between R_L and $\dot{V}'$ or between R_L and change in volume during inspiration or expiration in either species.

The influence of the tracheal catheter was examined by comparing the mechanics measurements made before the catheter was inserted with those made after. Based on the results of a paired t-test, the catheter did not significantly affect pulmonary mechanics. However, there were marked changes in breathing pattern in some of the cows as these individuals became quite agitated when the catheter was inserted.

The results describing the mechanics of the total (tot) and lower (low) pulmonary systems are presented in Tables VI and VII. Figure 7 is a scalar trace of flow, volume and changes in transpulmonary pressure of the total and lower pulmonary system during quiet breathing in a horse and a cow. As during the preceding measurements of the total pulmonary system, ΔP_{TP} max (tot) and W_b (tot) were significantly different between the two species. However, ΔP_{TP}max (low)

Table VI. Variables measured to describe the total and lower pulmonary mechanics in standing horses and cows. The values are presented as the mean ± 1 standard deviation and those marked with an asterisk (*) are significantly different ($P < 0.05$) between the two species.

Variable		Cows	Horses
n		4	4
Weight (kg)		600 ± 122	394 ± 46 *
$\Delta P_{TP}max$ (cm H_2O)	Total	7.38 ± 1.54	4.22 ± 0.87 *
	Lower	4.47 ± 0.44	3.20 ± 0.95
	Difference	3.01 ± 1.93	1.02 ± 0.14
W_b (kg·cm/L)	Total	4.32 ± 1.50	1.77 ± 0.12 *
	Lower	0.62 ± 0.10	0.50 ± 0.23
	low/tot (%)	15.30 ± 4.22	29.58 ± 13.52
$\Delta P_{\dot{V}=0}$ (cm H_2O)	Total	4.62 ± 0.82	3.17 ± 1.04
	Lower	3.61 ± 0.73	3.30 ± 1.12
	Difference	1.02 ± 0.90	-0.13 ± 0.10 *
C_{dyn} (L/cm H_2O)	Total	1.29 ± 0.44	1.82 ± 0.57
	Lower	1.72 ± 0.78	1.77 ± 0.57
	Difference	-0.43 ± 0.50	0.06 ± 0.02

Table VII. The resistances of total and lower pulmonary systems in standing horses and cows. The values are presented as the mean ± 1 standard deviation, and those marked with and asterisk (*) are significantly different between the two species.

Variable			Cows	Horses
n			4	4
Total:				
$R_L I$	25	% of V_I	0.61 ± 0.29	0.32 ± 0.15
	50		0.77 ± 0.38	0.32 ± 0.32
	75		0.81 ± 0.44	0.45 ± 0.26
$R_L E$	25	% of V_E	0.64 ± 0.42	0.61 ± 0.27
	50		0.63 ± 0.38	0.53 ± 0.23
	75		0.53 ± 0.31	0.45 ± 0.23
Lower:				
$R_L I$	25	% of V_I	-0.02 ± 0.05	-0.10 ± 0.17
	50		0.03 ± 0.10	-0.18 ± 0.25
	75		0.07 ± 0.11	-0.11 ± 0.23
$R_L E$	25	% of V_E	0.05 ± 0.11	0.19 ± 0.25
	50		0.09 ± 0.13	0.14 ± 0.12
	75		0.09 ± 0.09	-0.06 ± 0.18

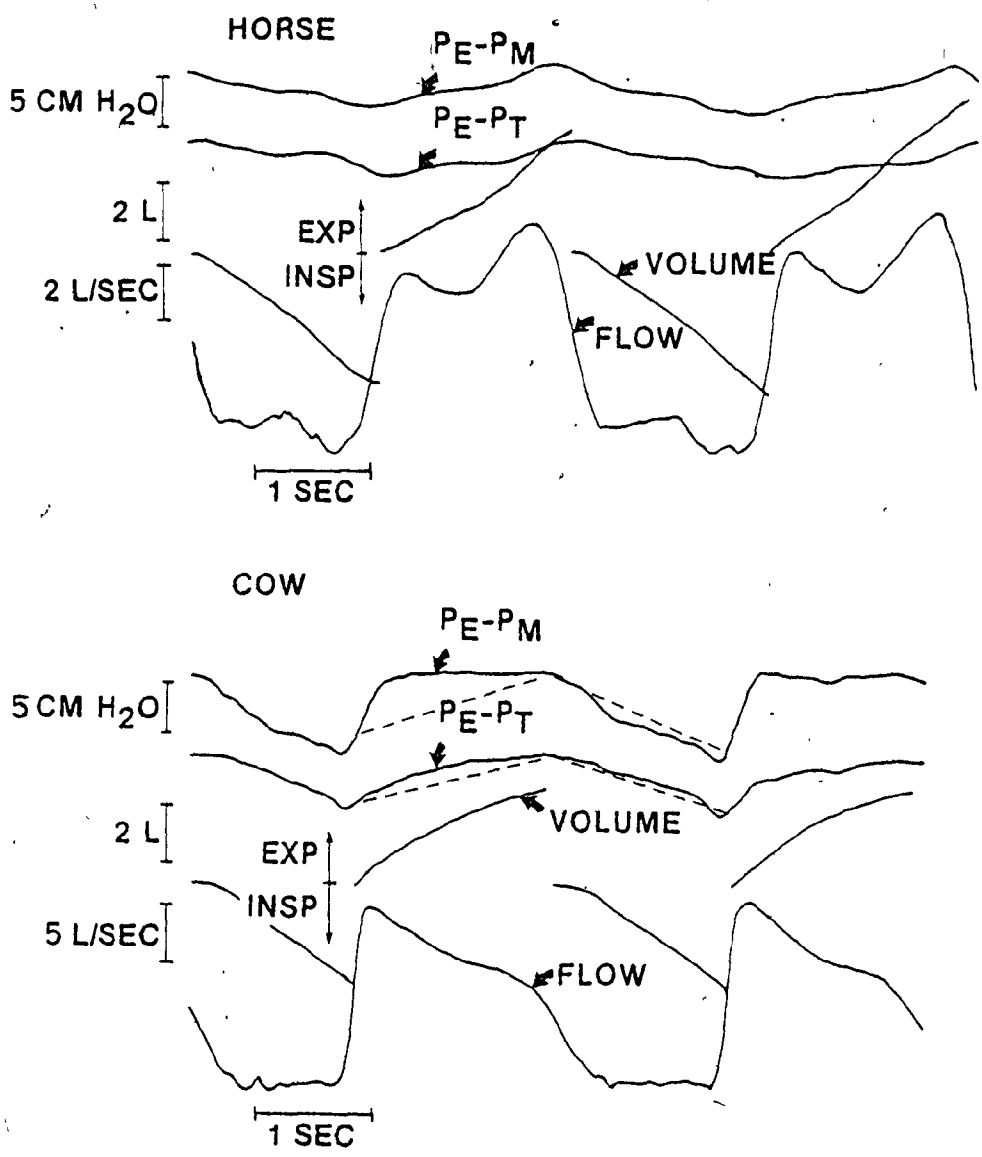


Figure 7. Representative scalar traces of flow rate, volume, and transpulmonary pressure of the total and lower pulmonary systems during normal breathing in a horse and cow. In the horse, the changes in $P_E - P_T$ lag behind the changes in $P_E - P_M$. In the cow, the slopes of the dotted lines indicate the change in $P_E - P_M$ is greater than the change in $P_E - P_T$ between end-inspiration and end-expiration.

and W_b (low) were not significantly different. The difference between ΔP_{TP}max (tot) and ΔP_{TP}max (low) was higher in the cows, and the ratio of W_b(low) to W_b(tot) was higher in the horses, but the differences between the two species were not statistically significant for either variable. In both cases the lack of significance was due to the high variability in one of the species.

In contrast to the earlier findings, C_{dyn}(tot) did not differ significantly between the two species even though C_{dyn}(tot) was lower in the cows. The $\Delta P_{\dot{V}=0}$(low) and C_{dyn}(low) were similar in both species. In the horses $\Delta P_{\dot{V}=0}$(low) and C_{dyn}(low) were the same as $\Delta P_{\dot{V}=0}$(tot) and C_{dyn}(tot), but in the cows $\Delta P_{\dot{V}=0}$(low) was less than $\Delta P_{\dot{V}=0}$(tot), and C_{dyn}(low) was greater than C_{dyn}(tot). These differences were not statistically significant, as the variability was large. But all of the cows showed the same trend. The differences were most marked in the cows which reacted most strongly to the insertion of the tracheal catheter and subsequently had a marked increase in $\dot{V}_E$.

The measurements of R_L(tot) and R_L(low) did not differ significantly between horses and cows. However, a consistent problem during the measurment of R_L(low) was the occurrence of negative resistances. During the preceding measurements of R_L(tot) in the horses negative resistance had occasionally been observed when the flow dropped during mid-inspiration. But in the measurements of R_L(low), negative resistances were observed during all phases of the breathing cycle in both species.

The differences between C_{dyn}(low) and C_{dyn}(tot) in the cows, and the negative resistances, prompted an examination of relationship

between the pressure changes in the total and lower pulmonary systems. It was noted that the changes in transpulmonary pressure in the lower pulmonary system usually lagged behind the changes in transpulmonary pressure in the total system (Figure 7). The transducers and recorder were tested for examination of phase lags (Materials and Methods), but both measuring systems responded at the same time. Thus, the difference was in the animals not in the measuring devices.

Lung Capacity and Quasistatic Compliance:

Representative loops obtained during the quasistatic P-V manoeuvres are presented in Figure 8, and the lung volumes and compliance values are presented in Table VIII. The lung volumes and P-V loops obtained during quasistatic manoeuvres were quite reproducible. The coefficient of variation of FRC in the anaesthetized animals was approximately 10%, which was similar to the coefficient of variation in FRC in the standing animals. Most of the variability in the P-V loops of the lungs was due to the changes in FRC_{an}. When FRC_{an} was standardized, the range of transpulmonary pressure at a given lung volume was usually less than 3 cm H_2O. The variability in the P-V loops of the chest wall and total respiratory system was much greater than the variability in the P-V loops of the lungs, and the variability was only slightly reduced by standardizing FRC. The variability was greater in the horses than in the cows, and appeared to be due to greater changes in the tone of the chest wall in the former species.

In the anaesthetized animals in sternal recumbency the lung volumes (FRC_{an}, IC_{an}, TLC_{an}, V_{Dan}) were significantly greater in the horses than in the cows. In both species, FRC_{an} was significantly less

Table VIII. Lung volumes obtained during the quasistatic P-V manoeuvres in anaesthetized horses and cows in sternal recumbency (prone). The values are presented as the mean ± 1 standard deviation, and those marked with an asterisk (*) are significantly different ($P < 0.05$) between the two species.

Variable	Cows	Horses
n	5	4
Weight (kg)	537 ± 151	394 ± 46
FRC_{an} (ml/kg)	31.96 ± 1.42	37.93 ± 2.68 *
IC_{an} (ml/kg)	52.51 ± 2.97	77.61 ± 4.78 *
TLC_{an} (ml/kg)	84.48 ± 3.91	115.54 ± 7.20 *
RV_{an} (ml/kg)	16.09 ± 2.66	-
VC_{an} (ml/kg)	68.38 ± 2.25	-
ER_{an} (ml/kg)	15.87 ± 2.12	-
CV_{an} (ml/kg)	13.74 ± 1.74	-
VC_{an} (ml/kg)	68.38 ± 2.25	-
V_{Dan} (ml/kg)	4.36 ± 0.70	8.94 ± 1.42 *
FRC_{an}/TLC_{an}	0.38 ± 0.01	0.33 ± 0.01 *
FRC_{an}/FRC	0.83 ± 0.09	0.76 ± 0.07
FRC/TLC_{an}	0.46 ± 0.05	0.44 ± 0.05

Table IX. The compliance values obtained during the quasistatic P-V manoeuvres in anaesthetized, paralyzed horses and cows in sternal recumbency (prone). C_L', C_W' and C_T' are the compliances calculated after FRC_{an} was adjusted to account for the decrease in FRC when the animals were anaesthetized. The values are presented as the mean ± 1 standard deviation, and those marked with an asterisk (*) are significantly different between the two species.

Variable		Cows	Horses
n		5	4
C_L	(L/cm H_2O)	1.02 ± 0.31	1.50 ± 0.46
C_W	(L/cm H_2O)	0.81 ± 4.85	3.14 ± 1.19
C_T	(L/cm H_2O)	0.89 ± 0.16	0.92 ± 0.21
C_L'	(L/cm H_2O)	1.00 ± 0.20	1.16 ± 0.10
C_W'	(L/cm H_2O)	2.64 ± 3.45	3.42 ± 1.35
C_T'	(L/cm H_2O)	0.88 ± 0.16	0.83 ± 0.10
C_L/FRC_{an}	(per cm H_2O)	0.061 ± 0.016	0.100 ± 0.024*
C_L'/FRC	(per cm H_2O)	0.050 ± 0.009	0.060 ± 0.020

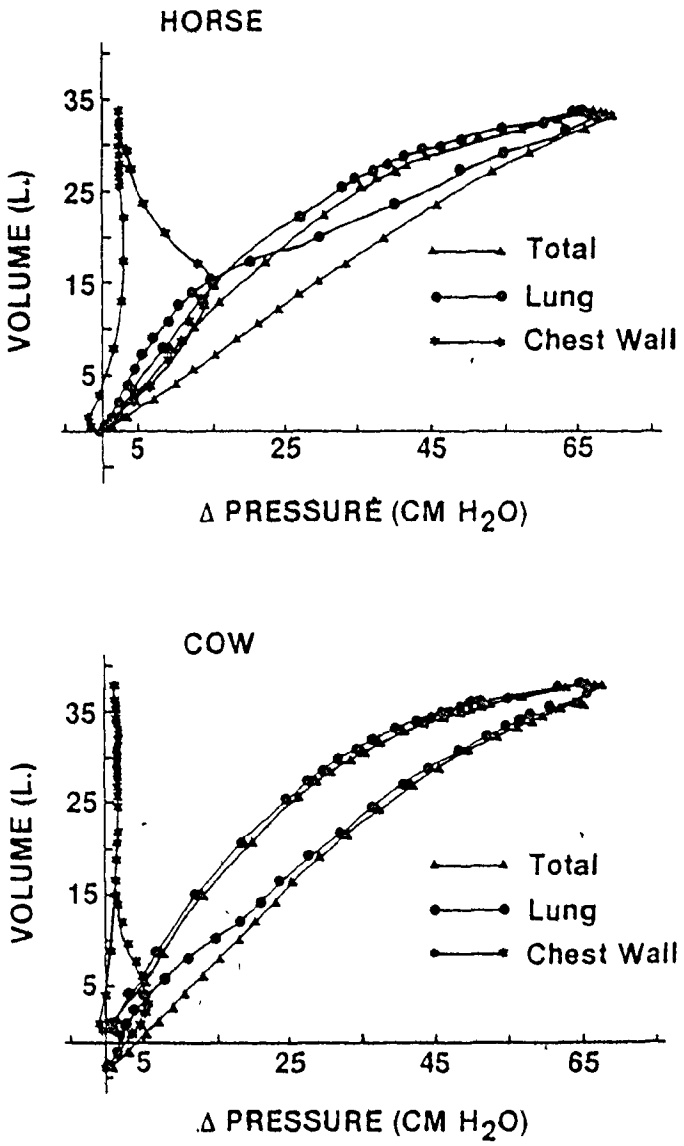


Figure 8. Representative pressure-volume (P-V) loops of the lung, chest wall and total respiratory system of anaesthetized, paralyzed horses and cows positions in sternal recumbency (prone). The volume indicates the inspiratory capacity (IC), or the volume above FRC. The pressure is expressed as the change in pressure above the resting end-expiratory pressure. The IC of the cow appears to be greater than that of the horse, however, the cow weighed 775 kg whereas the horse weighed 436 kg.

than FRC. The reduction in FRC (FRC_{an}/FRC) was slightly greater in the horses but the difference between the two species was not statistically significant. The FRC_{an}/TLC_{an} ratio was significantly greater in the cows, but the FRC/TLC_{an} ratio did not differ significantly between species. The V_{Dan} was calculated from the larynx to the lungs, and was significantly greater in the horses than the cows. The V_{Dan} was significantly less than V_{Dphys} in both species suggesting that during normal breathing a significant amount of the total dead space was in the upper airways.

The values for RV_{an}, ER_{an}, VC_{an} and CV_{an} are presented for the cows but not for the horses as there were technical problems in the measurement of these values in the latter species. The CV_{an} was slightly less than ER_{an} in the cows, and the relationship between these volumes appeared to be similar in the horses.

The compliances (C_L, C_W, C_T) and adjusted compliances based on FRC in the standing animal (C_L', C_W', C_T') were not significantly different between the two species. The difference between C_L and C_L' was 0.34 L/cm H_2O in the horses, but this was not statistically significant. The specific compliance of the anaesthetized animals (C_L/FRC_{an}) was significantly greater in the horses, but the adjusted specific compliance (C_L'/FRC) was not significantly different between the two species. In both species C_L and C_L' were less than C_{dyn}, and these differences were significant for the horses. When the specific dynamic compliances of the standing animals (C_{dyn}/FRC) were compared with specific the quasistatic compliances and adjusted specific

quasistatic compliances, C_L/FRC_{an} and C_L'/FRC respectively, the only significant difference was that C_L'/FRC was less than C_{dyn}/FRC in the horses.

The values obtained from the mathematical analysis of the deflation P-V curves using the methods of Pengelly (1977) are presented in Table IX. The only significant difference between the two species was the V_{max}, which was greater in the horses than in the cows. For all of the animals the coefficient of determiantion was very high (minimum $r^2 > 0.98$) indicating an excellent fit between the observed data and the calculated curves. This method of analysis appeared to be superior to the calculation of compliance in that the coefficient of variation of h was significantly less than the coefficient of variation of C_L and C_L'.

Pulmonary Structure:

Fixation:

The volume of the fixed lungs of the horses was significantly greater than the volume of the fixed lungs of the cows, and in both species the volume of the fixed lungs was significantly less than TLC_{an} (Table XII). The ratio of the volume of the fixed lung to TLC_{an} did not differ significantly between the two species. The relatively low value of the fixed lungs was due in part to the poor inflation, and consequently poor fixation, of areas within the lungs. During the instillation of fixative, the cranial and middle lobes inflated rapidly, while the caudal lobes did not begin to inflate until after the cranial

Table X. The parameters calculated from the mathematical analysis (Pengelly 1977) of the deflation P-V curves of the lungs of anaesthetized, paralyzed horses and cows in sternal recumbency. The values are presented as the mean ± 1 standard deviation, and those marked with an asterisk (*) are significantly different between the two species.

Variable	Cows	Horses
V_{max} (ml/kg)	97.95 ± 8.36	123.75 ± 4.37 *
V_{max}/TLC_{an}	1.16 ± 0.11	1.07 ± 0.03
h (cm H_2O)	21.48 ± 3.80	17.52 ± 1.44
P_z (cm H_2O)	-11.43 ± 1.43	-8.72 ± 2.48
REEP (cm H_2O)	0.74 ± 0.90	0.51 ± 2.16
k_1	0.033 ± 0.006	0.040 ± 0.003
r^2	98.83 ± 0.69	98.90 ± 0.84

and middle lobes were partially inflated. In the cows the pattern of lung inflation was obviously lobular. On examination of the 5 cm sections, there were unfixed lobules throughout the lung, but they were more frequent in the caudal lobes. In the cows with the lowest degree of inflation, often only the center of the lobule was fixed. The inflation of the equine lungs was generally better than that of the bovine lungs, and areas of poor fixation were more difficult to define. But as in the cows, the pattern of inflation appeared to be lobular, and the poorly fixed lobules were primarily in the caudal lobes.

Those areas of the lung which were obviously not fixed were avoided when the tissue samples were taken. However, even within the fixed tissue the pattern of inflation was not uniform. In the bovine lungs there were obvious differences in the degree of inflation of adjacent lobules (Figure 10E). In the equine lungs this pattern was less obvious, but the poorly inflated areas appeared to be associated with partially constricted bronchioles in which there was folding of the mucosa and lamina mucosa. This suggested that the pattern of inflation was lobular.

Morphology:

Quantitative Measurements:

Trachea:

The length of the trachea was similar in both species, but the volume of the trachea was significantly greater in the horses than in the cows (Table XI). The dimensions of the cross sections of the

trachea were measured just posterior to the larynx, midway from the larynx to the carina, and immediately anterior to the carina. In both species the trachea was ellipsoid in cross-section. In the horses the horizontal axis (width) was greater than the vertical axis (height), but in the cows the vertical axis was greater than the horizontal axis. The vertical axis was similar in both species, but the horizontal axis was greater in the horses.

Lungs:

The lungs of both species were approximately 45 cm in height. The bovine lungs were shorter than the equine lungs, and the diaphragmatic border was more vertical in the bovine lungs. The morphometric measurements of the lungs are presented in Table XII. The only significant difference between the two species was the greater amount of interlobular septa in the cows. The difference between the two species is apparent in Figures 9 and 10. There were no obvious dorso-ventral or cranio-caudal gradients in the volume and surface densities of any of the components.

As indicated previously, the tissue samples were taken from the inflated areas within the lungs. Thus, the measurements of volume and surface density are representative of those areas. Absolute values for the volumes and surface areas of the various components were not calculated from these measurements as the volumes of inflated lungs were less than TLC_{an}, and there were no measurements of the volume and surface densities of the various components in the unfixed tissue. Thus, there was the potential for considerable error in the determination of absolute volumes.

Table XI. Dimensions of the trachea of the horses and cows. The values are presented as the mean ± 1 standard deviation, and those marked with an asterisk (*) are significantly different ($P < 0.05$) between the two species. The width and height refer to the horizontal and vertical axes of the cross-section of the trachea. The terms larynx, mid and carina refer to locations of the measurements; immediately posterior to the larynx, midway between the larynx and the carina, and immediately anterior to the carina.

Variable		Cows	Horses	
n		4	4	
Weight		605 ± 117	394 ± 46	*
Volume (ml/kg)		1.40 ± 0.21	2.89 ± 0.27	*
Length (cm)		78.8 ± 2.5	78.5 ± 1.8	*
Width: (cm)	larynx	3.63 ± 0.23	4.63 ± 0.15	*
	mid	3.77 ± 0.21	5.13 ± 0.45	*
	carina	3.57 ± 0.31	4.63 ± 0.21	*
Height: (cm)	larynx	4.14 ± 0.25	3.93 ± 0.31	
	mid	4.34 ± 0.59	3.37 ± 0.55	
	carina	4.40 ± 0.40	4.13 ± 0.60	

Table XII. Morphometric measurements of the lungs of the horses and cows. The values are presented as the mean ± 1 standard deviation, and those marked with an asterisk (*) are significantly different ($P < 0.05$) between the two species.

Variable	Cows	Horses
n	4	4
Weight (kg)	605 ± 117	394 ± 46 *
Volume of Fixed Lungs (ml/kg)	48.0 ± 11.1	82.7 ± 5.2 *
Volume of Fixed Lungs/ TLC_{an} (%)	59.1 ± 14.1	71.7 ± 5.9
Lung:		
Parenchyma (cm^3/cm^3)	0.820 ± 0.032	0.840 ± 0.016
Non-parenchyma (cm^3/cm^3)		
Airways	0.097 ± 0.010	0.095 ± 0.006
Blood Vessels	0.047 ± 0.025	0.056 ± 0.015
Interlobular Septa	0.033 ± 0.002	0.005 ± 0.002*
Parenchyma		
$V_V(A)$ (cm^3/cm^3)	0.885 ± 0.013	0.909 ± 0.019
$V_V(c)$ (cm^3/cm^3)	0.067 ± 0.012	0.053 ± 0.011
$V_V(s)$ (cm^3/cm^3)	0.047 ± 0.009	0.038 ± 0.009
$S_V(A)$ (cm^2/cm^3)	1135 ± 100	1093 ± 131
$S_V(c)$ (cm^2/cm^3)	848 ± 96	766 ± 51
$S_V(A)/S_V(c)$ (cm^2/cm^2)	1.35 ± 0.13	1.43 ± 0.11
$V_V(c)/S_V(A)$ (cm^3/m^2)	0.59 ± 0.13	0.49 ± 0.12

Qualitative Observations:

During the gross and histological examinations of the lungs several observations were made which were not quantified. In some cases additional material would have been required as quantification would have compromised the tissue for the present study. In other cases, the differences were in the distribution, rather than the quantity, or the differences were obvious and the volume densities were so small that quantification would have required a disproportionate amount of effort.

The first observation was of the differences between the airways of the bovine and equine lungs. In the cows, the distance between successive branches, or the length of the airways, was less than in the horses (Figure 9). Also, in the cows the branching angles of the primary bronchi appeared to be more acute and the daughter bronchi were usually much smaller than the stem bronchi. The differences in branching angle and the relative sizes of the main and stem airways were not apparent at the level of the bronchioles, but the distal airways appeared to be much longer in the horses (Figure 10).

In the lungs of the horses and cows, the distal airway was usually a terminal bronchiole. Respiratory bronchioles were observed in both species, but were more common in the bovine lungs where they comprised approximately one-quarter of the distal airways. In both species the respiratory bronchioles were usually poorly developed (Figure 10A, 10C).

There were few obvious differences in the structure of the alveoli of horses and cows. In both species the tissue of the alveolar

septum was composed of squamous or Type I pneumocytes, granular or Type II pneumocytes, endothelial cells and fibroblasts (Figure 11, 12). The Type II pneumocytes were most commonly observed in the corners of alveoli, near the junctions with adjacent alveolar septae.

Erythrocytes were the most common blood cells in the capillaries, but granulocytes, lymphocytes and macrophages were also observed. The intravascular macrophages were more common in the bovine lungs, and appeared to aggregate in localized areas (Figure 11C).

Interalveolar pores were observed in the lungs of both species. They were larger and more common in the equine lungs.

Collagen was present in the lungs of both horses and cows. The greatest concentrations of collagen were associated with the airways and blood vessels. Large amounts of collagen were also observed in the interlobular septa. Collagen was distributed throughout the gas exchange parenchyma. In the gas exchange parenchyma the greatest concentrations were in the tips of the alveolar septa (Figure 12A, 12B), and the collagen appeared to be associated with the alveolar duct rather than the alveoli. Collagen was also observed throughout the alveolar septa. In the equine lungs it was distributed along one side of the capillaries, in the septa, between adjacent capillaries (Figure 12C, 12D), and at the junctions of alveolar septa. In the bovine lungs there appeared to be less collagen along the side of the capillaries than in the equine lung, and more collagen in the septum between adjacent capillaries. However, the amount of collagen in the alveolar septum did not appear to differ significantly between the two species.

In the horses and cows, smooth muscle surrounded the intra-

pulmonary airways from the primary bronchi to the alveolar ducts. The smooth muscle surrounding the alveolar ducts was located at the tips of the alveolar septa (Figure 11, 12A, 12B). There was a greater amount of smooth muscle surrounding the alveolar ducts in the bovine lungs. In well inflated sections the muscle appeared to form a continuous band around the openings to the alveoli, but in sections which were poorly inflated the smooth muscle was contracted and openings to the alveoli were occluded.

Figure 9. Photographs of 5 cm thick sections cut 10 cm from the top of the right lung of a horse and a cow. There is a greater amount of interlobular septa (→) in the bovine lung, and a shorter distance between airway branches. The differences in branching angle and size of the airways are not apparent due to the plane of the section. (Magnification: horse 0.33X; cow 0.45 X.

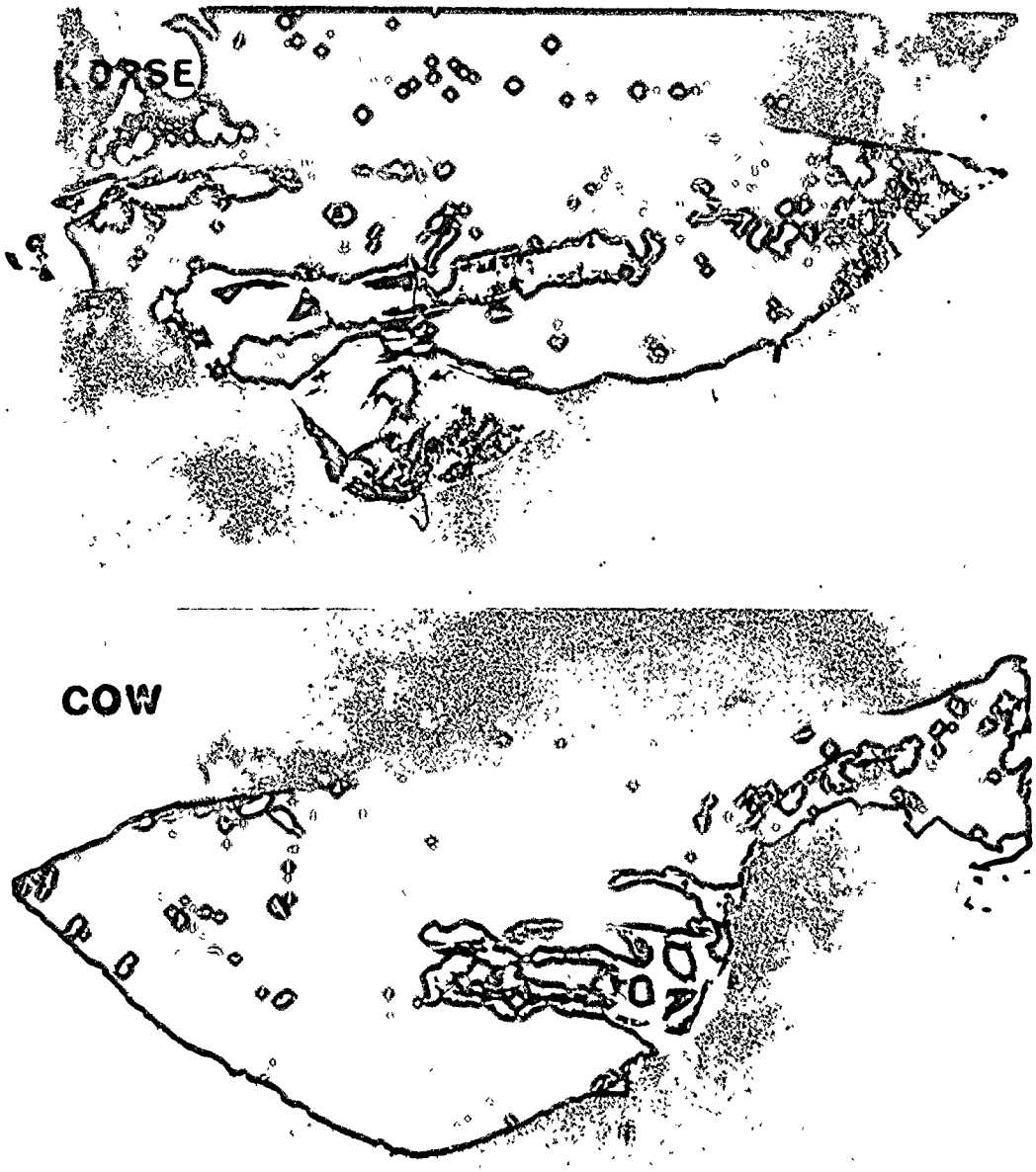

HORSE
COW

Figure 10. Low power (25X) photomicrographs of 6 µm sections of bovine and equine lungs stained with haematoxylin and eosin.

A and C. Sections of bovine lungs showing the structure of the gas exchange parenchyma and respiratory bronchioles (Rb). C also demonstrates the short distal airways and alveolar ducts (Ad) (S - interlobular septum).

B and D. Sections of the gas exchange parenchyma of equine lungs. D demonstrates the long terminal bronchioles (Tb) and alveolar ducts (Ad) in the horse.

E. Section of a bovine lung showing the differences in the degree of inflation of adjacent lobules (S - interlobular septa).

F. Section of a bovine lung at residual volume showing the alveolar atelectasis and airway collapse. The arrow indicates material in the collapsed airway.
(Magnification: A to E, 75X; F, 190X).

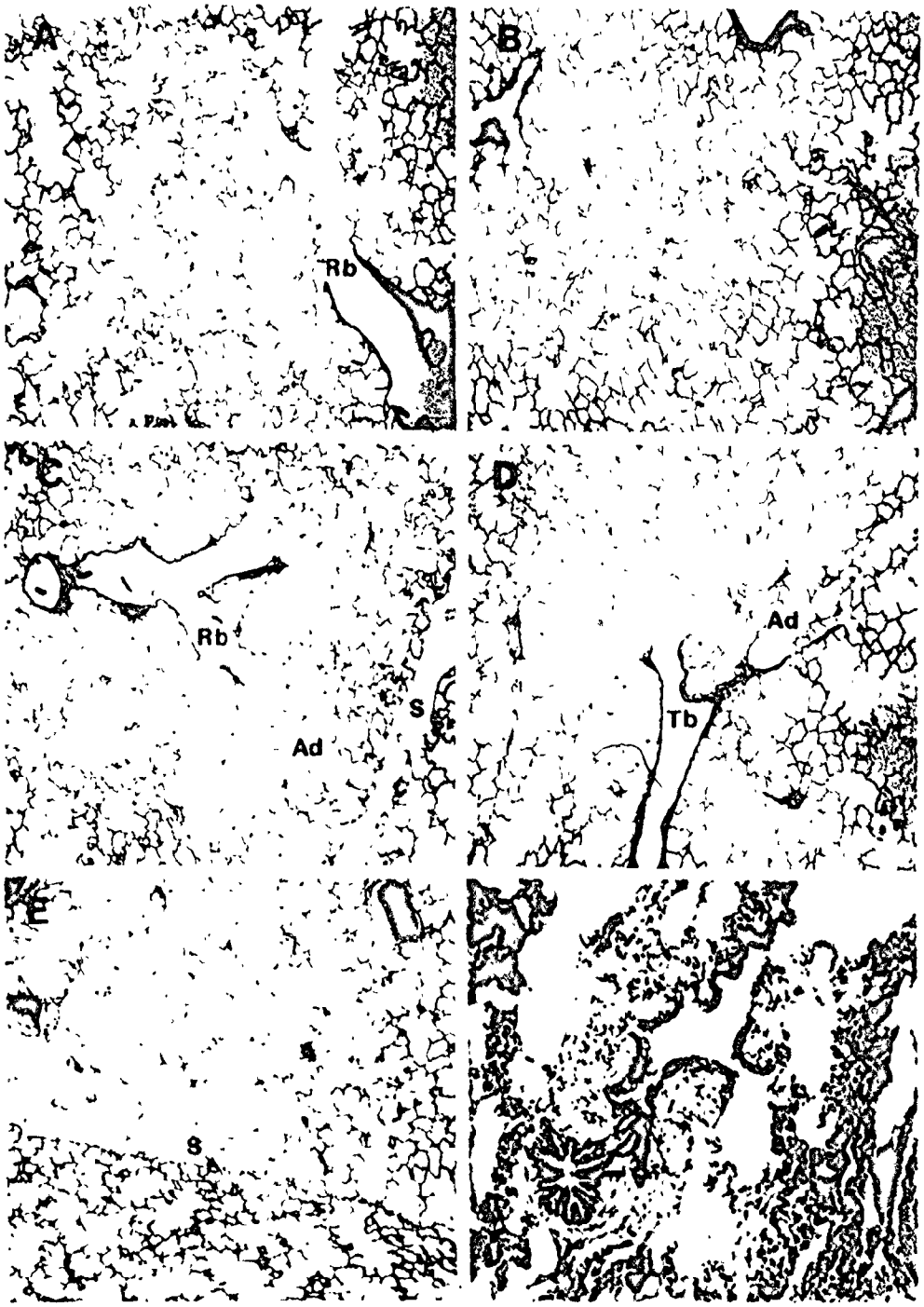

A
Rb
B
C
Rb
S
Ad
D
Ad
Tb
E
S

Figure 11. High power (400X) photomicrographs of 0.5 µm sections of equine (A, B) and bovine (C, D) alveoli stained with 1% methylene blue. AD - alveolar duct, Al - alveolus, Ca - capillary, Se - septal tissue, I - Type I pneumocyte, II Type II pneumocyte, Ma - macrophage. The large arrows indicate interalveolar pores, and the small arrows indicate the location of smooth muscle.
(Magnification: 1350X).

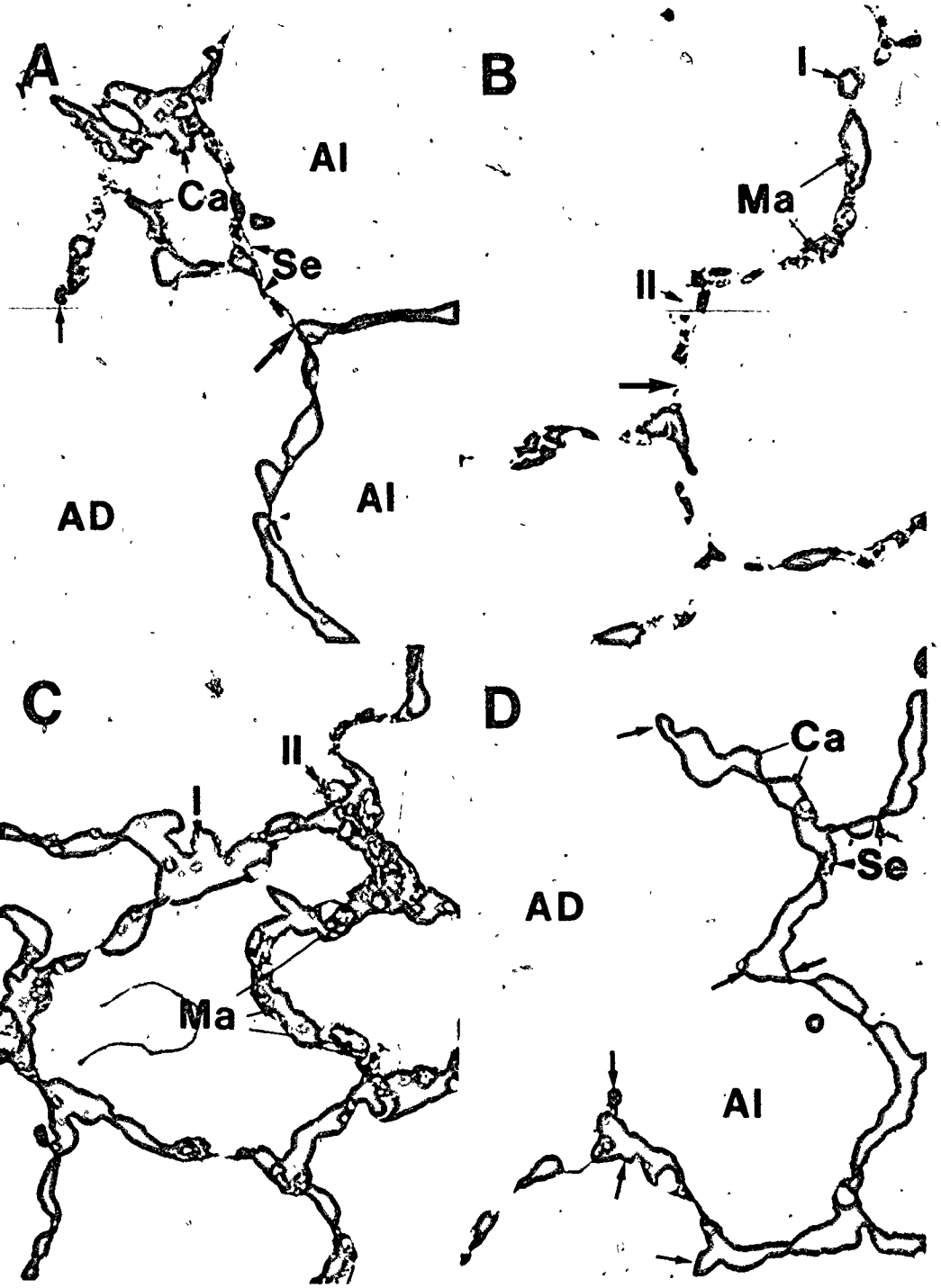

A
Al
Ca
Se
AD
Al
B
I
Ma
II
C
II
I
Ma
D
Ca
Se
AD
o
Al

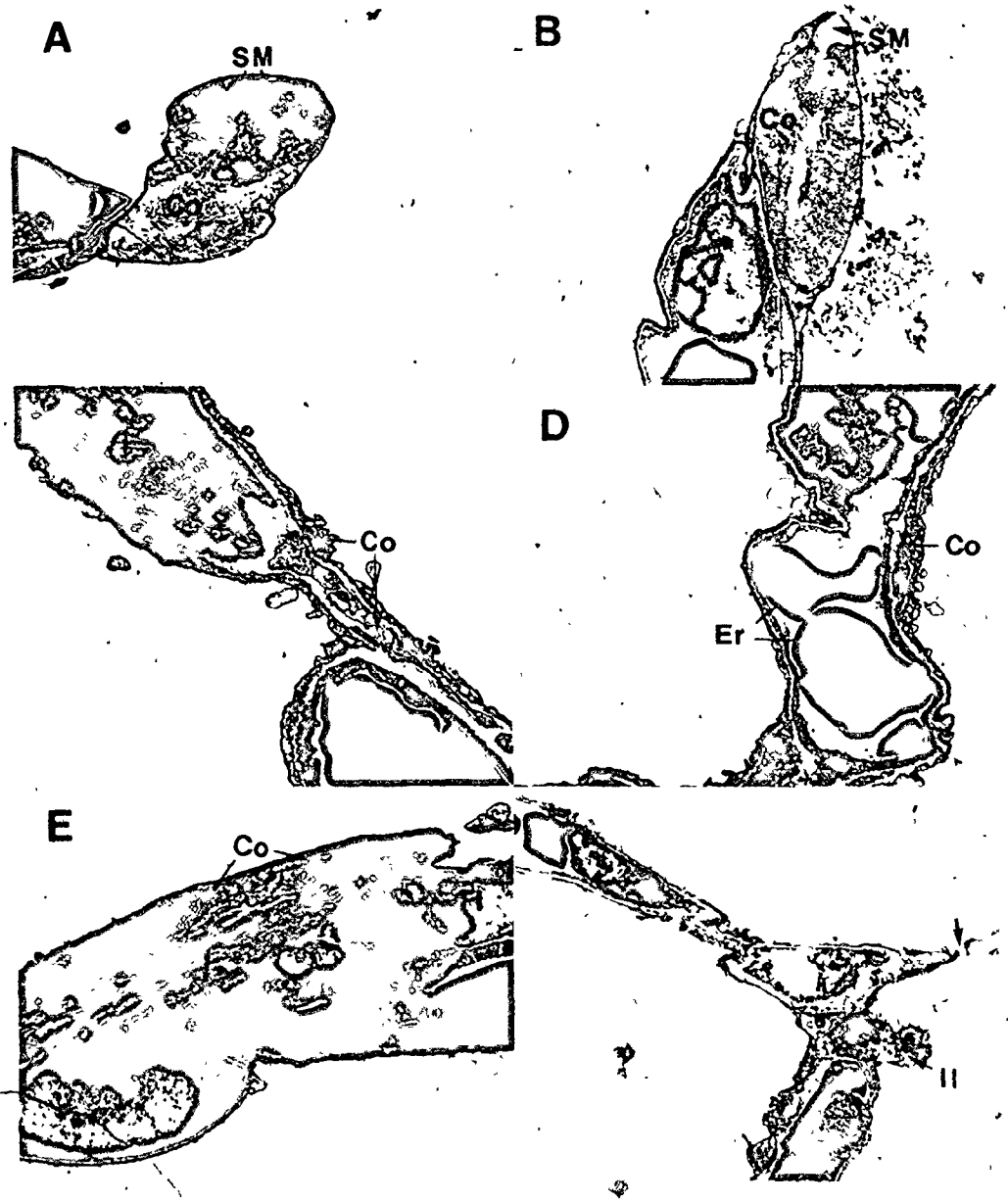
Figure 12. Photomicrographs of thin (1000 Å) sections of bovine and equine lungs.

A and B. Tip of the alveolar septa of bovine and equine lungs respectively. Co - collagen, SM - smooth muscle. (Magnification: A 2750X; B 5800X).

C and D. Capillaries in the bovine lung showing the location of collagen (Co) in the alveolar septa. The distribution is similar in the bovine lung, Er - erythrocytes, En - capillary endothelial cell. The cell in the upper capillary in section C may also be a macrophage. (Magnification: C 8850X; D 5700X).

E. Longitudinal section of smooth muscle cell (SM) in the parenchyma of the bovine lung. Co - collagen, Fi - fibrobast, Ma - intravascular macrophage. Magnification: 5500X.

F. Alveolar septa of the equine lung showing Type II pneumocyte (.||), and interalveolar pore at the tip of the arrow. (Magnification: 2700X).


A
SM
B
SM
Co
D
Co
Co
Er
E
Co
II

DISCUSSION

Lung Structure:

The morphological observations of the lungs of horses and cows largely confirm the reports of previous workers (Epling 1964, Hare 1975a, 1975b, Gillespie and Tyler 1967, Mariassy et al. 1975, McLaughlin et al. 1961a, Rybicka et al. 1974a, 1974b). The lungs of both species were of similar height, but the bovine lungs were shorter than the equine lungs and the diaphragmatic border formed a more acute angle with the dorsal border. The bovine lungs were divided into distinct lobes and the interlobular septa were well developed and complete. In contrast the equine lungs appeared to be a single lobe and had irregular interlobular septa.

Histological examination of the gas exchange parenchyma revealed few differences between the horses and cows, and the gas exchange parenchyma was similar to that of other mammals (Forrest and Weibel 1975, Gehr et al. 1978, Weibel 1973). As reported by Mariassy et al. (1975), there were relatively few inter-alveolar pores in the bovine lungs compared to the lungs of horses (Nowell and Tyler 1971, this study) and other mammals (Gehr et al. 1978, 1980, Siegwart et al. 1971). The pores, when present, were generally small. The observations in the present study also confirmed the finding of Rybicka et al. (1974b)

that most of the pulmonary macrophages in the bovine lung were in the capillaries rather than the alveoli.

Previous authors (Lodge 1969, Tenney and Bartlett 1967) have reported that the diameter of the bovine trachea is less than the diameter of the equine trachea. The results of the present study demonstrate that this difference is due to the greater width of the equine trachea. The mean diameter of the bovine and equine tracheas midway between the larynx and the carina were similar to the diameters reported by Tenney and Bartlett (1967), and the lengths of the bovine and equine tracheas were similar to values expected from tracheal length to body weight relationship reported for mammals ranging in size from the mouse to man (Tenney and Bartlett 1967).

Tenney and Bartlett (1967) suggested that the average linear velocity in the trachea was the same in all mammals. However, in the present study the mean diameter of the bovine trachea was less than the mean diameter of the equine trachea, and the flow rates during inspiration and expiration were higher in the cows. Thus, the average linear velocity was much higher in the trachea of the cows than in the trachea of the horses. Based on the mean linear velocities obtained in the present study, during inspiration, the Reynolds numbers of the horses and cows were 4700 and 7700 respectively. This indicates that flow was turbulent in the trachea of both species, and that there was greater turbulence in the bovine trachea.

The observations of the differences in the branching patterns of the intrapulmonary airways of horses and cows have not been reported previously. These observations need to be quantified in future

studies. Horsfield (1977, 1980) has suggested that in the lungs of dogs and humans, the branching pattern of the airways reduces entropy and minimizes airway resistance. But acute angles between airway branches, large differences in size between the main and stem bronchi, and short airways promote, or maintain, turbulent airflow (Pedley et al. 1977). Thus, the branching pattern of the airways in the bovine lung would tend to increase resistance as the pressure drop in turbulent flow varies as a function of the velocity squared. It is therefore somewhat surprising that despite the higher flow rates in the cows, R_L (low) did not differ significantly between the horses and cows. One possible explanation is that the higher resistance per unit airway length in the cows was counteracted by the decreased length of the airways in this species.

In contrast to the findings of McLaughlin et al. (1961a), respiratory bronchioles accounted for approximately 25% of the distal airways in the bovine lungs, but were seldom observed in the equine lungs. The respiratory bronchioles were, however, poorly developed in both species as previously reported (Mariassy et al. 1975, McLaughlin et al. 1961a).

Baltisberger (1921) described smooth muscle in the tips of the alveolar septa surrounding the alveolar ducts, and it has been suggested (Hammersen 1976) that the smooth muscle encircles the alveolar openings in a sphincter-like fashion. This distribution is similar to that observed in the present study. The significance of the increased amount of smooth muscle surrounding the mouths of the alveoli in the bovine lung is unknown. It is possible that the

increased amount of smooth muscle may have been an artefact as the degree of inflation of the bovine lungs was less than that of the equine lungs. But even when the lungs of both species were at residual volume, the smooth muscle is more obvious in the bovine lung (unpublished observations). Relaxation or contraction of the smooth muscle may alter the distribution of ventilation, or may regulate the pressure in the alveoli so as to prevent the development of equal pressure points and airway closure. However, until further studies are conducted on the changes in configuration of the smooth muscle during inflation, and on the pharmacological control of this muscle, the significance of smooth muscle in the gas exchange parenchyma will remain speculative.

Valid comparison of the morphometric measurements of the lungs between animals or species can only be made when the lungs are fixed at equivalent volumes. This volume has been defined as TLC, or the lung volume at a transpulmonary pressure of 30 cm H_2O (Gehr et al. 1978, 1980, Weibel 1973). Fixation of the lungs at a volume less than TLC will result in underestimation of the airspace volume, and an overestimation of $V_V(c)$ and $V_V(s)$ (Gehr and Erni 1980). In the present study the lungs were fixed at a transpulmonary pressure of 50 to 60 cm H_2O, the pressure normally required to reach TLC when the lungs were in situ. In every animal the volume of the fixed lungs was less than the measured TLC, and inflation and fixation were not uniform throughout the lung. Nevertheless the ratio of the fixed lung volume to TLC was similar to the ratios reported from other studies (Weibel et al. 1981). By random sampling it would have been possible

to obtain comparable values in the present study. However, as poorly fixed areas, which have a high $V_V(c)$ and $V_V(s)$ were not sampled, comparisons of the absolute values from the present study with those of other studies would be somewhat tenuous.

There are two possible reasons for the incomplete inflation of the lungs in the present study; 1) the inability to overcome surface tension in collapsed airways, and 2) the volume of the lung after fixation varies with the rate of instillation of fixative (Hayatdavoudi et al. 1980). The first reason may account for the unfixed lobules, while the second accounts for the nonuniform inflation of the fixed tissue.

The hypothesis that surface tension in the collapsed airways prevented the inflation of lobules is based on observations made on material obtained from the post-mortem room of the Department of Pathology, and from the abbatoir of the Department of Animal Science, University of Guelph. During the movement of carcasses within these areas the carcasses are often suspended so that the abdominal contents compressed the thoracic viscera. Also, when the lungs are removed from the thorax they are allowed to collapse to residual volume. Only the cranial lobes of bovine lungs obtained from the abbatoir could be reinflated, even though pressures of up to 150 cm H_2O were used for the instillation of buffered saline. In equine and bovine lungs obtained from the post-mortem room, there was extensive alveolar atelectasis. The bronchioles were collapsed and there was often a proteinaceous material in the lumen (Figure 10F). According to the Laplace equation, the pressure inside a bubble is inversely

proportional to the radius. Thus, considerable pressure would have been required to overcome the surface tension in the narrow lumen of the collapsed bronchioles. Theoretically, the instillation of a liquid would have eliminated the air-liquid interface, and overcome this problem. However, a considerable volume of air is retained in the airways even at residual volume. It is probable that air was trapped between the instilled liquid and the collapsed bronchiole and the air-liquid interface was maintained. Thus, the collapse of the bronchioles probably prevented the inflation of the lobules.

During the post-mortem procedures in the present study, the endotracheal tube was capped to maintain the lungs at FRC to prevent the total collapse of the lungs and airways when the thorax was opened. But the FRC would have been reduced as the abdominal viscera tended to shift forward when the carcasses were transferred to the table. The weight of the abdominal viscera would have compressed the caudal lobes of the lung (McDonell 1974, McDonell et al. 1979). Most of the uniflated lobules were at the periphery of the caudal lobes of the lung where the compression of the lung and collapse of the airways would have been most severe.

Hayatdavoudi et al. (1980) have shown that the degree of inflation of the fixed lung was independent of the volume of air in the lungs at the beginning of inflation. However, their study was conducted with rats. This species has a Type II lung, which has no interlobular septa and a large number of interalveolar pores (McLaughlin et al. 1961a). This type of lung has a low collateral resistance (Robinson and Sorenson 1978, Woolcock and Macklem 1971)

hence it is possible to inflate areas of lung distal to occluded airways. Collateral resistance in the Type III (equine) lung is much higher than in the Type II lung (Robinson and Sorenson 1978, Woolcock and Macklem 1971), and is extremely high in the Type I (bovine) lung (Van Allen et al. 1931, Woolcock and Macklem 1971). Thus inflation of the bovine lungs must be via the airways, and inflation of the equine lungs is primarily via the airways. Therefore it seems reasonable to conclude that airways collapse could alter the inflation of the lungs of horses and cows.

The second reason suggested for the incomplete inflation of areas of lung parenchyma was that at low rates of instillation of fixative, fixation occurs prior to complete inflation (Hayatdavoudi et al. 1980). This might account for the differences in the degree of inflation of adjacent lobules (Figure 10E). The lobules with the shortest time constant expanded rapidly and were fixed at a volume equivalent to TLC, while the lobules with longer time constants expanded more slowly were often fixed at a lower volume. The degree of inflation of the lobules with the longer time constants was probably improved by the interdependence of adjacent lobules (Mead et al. 1970). But, complete inflation was not possible as the interdependence appeared to decrease as most of the lobules became inflated. Also, the rate of inflation declined as the pressure gradient between the lungs and the reservoir was reduced.

Other workers (Gehr and Erni 1980, Gehr and Weibel 1974) have reported differences in the degree of inflation within fixed lungs. However, they reported that there were dorso-ventral or cranial-caudal

gradients. In the present study the degree of inflation varied between adjacent lobules within the lobes of the lungs and there were no obvious dorso-ventral or cranial-caudal gradients. The differences between the findings in the present study and those of the previous studies (Gehr and Erni 1980, Gehr and Weibel 1971) are most likely due to differences in method of fixation. In the studies reported by Gehr and coworkers the lungs were fixed in situ, whereas in the present study the lungs were removed from the thorax and immersed in buffered saline. In situ there is a vertical gradient of pressure due to the weight of the fixative, and there is interaction between the lung and chest wall which increases the interdependence of lung units (Zidulka et al. 1979). When the lungs were immersed in buffer in the present study, the pressure gradient was abolished and there was anisotropic expansion as there was no longer any interaction between the lung and chest wall.

Hayatdavoudi et al. (1980) found that lungs fixed in situ had a greater volume than excised lungs, and postulated that this was due to circulating blood in the lungs fixed in situ. The volumes of the fixed lungs in the present study were greater than those previously reported for equine and bovine lungs fixed in situ (Gehr and Erni 1980). The relatively low lung volumes reported by Gehr and Erni (1980) were most likely due to their positioning of the animals in dorsal recumbency during the fixation procedures. In horses in dorsal recumbency, the abdominal contents compress the caudal lobes of the lung (McDonell 1974, McDonell et al. 1979) and TLC is significantly reduced (Sorenson and Robinson 1980). The reduction in TLC has not

been demonstrated in the cow, but the FRC is significantly reduced when the abdominal pressure is increased (Musewe et al. 1979), and the abdominal viscera tend to shift forward onto the thoracic cavity when these animals are positioned in dorsal or lateral recumbency. Given the large mass of the abdomen in the cow, it is probable that TLC is also significantly reduced when this species is positioned in dorsal recumbency.

The volume of the fixed lungs reported by Gehr and Erni (1980) is greater than the TLC reported by Sorenson and Robinson (1980). However, Gehr and Erni (1980) inflated the lungs to a pressure of 80 cm H_2O above mid-lung height. At this inflation pressure, the hydrostatic gradient would have produced pressures in excess of 100 cm H_2O in the lowermost regions of the lung. These latter pressures are more than three times greater than the inflation pressure used by Sorenson and Robinson (1980), and would have counteracted the pressure exerted on the lung by the abdominal contents.

There would appear to be problems in the fixation of the lungs in all of the morphometric studies of the lungs of horses and cows (Gehr et al. 1977, Gehr and Erni 1980, the present study), nevertheless it is of interest to compare the results. In the present study the volumes of the fixed lungs of the horses and cows respectively were 1.12 and 1.50 times greater than those reported by Gehr and Erni (1980). The parenchymal fraction of the equine lung was similar to that reported by Gehr and Erni (1980), but $V_V(A)$ was higher, and $V_V(c)$ and $V_V(s)$ were lower. These differences can be explained by the greater degree of inflation obtained in the present study. The $S_V(A)$

and $S_V(c)$ were higher than the values reported by Gehr and Erni (1980) but the ratio of $S_V(c)$ to $S_V(A)$ was the same. The ratio of $V_V(c)$ to $S_V(A)$ in the present study was less than one-half of the value reported by Gehr and Erni (1980). The $S_V(A)$ of the bovine lung was twice that reported by Gehr et al. (1977) and the $V_V(c)$ to $S_V(A)$ ratio was about one fourth of the value reported by Gehr and Erni (1980).

Gehr and Erni (1980) have suggested that the maximum diffusing capacity of the equine lung is 2.75 times greater than that of the bovine lung, and that the resting $\dot{V}_{O_2}$ of the horse is 2.81 times greater than that of the cow. In the present study the measured $\dot{V}_{O_2}$ of the cows was higher than the $\dot{V}_{O_2}$ of the horses. The relative amount of gas exchange parenchyma within the lung did not differ between the two species, nor did the volume and surface densities of the alveoli and capillaries within the parenchyma. The only significant difference between the horses and cows was the volume of the fixed lungs, which was higher in the horses than in the cows. As there were no significant differences in the density of the components of the gas exchange region of the lungs, and the harmonic mean thickness of the alveolar-capillary barrier does not appear to differ significantly between the two species (Gehr et al. 1981), it may be assumed that $D_L$max is proportional to the difference in TLC. Thus $D_L$max would only be 1.35 times greater in the horse than in the cow.

Pulmonary Function:

Gas Exchange and Metabolic Rate:

The metabolic rates of the horses and cows were similar to the metabolic rates reported for these species by Brody (1945). For the horses and cows respectively, the metabolic rates were 1.45 and 1.85 times higher than the values predicted by Kleiber's (1975) equation for the basal metabolic rate of mammals. The difference between the measured and expected basal metabolic rate is assumed to represent the metabolic cost of standing (Hall and Brody 1933, 1934). In the present study the metabolic rate of the cows was higher than that of the horses. This difference may be in part due to excitement, as the cows were less accustomed to being handled than the horses. The higher $\dot{V}_E$ to $\dot{V}_{O_2}$ ratio, and lower P_{ACO_2} of the cows suggested that they were hyperventilating, which would be consistent with this hypothesis.

The values for V_T, f_R and $\dot{V}_E$ were within the range of values reported previously (Figure 2). The V_T of the cows was similar to the value predicted by the equation of Stahl (1967) while the V_T of the horses was higher. In contrast, the f_R of the horses was similar to the predicted value while the f_R of the cows was higher. The $\dot{V}_E$ of both species was higher than the value predicted from the equations of Stahl (1967). In the horses the increase in V_E was similar to the increase in $\dot{V}_{O_2}$ over the value predicted by Kleiber's (1975) equation, but in the cows the relative increase in $\dot{V}_E$ was greater than the increase in $\dot{V}_{O_2}$.

The arterial blood gases (Table III) were within the range of values reported for normal horses and cows at sea level by previous

authors (Bisgard et al. 1973, Gillespie et al. 1964, Hales and Findlay 1968a, Hilledge and Lees 1975, Hilledge et al. 1975, Kiorpes et al. 1978, Meister et al. 1976, Musewe et al. 1979, Willoughby and McDonell 1979). The only significant differences between the two species were the pH_a and P_{aCO_2}. The $P_{(A-a)O_2}$ of the horses was similar to values reported previously for this species (Garner et al. 1971, Mauderley 1974, Viel 1980) and close to the ideal $P_{(A-a)O_2}$ of man (Comroe et al. 1962). Both P_{AO_2} and $P_{(A-a)O_2}$ were higher in the cows than in the horses, however, these differences were not statistically significant. The higher P_{AO_2} and $P_{(A-a)O_2}$ in the cows is consistent with the hypothesis of hyperventilation in this species.

The V_D/V_T ratio was similar in both species but V_{Dphys} was higher in the horses while $\dot{V}_E$ was higher in the cows. These differences reflect the respective differences in V_T and $\dot{V}_E$. The V_D/V_T ratio of the horses was similar to the value reported by Forster et al. (1976) while the values for V_D/V_T and $\dot{V}_A$ in the cows were similar to those reported by Hales and Findlay (1968a).

In the horses and cows the V_D/V_T ratio was approximately 0.63. This value is much higher than the V_D/V_T ratio of 0.36 proposed by Stahl (1967), and is almost three times higher than the value (0.22) calculated separately from Stahl's (1967) equations for V_D and V_T. Although they are somewhat tenuous, calculations of the anatomical dead space distal to the larynx, indicate that the anatomical dead space accounts for most of the increased V_{Dphys} in horses and cow. As indicated previously, the volume of the trachea was similar to the

volume reported by Tenney and Bartlett (1967), however, their assumption that the trachea is a constant proportion of the dead space is probably invalid. In previous morphometric studies of the lungs, the volume density of the intrapulmonary airways (V_V(air)) has not been reported. The volume density of non-parenchyma fraction of the lung (V_V(np)) was assumed to be 0.1 (Weibel et al. 1981), the value for the human lung (Weibel 1963). Measurements of V_V(np), show that V_V(np) is 0.04 in the 3 kg suni (Nasotragus moschatus), 0.10 in the 100 kg wildebeest (Connochaetes taurinus) (Weibel et al. 1981), and 0.18 in the 600 kg cow (present study). Even if the airways accounted for the total V_V(np), the V_V(air) would be less in the suni than in the cow, indicating that the relative volume of the intrapulmonary airways increases with increasing body size.

The mechanical factors related to the dead space are: a) the cost of increasing ventilation to compensate for the increase in dead space, and b) the cost of airway resistance (Tenney and Bartlett 1967). The former is a function of the radius squared, and the latter is a function of the radius to the fourth power. Guyton (1947) postulated that the pressure drop per centimeter varies inversely as the square of the radius, but the total drop is similar in all species as the length of the airways increases with body size. This suggests that the volume of the airways is a linear function of body weight. However Guyton's (1947) hypothesis is based on the assumption that the mean linear velocity in the airways does not differ between species. Crude approximations suggest that mean linear velocity is 2.2 and 3.9 times higher in the trachea of horses and cows respectively

than in the trachea of man, rendering Guyton's assumption invalid. The relative differences in the mean linear velocity would not change if the tracheal diameter of the horses and cows was reduced to eliminate the excess dead space. However there would be a disproportionate increase in the airway resistance. Thus, it is probably more efficient for large mammals to accept the cost of increased ventilation, and minimize the cost of airway resistance.

Pulmonary Mechanics and Lung Volumes:

All of the measured lung volumes of the horses were greater than those of the cows. The FRC of the horses was similar to the values reported by Willoughby and McDonell (1979) and Viel (1980), while the FRC of the cows was similar to the value reported by Peters et al. (1973). The FRC of the horses was similar to the value predicted from the equation of Stahl (1967) whereas the FRC of the cows was less than the predicted value.

Anaesthesia and positioning in sternal recumbency caused a 24% drop in FRC in the horses and a 17% drop in the cows. The FRC_{an} of the horses was similar to the value reported by Sorenson and Robinson (1980) for horses in the sternal recumbency. The TLC_{an} was greater than the previously reported values (Sorenson and Robinson 1980, Leith and Gillespie 1971). This difference is most likely due to the higher inflation pressures used in the present study. For both species TLC_{an} was higher than the predicted value (Stahl 1967) while the FRC_{an}/TLC_{an} ratio was less than the predicted value. However, when FRC_{an} was adjusted to account for the drop in FRC during anaesthesia,

the FRC/TLC_{an} ratio was similar to that of other mammals.

Due to technical difficulties, RV_{an}, ER_{an}, VC_{an} and CV_{an} were not reported for the horses. However, these values have been reported for the horse by Sorenson and Robinson (1980). For all of these volumes, the values obtained for the cows in the present study are less than the values reported by Sorenson and Robinson (1980).

Most investigators have arbitrarily defined TLC as the lung volume at a P_{TP} of 30 cm H_2O, even though further increase in P_{TP} may result in significant increases in lung volume (Leith 1976). The P_{TP} in the present study was approximately 60 cm H_2O. The choice of the higher inflation pressure was based on the characteristics of the apparatus used to inflate the lungs. During the inflation and deflation manoeuvres it was not possible to determine P_{TP} as P_{TP} was not measured directly. Therefore the limits to inflation were determined by observing the rate of change in the mask pressure and volume, so that when the rate of change in the volume decreased relative to the rate of change of the mask pressure, inflation was terminated. This normally corresponded to the plateau of the P-V curve. While these measurements may not be directly comparable to those reported for other species, they are probably more accurate measurements of the true TLC of horses and cows.

The purpose of the P-V manoeuvres was to calculate the quasi-static lung compliance. The original hypothesis was that given the greater amount of connective tissue in the bovine lung, the bovine lung would be less compliant than the equine lung. However, C_L/FRC_{an} was the only index calculated to describe the lung compliance which

differed significantly between the two species. This difference was due to the greater reduction in FRC in the horses. The C_L of the horses was similar to the value reported by Sorenson and Robinson (1980), and the value predicted by the equation of Stahl (1967). The C_L of the cows was much lower than the predicted value.

The C_L of the horses was much lower than the value of 3.4 L/cm H_2O reported by Leith and Gillespie (1971), and the stiffness constant (k_1 of equation 4) was one-quarter of that calculated by Schroter (1980) from a curve presented by Leith (1976). Unfortunately it is difficult to fully compare these values as Leith and Gillespie (1971) did not describe their methods for obtaining the P-V curves. Potentially, the most serious problem in the estimation of compliance using quasistatic manoeuvres is that the change in volume will lag behind the change in pressure, and compliance will be overestimated (Gibson and Pride 1976). In the present study, the expiratory flow rates were similar to those reported by Sorenson and Robinson (1980) for use in horses, and were well below the value of 0.1 VC/sec suggested for use in closing volume manoeuvres in man (Hyatt et al. 1973, Rodarte et al. 1975). As there was no correlation between C_L or C_L' and flow rate it was assumed that the deflation procedure was slow enough to compensate for any inertia in the lung tissue. Thus it seems reasonable to conclude that the values for C_L are close to the "true" values.

In this study the lungs did not exhibit the marked hysteresis typical of mammalian lungs (Leith 1976, Sorenson and Robinson 1980). The P-V curves of the equine lungs formed a double loop with the

inflation P-V curve crossing over the deflation curve (Figure 8). The P-V curve of the chest wall indicates that even though the animals were paralyzed the chest wall exerted a significant influence on the mechanics of the respiratory system. During the initial stages of inflation a significant increase of the pressure was needed to overcome the force exerted by the weight of the chest wall. Due to the different shape of the thoracic cavity this problem was not as obvious in the cows as in the horses. The lungs of the horse tend to sit on top of the abdominal cavity (Figure 1), whereas the lungs of the cow sit in front of the abdominal cavity. In the paralyzed horses the weight of the shoulders rested on the lungs and this weight had to be lifted before the lungs could expand. In contrast there was no equivalent weight to interfere with the expansion of the bovine lungs, although the abdominal contents and chest wall still had to be moved when the lungs were inflated. Sorenson and Robinson (1980) did not report this problem in their study of the lung mechanics of anaesthetized horses. However, their horses were not paralyzed, and still retained muscle tone as evidenced by the excursions on the P-V loop which they presented.

Respiratory Mechanics During Normal Breathing:

The patterns of airflow during inspiration and expiration were similar to those reported for horses and cows by Amoroso et al. (1963). In both species the peak flows usually occurred during the latter part of inspiration and the early part of expiration. The peak flow rates in both species were higher than those reported by Amoroso et al.

(1963), with the peak flow rates of the cows being similar to those reported by Purchase (1965). For the horses the mean inspiratory and expiratory flow rates were lower than those reported previously (Gillespie et al. 1966, Muylle and Oyaert 1973, Sasse 1971). The flow rates at 25, 50 and 75% of inspiration and expiration were higher than those reported by Viel (1980), but the differences were not statistically significant. Throughout inspiration and expiration, the flow rates of the cows were higher than those of the horses.

The $\Delta P_{TP}max$ of the horses was lower than the $\Delta P_{TP}max$ of the cows. The value for the horses was similar to the values reported by some authors (Gillespie and Tyler 1969, McPherson et al. 1978, Viel 1980) and lower than those reported by others (Derkson and Robinson 1980, Muylle and Oyaert 1973, Sasse 1971, Willoughby and McDonell 1979). The $\Delta P_{TP}max$ of the cows was lower than the previously reported values (Amoroso et al. 1963, Musewe et al. 1979). In both species $\Delta P_{TP}max(low)$ was significantly less than $\Delta P_{TP}mas(tot)$ indicating a significant drop in pressure between the nares and the trachea.

In the horses W_b was less than the previously reported values (Muylle and Oyaert 1973, Sasse 1971, Viel 1980, Willoughby and McDonell 1979). In the cows W_b was significantly higher than in horses, and was similar to the predicted value (Stahl 1967) while the W_b of the horses was much lower. In both species $W_b(low)$ was significantly less than $W_b(tot)$. The ratio of $W_b(low)$ to $W_b(tot)$ was similar to that previously reported for horses (Viel 1980).

The higher $W_b(tot)$ in the cows was related to the higher flow

rates in this species. Earlier authors (Agostoni et al. 1959, Crosfill and Widdicombe 1961) have suggested that the natural frequency of breathing is determined by the minimum rate of work. If this is so, the cow appears to be an exception in that it has a high cost of breathing and an increased f_R relative to the horse.

The C_{dyn} of the horses was similar to that reported previously (Muylle and Oyaert 1973, Sasse 1971, Viel 1980, Willoughby and McDonell 1979), while the C_{dyn} of the cows was higher than the value reported by Musewe et al. (1979).

There were two interesting findings with respect to the measurement of C_{dyn} in horses and cows. The first was that in cows $C_{dyn}(low)$ was higher than $C_{dyn}(tot)$, and the second was that in the horses C_{dyn} was significantly higher than C_L. The differences between $C_{dyn}(low)$ and $C_{dyn}(tot)$ in the cows was not statistically significant. However, the sample size was small and the variability was large. All of the cows followed the same trend, and in all of the animals changes in $P_{TP}(low)$ lagged behind the changes in $P_{TP}(tot)$. The measurements indicate that there is a significant inertial component in normal breathing in the cow, and that even though there was zero flow at the nares air continued to move within the pulmonary system. Ignoring the pressure changes due to inertia will lead to an overestimation of C_{dyn} (Dosman et al. 1975), and this would explain the difference between $C_{dyn}(low)$ and $C_{dyn}(tot)$. Tissue inertia would also explain the paradox that $C_{dyn}(tot)$ is markedly increased in cows with interstital pulmonary fibrosis (unpublished observations). However, it

does not explain why the measurements of C_{dyn}(tot) indicate that the pulmonary system is behaving in accordance with the elastic forces within the lung (i.e. $C_{dyn}(tot) \simeq C_L$).

The second observation was that in the horses C_{dyn} exceeded C_L. This can also be explained on the basis of inertia. However, this explanation would initially appear to exacerbate the problems encountered in the measurements of R_L.

A priori, one would expect R_L to be higher in the cows due to the narrower trachea and branching pattern of the airways in this species. Yet, R_L did not differ significantly between the horses and cows. In the horses, R_L was lower than the previously reported values (Gillespie et al. 1966, Robinson et al. 1975, Viel 1980). The observation that R_L(low) was significantly less than R_L(tot) was in agreement with previous observations (Ferris et al. 1964, Viel 1980, Willoughby and McDonell 1979) that most of the resistance is in the upper airway.

A major problem during the measurement of R_L, and particularly during the measurement of R_L(low), was the finding of negative resistances. Although negative values occurred in both species they were more common in the horses, and have been a consistent problem in the measurement of resistance in this species (McDonell and Viel, pers. comm.). These negative values are difficult to explain as they are "impossible" on a mechanical basis. One explanation for this finding might be that the change in pressure due to the elastic properties of the lung was overestimated. However, if this was the case, then

the difference between C_{dyn} and C_L in the horse would have been increased. A more likely explanation is that the change in pressure due to inertia damped the pressure changes due to elasticity and resistance. This would explain the differences between C_{dyn}(tot) and C_{dyn}(low) in the cows, and the phase lags between ΔP_{TP}(tot) and ΔP_{TP}(low), and the findings of negative resistance in both species. There were no differences between C_{dyn}(tot) and C_{dyn}(low) in the horses because, at the lower flow rates in this species, there was sufficient time for the total and lower pulmonary systems to equilibrate at end-inspiration and end-expiration. The fact that C_{dyn}(tot) and C_L are approximately equal in the cow is probably due to a fortuitous interaction of forces rather than a true reflection of the elastic properties of the lung.

In man the pressure changes due to inertia are not considered to be important until the respiratory rate is 100 per minute (Mead 1956), though it has been suggested that inertia is important at lower frequencies (Dosman et al. 1975). During normal respiration the change in pressure due to inertia is considered to be negligible (Mead and Whittenberger 1953), and this convention was adopted in the present study and in other studies of pulmonary function in horses and cows (Dewes et al. 1974, Gillespie et al. 1966, Musewe et al. 1979, Viel 1980). In the normal adult human, V_T is 500 ml and f_R is 12 per minute. In contrast, the V_T of horses is 5 to 6 L. and f_R is 12 per minute, while the V_T of cows is 4 to 5 L. and f_R is 25 per minute. The lungs of these latter species weigh approximately 10 kg, and they are in

series with a substantial chest wall and large abdomen. In order to breathe horses and cows must not only move large volumes of air relative to man, but must also accelerate and decelerate the relatively large tissue mass of the lungs, chest wall and abdomen. Sharp et al. (1964) have shown that the inertance of the respiratory system is increased in obese men, and that this increase can be attributed to the greater tissue mass in the obese subjects. Thus, in the horse and the cow, it is likely that the tissue mass does contribute significantly to the inertance of the respiratory system, and inertia was not a negligible factor as was assumed.

Pulmonary Structure Versus Pulmonary Function:

The basic hypothesis of this thesis was that the differences in pulmonary function between horses and cows were due to differences in lung structure, particularly the structure of the lung parenchyma. While there were several differences in pulmonary function between these two species, there were no significant differences in the structure of the gas exchange parenchyma. The significant differences in structure were; 1) the size and shape of the lungs and chest wall, 2) the dimensions and branching patterns of the airways, and 3) the amount and distribution of interlobular septa. This latter difference did not appear to significantly influence the mechanical properties of the lungs in normal animals, although it may exert a significant influence on pathological processes.

The differences in the size and shape of bovine and equine lung

airways are probably due to the differences in size and shape of the abdominal viscera rather than inherent differences in the demands on the lung. Although the horse and the cow are both herbivores, they utilize different digestive processes. The horse is a monogastric and the digestion of plant fibres is primarily in the large hindgut which loops around the abdominal cavity (Getty 1964). Gravity would tend to pull the abdominal contents ventrally, and in the evolution of the horse this has allowed the lungs to expand caudally over the top of the abdomen. During this process, the airways have increased in size to compensate for the increased lung growth. In contrast, the cow is a ruminant, and the digestion of plant fibres takes place in the bulky rumen which is situated immediately caudal to the diaphragm (Getty 1964). The mass of the rumen and pressure exerted by the rumen gas cap tend to compress the lungs (Musewe et al. 1979). Thus, expansion of the bovine lung was restricted during the course of evolution. The differences in the forces exerted on the lung by the abdomen could explain why the TLC of the horse is 50% larger than that predicted for a mammal of equivalent size (Stahl 1967), whereas the TLC of the cow is similar to the predicted value.

The differences in the size and shape of the abdominal viscera have not only influenced the size and shape of the equine and bovine lungs, but probably also influences the pattern of breathing in these species. When a horse inspires the abdomen moves caudally and ventrally, and the force of gravity and inertia of the abdominal mass must be overcome to lift the abdomen during expiration. In the cow, the respiratory movements are most obvious on the flank, and ventilation

is distributed uniformly from the top to the bottom of the lung (Ruiz et al. 1974). This would suggest that the abdominal viscera are moved caudally during inspiration. During expiration the abdominal viscera would tend to return to the position from which they were displaced, and expiration would be passive as is indicated by the pattern of airflow. In contrast, both inspiration and expiration would appear to be active processes in the horse.

It was originally suggested in this thesis that cows have a high work of breathing due to the increased frequency and resistance in the airways. Yet the work of breathing in the cows was similar to that predicted by Stahl's (1967) equation. Thus, an alternative hypothesis is that the horse has an abnormally low work of breathing. However, a more probable hypothesis is that the true cost of breathing is similar in both species, but that the distribution of the cost is different.

When a cow inspires the abdominal viscera must be moved caudally if previous suggestions are correct. Given the weight of the abdominal contents, this would be very costly, and to increase V_T would markedly increase in the total cost of breathing. Thus, it is energetically more efficient to accept the increased work of breathing associated with increase in frequency. In the horse the abdominal viscera are falling away from the lung during inspiration, and expiration requires that the viscera be lifted. The unit cost of lifting probably doesn't vary with the distance moved, but the displaced volume is proportional to the distance cubed. Therefore, it

is energetically more efficient for the horse to maintain ventilation by utilizing a higher tidal volume at a lower frequency.

Agostoni et al. (1959) and Crosfill and Widdicombe (1961) have suggested that the natural frequency of breathing is determined by the minimum cost of ventilation. Their studies were conducted with small mammals, and the cost of ventilation was determined by the compliance of the lungs and the airway resistance. The hypothesis arising from the present study is that in large mammals the natural frequency of breathing is also determined by the minimum cost of ventilation. But, that the size and shape of the "chest wall" is more important than the characteristics of the lungs.

CONCLUSIONS

The hypothesis of this thesis was that the reported differences in pulmonary function between horses and cows were due to differences in the structure of the lung parenchyma. In the standing animals there were differences in FRC, V_T, f_R and the pattern of airflow. The cows had a lower C_{dyn} and a higher W_b than the horses. The increase in W_b in the cows was due to increased resistance in the upper airway, as W_b(low) did not differ significantly between the two species. In anaesthetized, paralyzed horses and cows in sternal recumbency the lung volumes of the horses exceeded those of the cows, while C_L did not differ significantly between the two species. The compliance measurements, and the measurements of the lower pulmonary system in the standing animals suggest that there are few differences in lung function, per se, in these animals. Quantitative and qualitative observations of the lung parenchyma also revealed few significant differences between the two species. The structural differences were in the size and shape of the lungs and airways, and the amount of interlobular septa. The latter did appear to influence lung function in normal animals.

The differences between C_{dyn} and C_L in the horses, and between C_{dyn}(tot) and C_{dyn}(low) in the cows, the measurements of negative

resistance, and the observations of phase lags between ΔP_{TP}(tot) and ΔP_{TP}(low) prompted a re-examination of the assumptions made in the mechanics measurements. The assumption that inertia is a negligible factor during normal breathing may be valid in man, but probably is invalid in large mammals such as the horse and the cow, due to the large abdomen in these species. It is suggested that the size and shape of the abdominal viscera has influenced the size and shape of bovine and equine lung during the course of evolution, and that abdominal movements are an important factor in determining the breathing patterns of horses and cows. The natural pattern of breathing in horses and cows probably minimizes the cost of ventilation. In small mammals the cost of ventilation is determined by the characteristics of the lungs, but in large mammals, such as the horse and the cow, the "chest wall" is the predominant factor.

ADDENDUM

Following the preparation of this thesis questions were raised as to the accuracy of the oesophageal pressure measurements, particularly those made during the quasistatic P-V manoeuvres. In making the measurements for this thesis oesophageal measurements were preferred as they were easier to obtain and are essentially non-invasive. As indicated in the Materials and Methods (p. 36) the changes in oesophageal and intrapleural pressures were similar in both standing horses and standing cows (Figure 13A). A comparison of the changes in oesophageal and intrapleural pressures in anaesthetized animals (Figure 13B) also indicated that both measurements yielded similar results.

The potential problems in making oesophageal pressure measurements are incorrect positioning of the balloon and under-inflation of the balloon. Both of these factors will cause the true pressure change to be underestimated. In performing the intrapleural pressure measurements the problems encountered were kinking of the catheter, blood clots in the catheter and bleeding, loss of the air bubble around the catheter tip, and in one case, an aseptic pleuritis most likely due to the abrasion of the pleural surface of the catheter. In all of the comparisons made by the author (and by others in the laboratory), it was much more difficult to obtain reproducible intrapleural measurements than oesophageal measurements, thus the oesophageal measurements were preferred.

In the standing animals the underestimation of the change in intrathoracic pressure would cause an overestimation of C_{dyn} and an

underestimation of R_L. However, altering the volume of the balloon between 1.0 and 5.0 ml did not affect C_{dyn} or R_L, and negative values for R_L(low) were as common at high balloon volumes as at low balloon volumes. Potential errors due to balloon volume would have no effect on the differences between $\Delta P_{\dot{V}=0}$(tot) and $\Delta P_{\dot{V}=0}$(low), or on the phase lags as these latter measurements relate the tracheal pressure and mask pressures to the same oesophageal pressure determinations. Thus, the measurements of oesophageal pressure in the standing animal and their interpretation are assumed to be correct.

The oesophageal and intrapleural pressure measurements in the anaesthetized animals, and oesophageal pressure measurements during subsequent P-V manoeuvres (Figure 14) differed markedly from the oesophageal pressure measurements presented in the thesis. During these latter studies the oesophageal pressure increased throughout inflation and decreased gradually during deflation. Repeated checks of the system suggested that the shape of the P-V curves for the lung and chest wall presented in the thesis was most likely due to a leak, possibly at the stopcock or at the junction between the tubing and a connector. The leak did not appear to affect the oesophageal pressure measurements at low or negative pressures, and at negative pressures the balloon volume tended to increase. However, at higher pressures, such as those obtained during the P-V manoeuvres, air was forced from the balloon and the pressure response was damped or eliminated. It seems somewhat incongruous that this would have been missed, but "normal" pressure traces were obtained prior to paralysis, and although the absolute values varied the shape of the oesophageal pressure curves was similar between

loops and between animals.

After this problem had been recognized quasistatic P-V manoeuvres were repeated with a normal horse and a normal cow. The P-V loops are presented in Figure 14 and the calculated compliances are presented in Table XIII. The total compliance and adjusted total compliance (C_T and C_T') were similar to the values obtained previously, and the chest wall compliance (C_W) was within the previous range, but the lung compliance (C_L) was much higher than the earlier estimates, and the half-inflation pressure (h) was much lower than the previous values (see Table IX and X, in the thesis). As with the earlier findings, the compliances and half-inflation pressure did not appear to differ between the horse and cow.

The pattern of results obtained from the latter P-V manoeuvres is similar to that reported by Leith and Gillespie (1971), although the values for compliance are somewhat higher. This difference may be due to the paralysis of the chest wall in the present study. C_W tended to decrease as the muscle relaxant wore off. While the new results differ from those obtained earlier, they would appear to support rather than detract from the ideas presented in the thesis. As C_L is much greater than C_W, these new results emphasize the importance of the chest wall of horses and cows.

Table XIII. Values for compliance and half-inflation pressure derived from quasistatic P-V manoeuvres without a leak.

Variable	Cow	Horse
Weight (kg)	500	520
C_L (L/cm H_2O)	4.92	5.07
C_W "	1.50	1.76
C_T "	1.15	1.30
C_L' "	2.38	5.99
C_W' "	1.88	1.23
C_T' "	1.05	1.02
h (cm H_2O)	8.59	6.65
k_1	0.081	0.104
C_L/FRC_{an}	0.031	0.210
C_L'/FRC	0.106	0.189

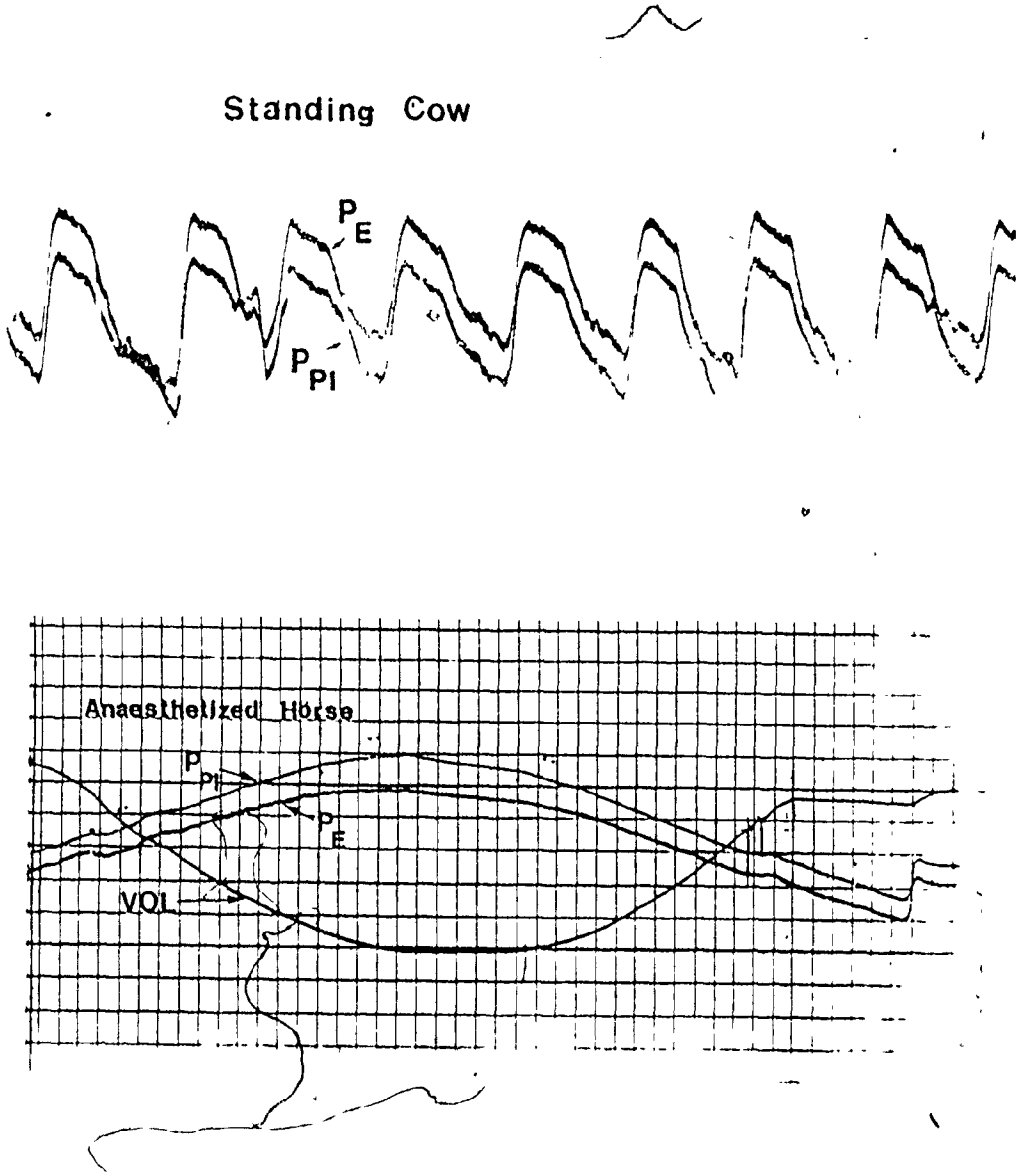


Figure 13. Comparison of intrapleural (P_{pl}) and oesophageal (P_E) in a standing cow (A) and an anaesthetized horse (B).

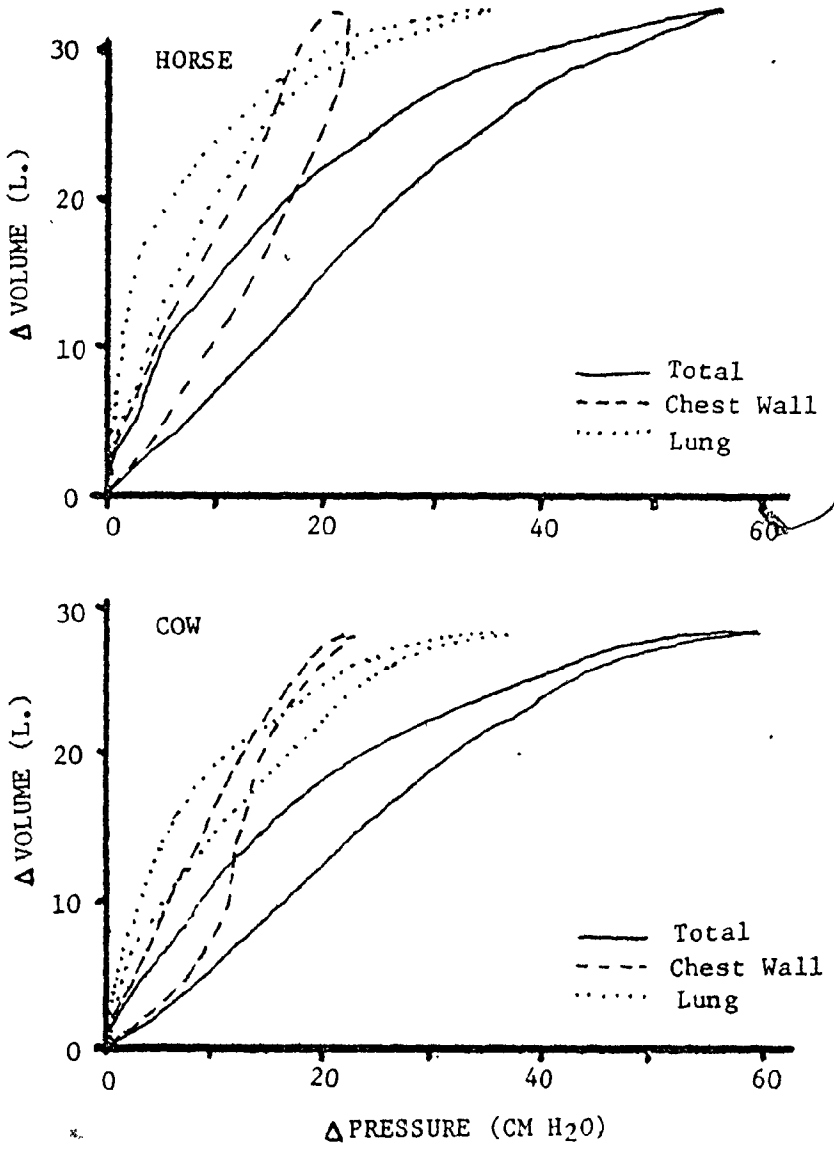


Figure 14. Pressure-volume loops of the lung, chest wall and total respiratory system without a leak in the oesophageal pressure.

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APPENDIX I

Equations used in the Calculations of Gas Exchange

Symbols:

- V = volume (L)
- $\dot{V}$ = rate of change in volume (L/min)
- F = concentration (%)
- F' = concentrate O_2 corrected for apparatus dead space (%)
- P = pressure (torr)
- t = time (min)
- nb = number of breaths

Subscripts:

- BTPS = body temperature, ambient pressure, saturated
- STPD = standard temperature and pressure, dry
- I = inspired
- E = expired
- a = arterial
- A = alveolar
- g = gas
- Dap = apparatus dead space

Equations:

1) Correction for dead space (F')

$$F'_{Eg} = ((V_E \times F_{Eg}) + (V_{Dap} \times (F_{Eg} - F_{Ig})))/V_E$$

2) Oxygen consumption ($\dot{V}_{O_2}$)

$$\dot{V}_{O_2} = V_{STPD} \times (F_{IO_2} - F'_{EO_2}) \times F'_{EN_2}/(F_{IN_2} \times t)$$

3) Carbon dioxide production ($\dot{V}_{CO}$)

$$(\dot{V}_{CO_2} = V_{STPD} \times (F'_{ECO_2} - F_{ICO_2})/t$$

4) Respiratory Exchange Ratio (R)

$$R = \dot{V}_{O_2}/\dot{V}_{CO_2}$$

5) Tidal Volume (V_T)

$$V_T = V_{BTPS}/nb$$

6) Respiratory Rate (f_R)

$$f_R = nb/t$$

7) Minute Ventilation ($\dot{V}_E$)

$$\dot{V}_E = V_{BTPS}/t$$

8) Physiological Dead Space (V_{Dphys})

$$V_{Dphys} = V_T \times (P_{aCO_2} - P_{ECO})/P_{aCO_2}$$

9) Alveolar Ventilation ($\dot{V}_A$)

$$\dot{V}_A = \dot{V}_E \times P_{ECO_2}/P_{aCO_2}$$

10) Alveolar O_2 Pressure (P_{AO_2})

$$P_{AO_2} = P_{IO_2} - (P_{aCO_2}/R)$$

APPENDIX II

Equations used in the Calculation of the Functional Residual Capacity

The functional residual capacity (FRC) was calculated as:

$$FRC = BTPS \times ((F'_{EN_2} \times V_E) - V_{N_2el}) \div (0.81 - F''_{AN_2})$$

where: BTPS = correction factor to convert the measured volumes to the volumes at body temperature, ambient pressure, saturated.

F'_{EN_2} = concentration of expired N corrected for apparatus dead space* (%).

V_E = expired volume (L).

V_{N_2el} = volume of N_2 eliminated from the body when 100% O_2 was breathed** (L).

0.81 = concentration of alveolar N_2 at the beginning of the washout (%).

F''_{AN_2} = concentration of alveolar N_2 in the final breath of the washout (%).

* F'_{EN_2} was determined using equation 1 in Appendix I.

** The volume of N_2 eliminated from the body was determined by the equation:

$$V_{N_2el} = 0.035 + (0.095 \times BSA)$$

where: BSA = body surface area (m^2)

In the cows, BSA was calculated by the equation $BSA = 0.15W^{0.56}$, and in the horses the equation $BSA = 0.01W^{0.63}$ was used. W = weight (kg)

APPENDIX III

Formulation of the Glutaraldehyde Fix and Phosphate Buffer Wash

Approximately 100 litres of fixative and buffer wash were required for each animal in this study, and the fixative and wash were made in 50 L. lots. The formulae were based on formulae provided by Dr. R. Lee.

Glutaraldehyde Fix:

To make 50 litres of 2.5% glutaraldehyde in phosphate buffer required 247.84 gm of K_2HPO_4, 32.48 gm of KH_2PO_4, 2.5 litres of 50% glutaraldehyde and 47.5 litres of water. The pH was adjusted to 7.4 by adding the appropriate amounts of K_2HPO_4 or KH_2PO_4. The osmolarity was adjusted to 400 mosmol by adding water or sucrose.

Phosphate Buffer Wash:

To make 50 litres of phosphate buffer wash required 1470.47 gm of K_2HPO_4, 192.72 gm of KH_2PO_4 and 50 litres of water. The pH was adjusted to 7.4 by adding small amounts of K_2HPO_4 or KH_2PO_4, and the osmolarity was adjusted to 400 mosmol by adding water or sucrose.

APPENDIX IV

Procedure for the Embedding of Tissue For Electron Microscopy

Medium	Duration
1% osmium tetroxide in sodium cacodylate buffer	1.5 hr
0.5% uranyl acetate	1 hr
70% alcohol (2 changes)	10 minutes per change
80% alcohol (2 changes)	10 minutes per change
95% alcohol (2 changes)	10 minutes per change
100% alcohol (4 changes)	10 minutes per change
Spurr (E.F. Fullam, Inc. Schenectady, N.Y.)	
1st change	1 hr
2nd change	8 hr
3rd change	12 hr
4th change	24 hr
Embedding	14 hr at 60°C