INVESTIGATION INTO PULMONARY FUNCTION DERANGEMENTS AND THE ROLE OF VAGAL MECHANISMS IN MODELS OF EQUINE LUNG DISEASE

By

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ABSTRACT

INVESTIGATION INTO PULMONARY FUNCTION DERANGEMENTS
AND THE ROLE OF VAGAL MECHANISMS IN MODELS
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After a standard method for the measurement of pleural pressure was determined, a technique for reversible vagal blockade was developed and reproducibility of pulmonary function tests was established, I studied pulmonary function derangements in 3-methylindole induced pulmonary toxicosis and ovalbumin aerosol challenge induced allergic lung disease before and after vagal blockade in standing ponies.

Oral administration of 3-methylindole (3MI) increased respiratory rate (RR), minute ventilation ($\dot{V}_{min}$), functional residual capacity (FRC) and minimum volume (MV), decreased arterial CO_2 tension ($PaCO_2$), dynamic compliance (C_{dyn}) and specific conductance (SG_{tot}) and did not alter arterial oxygen tension (PaO_2), total lung capacity (TLC) and quasistatic compliance (C_{stat}). Vagotomy following 3MI treatment decreased RR, and increased SG_{tot} but did not change the other variables. Histopathologic examination showed that 3MI treatment resulted in necrotizing bronchiolitis and alveolar emphysema. I concluded that tachypnea was caused by stimulation of pulmonary receptors with afferents in the vagus nerve, and that 3MI pulmonary toxicosis was characterized by small airway obstruction, occurring independent of vagal mechanisms.

Bilateral ovalbumin aerosol challenge of locally and systemically sensitized ponies increased RR, $\dot{V}_{min}$, total respiratory resistance (R_{tot}) and MV, decreased C_{dyn} and PaO_2 and was without effect on FRC and $PaCO_2$. In the locally sensitized ponies, TLC and C_{stat} decreased following challenge but in systemically sensitized ponies these variables did not change. Following aerosol ovalbumin challenge of the left lung, RR and left lung resistance (R_{totL}) increased while right lung resistance did not change. Vagal blockade following challenge failed to decrease R_{totL}. Histopathologically ovalbumin challenge resulted in bronchitis, bronchiolitis and pulmonary edema. I concluded that like the 3MI model, tachypnea was mediated via pulmonary receptors with their afferents in the vagus nerve, and that local mechanisms were of primary importance in the mechanism of disease. Increased sensitivity to normal bronchomotor tone may have played a minor role in the pathogenesis of pulmonary disease in this model.

DEDICATION

This dissertation is dedicated to my wife Jano, because without her guidance and support this study would not have been conducted.

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INTRODUCTION

Pulmonary diseases in persons and domesticated animals are a major cause of morbidity and poor performance, resulting in enormous financial and social losses. Numerous etiologic agents including bacterial and viral agents, pollutants such as dust, ozone and allergens, and enzyme deficiencies have been identified as causes of some respiratory diseases while the etiology of other respiratory diseases remains unknown. Because of the large variety of etiologic agents and their preponderance in the daily environment, control and treatment measures directed at the agents themselves have enjoyed only partial success. Since it has become apparent that the lung uses only a limited number of mechanisms in response to insults, control and treatment measures aimed at mechanisms whereby lung diseases are expressed rather than at etiologic agents may have greater chance of success. Presently, this approach to the control of pulmonary disease in persons and animals is not widely used due to deficiencies in understanding of basic mechanisms. Because of this lack of understanding and because vagal mechanisms are thought to be important in the pathogenesis of allergic as well as nonallergic pulmonary diseases in man and experimental animals, a major goal of this investigation was to elucidate the role of vagal mechanisms in the derangement of pulmonary function in an allergic and a nonallergic model of equine lung disease. The study of respiratory disease in the horse is appropriate because of the economic and social importance of respiratory disease in this species. In addition the horse may be a good

model of some forms of respiratory disease in persons because anatomically human and equine lungs are similar and because the horse is the only domestic species that commonly suffers from a naturally occurring asthma-like syndrome.

Since at the outset of this investigation there was concern about the validity of pleural pressure measurement in the horse the first study was designed to compare esophageal pressures, measured at different sites in the thoracic portion of the esophagus with pleural pressures measured at various sites in the pleural space. In order to facilitate the study of vagal mechanisms in control of pulmonary function in health and disease I developed a technique to reversibly block the cervical vagus nerves in the standing conscious horse. Subsequently I evaluated the reproducibility of pulmonary function tests used in this investigation, and determined the effect of vagal blockade on pulmonary function in normal ponies. Once these preliminary studies were completed I studied the role of vagal mechanisms in the pathogenesis of 3-methylindole induced pulmonary toxicosis and ovalbumin induced allergic lung disease in the horse.

LITERATURE REVIEW

This literature review will first briefly describe the pulmonary innervation in order to provide a basis for subsequent discussion of the role of vagal mechanisms in asthma in persons and experimental lung diseases in animals. Experimental allergic lung diseases are most commonly developed as models for asthma in humans and therefore I will describe the derangement in pulmonary function in both asthma and the available experimental lung diseases. This background information is necessary to develop an understanding of the range of functional abnormalities that occur in various lung diseases in mammals.

Subsequently I will review our current knowledge of "heaves", a naturally occurring asthma-like disease in the horse, because its occurrence is one of the most persuasive reasons why the study of models of lung disease in the horse might add new basic knowledge.

Finally, 2 new models of equine lung disease will be introduced and I will pose the questions investigated by this study.

Pulmonary Innervation

The lung has both an afferent and efferent nerve supply which has been studied with anatomical, histochemical and physiological techniques. This portion of the review will be restricted to the major physiologic studies designed to elucidate the functional importance of the pulmonary innervation.

As reviewed by Paintal (1973) three kinds of pulmonary afferent receptors have been identified. They are the bronchopulmonary stretch receptor, the irritant receptor and the interstitial type J receptor.

Pulmonary stretch receptors are those endings whose activity increases rhythmically in phase with each inspiration (Adrian, 1933). When a sudden and maintained inflation is applied, they respond with a discharge of impulses that adapts slowly, distinguishing them from the much more rapidly adapting irritant receptors which also respond to lung inflation with increased afferent activity (Mills et al, 1969). The histologic characteristics of pulmonary stretch receptors have not been established although it appears that these endings are associated with the smooth muscle of bronchi and bronchioles (Larsell et al, 1933, Elftman, 1943, Widdicombe, 1954B).

In all species studied so far, stretch receptor neurons are myelinated and run in the vagus nerve (Paintal, 1963). The natural stimulus for stretch receptor activation is the volume of air entering the lung. Hering and Breuer (1868) showed that inflation of the lungs of dogs led to a decrease in frequency and force of expiratory effort (Hering-Breuer reflex) and that deflation of the lung caused stronger and more frequent inspiratory efforts (inflation reflex). In 1933 Adrian showed that pulmonary stretch receptors were responsible for these reflexes. The importance of these reflexes in control of breathing varies between species. Adrian (1933) showed that in cats the inflation reflex modifies the respiratory cycle substantially while Marshall et al (1958) showed that in man the reflex is weak and does not substantially modify the respiratory cycle. Widdicombe (1961) studied the inflation reflex in persons, monkeys, dogs, cats, rabbits, guinea pigs, rats, and mice and

showed that the reflex was weakest in persons and strongest in rabbits. The Hering-Breuer reflex is so strong in rabbits that on occasion lung inflation induced apnea causes asphyxiation. The relative importance of the Hering-Breuer reflex in control of respiration in the horse is presently unknown.

In cats the effect of lung inflation on respiratory pattern is dependent upon bronchomotor tone and rate of inflation (Widdicombe, 1954A, Davis et al, 1956). When bronchomotor tone is increased (by administration of acetylcholine or histamine), the effect of pulmonary inflation on respiratory pattern is decreased, while following bronchodilation (using adrenaline) the Hering-Breuer reflex is enhanced (Widdicombe, 1954A). Davis et al (1956) showed in cats that an increase in rate of inflation enhances the Hering-Breuer reflex and that changes in stretch receptor activity follow pleural pressure changes closely. Data from these studies support the hypothesis of Christie (1953) that the inflation and deflation reflexes adjust the rate and depth of breathing to be mechanically the most economical.

When studying the effects of pulmonary inflation and deflation on vagal afferent activity in cats, Knowlton et al (1946) described a group of afferent fibers whose activity was increased with lung inflation, but adapted rapidly to this stimulus. They called the receptors involved "rapidly adapting pulmonary stretch receptors", now known as irritant receptors. Widdicombe (1954A) found that in cats mechanical stimulation of the tracheal mucosa activated irritant receptors, and Mills et al (1969) in rabbits found similar receptors in the intrapulmonary airways, concentrated in the carina. Since the intrapulmonary irritant receptors are tonically active in the rabbit, while in the cat

tracheal irritant receptors are inactive during normal breathing, these two types of receptors were thought to be different (Mills et al, 1969). It is now known that species differences, not differences between receptor types, are responsible for the differences in activity of irritant receptor (Paintal, 1973).

Another difference between cat and rabbit irritant receptors is that the cat shows no increased activity after intravenous injection of phenyldiguanide, whereas the response in rabbits is marked (Mills et al, 1969, Paintal, 1953). Because species differences in irritant receptor activity exist, it is important to note that presently no reports on equine pulmonary irritant receptors are available.

In addition to sensitivity to lung inflation and mechanical stimulation, irritant receptors increase firing frequency in response to chemical stimulation of airway mucosa, anaphylaxis and microembolism (Nadel et al, 1965, Mills et al, 1969, Sellick et al, 1969, 1971, Karczewski et al, 1969) resulting in tachypnea, bronchoconstriction and coughing. Mills et al (1969) and Paintal (1973) suggested that irritant receptor activity in response to various stimuli is enhanced by bronchoconstriction induced by histamine, or anaphylaxis, thereby producing a positive feedback system. However, it is not clear how the effect of bronchoconstriction and the direct effects of histamine or anaphylaxis on irritant receptor activity can be separated. Since the direct effects of histamine or anaphylaxis on irritant receptors can explain experimental data, it is not necessary to postulate that bronchoconstriction enhances irritant receptor activity.

In contrast with the previous two receptor types, the type J pulmonary receptor is served by nonmyelinated C fibers (Paintal, 1954). They

were discovered by Paintal in 1954 when studying gastric receptors. The type J receptor is normally inactive and is located adjacent to pulmonary capillaries (Paintal, 1969). Afferent activity is increased by a variety of chemical substances including halothane and phenyldiguanide, but the only physiologic stimulants capable of eliciting consistent responses are pulmonary vascular congestion and edema, produced by either chemical vasculitis or increased left atrial pressure (Paintal, 1973). It has been postulated that type J receptors function as interstitial stretch receptors, because they are located in series with the collagen elements of the pulmonary interstitium (Paintal, 1973).

Chemical stimulation of J receptors, using phenyldiguanide results in apnea (Paintal, 1955). In addition, type J receptor activity inhibits somatic motor activity and produces the sensation of breathlessness (Kalia, 1969).

Efferent innervation of the lung is via the parasympathetic and sympathetic diversions of the autonomic nervous system. Parasympathetic neurons travel in the vagus nerve, while pulmonary sympathetic fibers originate in the cervical and first 5 thoracic sympathetic paravertebral ganglia (McKibben, 1975). Both autonomic divisions enter the lung via the hilum and exert a tonic influence on airway smooth muscle. Longet (1842) showed that the vagus nerve contained constrictor fibers to the bronchial muscle. He directly observed the bronchi, exposed by cutting through the lung of freshly killed horses and oxen and found that they contracted when the vagus was excited electrically. Since that time, many investigators have confirmed that vagal stimulation results in bronchoconstriction, while vagotomy is followed by bronchodilation (Dixon et al, 1903, 1912, Woolcock et al, 1969, Hahn et al, 1976).

As first demonstrated by Dixon et al (1912) sympathetic stimulation causes bronchodilation. This is attributed to the predominant presence of β-adrenergic receptors in the bronchial and bronchiolar smooth muscle (Castro de la Mata et al, 1962, Guirgis et al, 1969). Some authors have reported a paradoxical bronchoconstriction following sympathetic stimulation, but attributed this phenomenon to contamination of sympathetic nerves with parasympathetic fibers (Dixon et al, 1912). However, Castro de la Mata et al (1962) showed in dogs that following β-adrenergic blockade, sympathetic stimulation resulted in bronchoconstriction, and suggested that this could be explained by the presence of α-adrenergic receptors in the lung. His conclusion was supported by Fleisch et al (1970) who studied rats, guinea pigs, cats and rabbits but refuted by Foster (1966), Guirgis et al (1969), and Cabesas et al (1971) who studied guinea pigs, persons and dogs, respectively. Since these studies use pharmacologic techniques, differences in results may be due to the lack of specificity of various blocking agents at different dosages.

The distribution of autonomic fibers to various airway generations is not well established. Macklem et al (1967) introduced the retrograde catheter technique which was subsequently used to study distribution of autonomic innervation in the lung (Macklem et al, 1969). The technique uses a catheter inserted into a lung via the airway opening and exited through the pleural surface. Pulmonary resistance, calculated as the difference between airway opening pressure and pleural pressure, divided by air flow, measured at the airway opening, is separated in central and peripheral components. Peripheral resistance is defined as the pressure difference between the retrograde

catheter and pleural space divided by airflow, measured at the airway opening, while central resistance is the difference between pulmonary resistance and peripheral resistance. Macklem et al (1969) showed that in dogs, using the retrograde catheter technique, vagotomy resulted in preferential dilation of 3-8 mm bronchi, while Woolcock et al (1969) using the same technique found that vagal stimulation increased central resistance in some dogs and peripheral resistance in others. In the latter study, catheters were wedged in bronchi between 2.7-0.8 mm diameter. Therefore if vagal innervation was predominantly in 3-8 mm bronchi, an increase in central resistance would be expected following vagal stimulation. Since the variable results of Woolcock et al cannot be explained based on retrograde catheter position, they are most likely caused by animal to animal variability in distribution of parasympathetic efferent innervation. Severinghaus et al (1955) reported that atropine increased dead space volume, suggesting that vagal tone has an important bronchoconstrictor effect on central airways. Using a radiographic technique, Cabesas et al (1971) confirmed Macklem's findings and reported that vagal stimulation reduced airway diameter from the trachea to bronchioles 0.5 mm in diameter and had the greatest effect on airways 1-5 mm in diameter. Thus, although individual variation may be great, the majority of evidence suggests that in the dog vagal bronchomotor tone preferentially effects airways between 1-8 mm diameter.

The distribution of sympathetic pulmonary innervation is also in dispute, although the majority of evidence suggests that sympathetic fibers influence peripheral airways more than central airways. Hensly et al (1978) reported an increase in closing volume and decrease in

airway resistance without changes in dead space volume following treatment with a β-adrenergic agent, suggesting peripheral bronchodilation. This conclusion was suported by Ingram et al (1975) who showed that following isoproterenol treatment in persons maximum expiratory flow increased while elastic recoil did not change suggesting that sympathetic innervation predominantly influences peripheral airways. In contrast, Cabesas et al (1971) using a radiographic technique, showed that sympathetic stimulation dilated airways from 0.5-5 mm in diameter with the greatest effect on airways with diameters between 1-5 mm. These findings were recently confirmed by Russell (1980) who studied airways in vitro. The discrepancy between these studies is presently unexplained.

Recently a third division of the autonomic nervous system (purinergic nervous system) was shown to be present in guinea pig trachealis muscle (Coburn et al, 1973). Purinergic innervation of guinea pig airways was confirmed by others and the finding extended to human airways (Bando et al, 1973, Coleman, 1973, Richardson et al, 1976). The purinergic system, is also present in the gastrointestinal tract, uterus and guinea pig vas deferens (Burnstock, 1972). In the gastrointestinal tract and the lung following muscarinic and adrenergic blockade, vagal stimulation causes smooth muscle relaxation, suggesting that the purinergic system is inhibitory in nature. The chemical mediator of this inhibitory nervous system is not known, but there is extensive evidence to support adenosine triphosphate or another purine nucleotide as the mediator (Burnstock, 1972). The role of the purinergic nervous system in control of airway caliber in health and disease is presently unknown, although malfunction of this system may be

important in the pathogenesis of asthma-like syndromes in man and other animals.

Allergic lung diseases: Derangements in pulmonary function and pathogenesis

In this section I will describe the derangement in pulmonary function occurring in asthma in persons and the available experimental models of allergic lung disease. In addition, possible mechanisms involved in the pathogenesis of these diseases will also be discussed.

In the last ten years there has been a great interest in models of allergic lung disease because of the lack of knowledge about and the prevalence of asthma in persons. Asthma has been defined as "widespread narrowing of the bronchial airways which changes in severity over short periods of time either spontaneously or under treatment and is not due to cardiovascular disease" (CIBA symposium, 1959). As reviewed by Alexander et al (1921) an attack is triggered by a variety of stimuli, including allergens, chemical irritants, dust, smoke, cold, exercise, coughing, hyperinflation, laughter and excitement. Animal models have been developed to study asthma induced by allergens, i.e., asthma with a major allergic or immunologic component. Although approximately 75% of asthma cases have no major immunologic component, no animal model has been developed for these types of asthma (Stevenson, 1975).

As reviewed by McFadden (1975) arterial oxygen tension (PaO_2) decreases, while alveolar-arterial oxygen difference increases during an attack of asthma. In addition, specific conductance is increased, while dynamic compliance is decreased, suggesting bronchoconstriction involving both large and small airways. Small airway obstruction is also indicated by a depression of maximal expiratory flow rates

throughout the vital capacity (Despas et al, 1972, McFadden et al, 1973, 1975). Residual volume and functional residual capacity increase, probably because of airway closure (Hurtado et al, 1934). After symptomatic improvement, specific conductance increases but maximum expiratory flow rates, lung volumes and PaO_2 are still abnormal, suggesting central airway bronchodilation with persistence of peripheral airway narrowing (McFadden et al, 1969, 1973, 1975). It was postulated by McFadden (1975) that the persistent small airway narrowing may serve as a basis for recurrent attacks of airway obstruction.

The mechanism of allergen induced bronchoconstriction may have several components. Combinations of antigen and antibody on the bronchial epithelial surface releases mast cell mediators which act on irritant receptors in the epithelium and elicit vagally mediated reflex bronchoconstriction (Weber, 1914). Alternatively, components of the complement cascade, lymphokines or polymorphoneuclear lysozymes could also stimulate these receptors (Cohen et al, 1979). The importance of vagal reflex bronchoconstriction in human asthma is presently in dispute. Arborelius et al (1962) administered specific antigen to only one lung in each of two patients with allergic asthma. In both cases bronchoconstriction, indicated by delayed nitrogen washout, was observed in the challenged lung, while in the unchallenged lung, nitrogen washout characteristics remained normal. The authors concluded that vagal reflex mechanisms were not important in the pathogenesis of bronchoconstriction in the two patients studied. A similar conclusion was reached by Rosenthal et al (1976) who showed that in a group of asthmatics, atropine pretreatment did not reduce the dose of antigen required to produce a 35% fall in specific conductance, although atropine did

increase baseline specific conductance. Yu et al (1972) arrived at an opposite conclusion. They reported that in 5 of 7 asthmatics, increased airway resistance due to antigen challenge was reversed or prevented by atropine treatment and pretreatment, respectively. They concluded that the parasympathetic nervous system was critically important in antigen induced bronchoconstriction is asthmatic patients.

One explanation of the differences between various studies is that asthmatics are a heterogeneous population, with various mechanisms contributing to bronchoconstriction to various degrees. In support of this hypothesis, Orehek et al (1975) showed that in 10 asthmatic patients, pretreatment with scopolamine prevented increases in specific resistance in 5 patients and had no effect in 3 subjects and provided partial protection in two others.

Other mechanisms that may be important in allergen-induced airway narrowing in asthmatics includes direct action of antigen-antibody complexes or chemical substances on smooth muscle, causing bronchoconstriction, excess mucus production, edema, and inflammatory exudate (Huber et al, 1922, Rebuck et al, 1971, Bardana, 1976, Nadel, 1977). Alternatively, bronchoconstriction may be due to bronchial hyperreactivity, characteristic of the asthmatic patient. As reviewed by Boushey et al (1980), the hypothesized mechanisms of bronchial hyperreactivity include decreased baseline airway caliber, alterations in the amount or reactivity of smooth muscle, exaggerated parasympathetic response to stimulation of pulmonary mechanoreceptors, abnormalities of the sympathetic nervous system and changes in epithelial permeability which allow greater concentrations of antigen to contact subepithelial irritant receptors. Presently no animal model for bronchial hyperreactivity

is available although recent reports suggest that the Basenji-Greyhound, sensitized to Ascaris suum antigen has hyperreactive airways (Hirshman et al, 1980, 1981).

A spontaneously occurring disease syndrome with clinical characteristics similar to those of human asthma is uncommon in other mammalian species except the equid (Cook, 1976). Since the horse is an unusual laboratory animal and since few baseline data on equine pulmonary function exist, most work with animal models has been done using experimentally induced allergic lung disease in other species. Experimental models of asthma have been developed in the dog, cat, rhesus monkey, guinea pig and rabbit (Dain et al, 1975, Drazen et al, 1975, Karcsewski et al, 1969, Mills et al, 1970). Sensitized animals are challenged by aerosol or intravenous administration of antigen, resulting in immediate bronchoconstriction.

The mongrel dog, naturally sensitized to Ascaris spp. was first studied by Booth et al (1970) who reported an increased respiratory rate, decreased tidal volume and decreased peak expiratory flow rate, associated with abnormalities in gas exchange following challenge. Dain et al (1975) and Gold et al (1972A) characterized the pulmonary mechanical abnormalities in this model and showed that following aerosol challenge respiratory resistance increased, dynamic compliance and PaO_2 decreased without a change in functional residual capacity or CO diffusion capacity. Tantalum bronchograms showed bronchoconstriction in all airways down to 1 mm diameter bronchi. Significantly, bronchodilators reversed the resistance and compliance changes, but hypoxia persisted suggesting that, like in asthma, gas exchange remains impaired after symptomatic improvement. A more detailed bronchographic study by

Kessler et al (1973) showed that following antigen exposure airway narrowing was slight in airways larger than 12 mm in diameter, moderate in airways 8-12 mm and maximal in airways 1-8 mm, but less in airways 0.5-1 mm. Since the distribution of airway constriction following antigen inhalation was identical to that observed during vagal stimulation and since atropine inhibited antigen-induced bronchoconstriction, it was concluded that antigen-induced airway constriction is mediated by the parasympathetic nervous system. The reflex nature of this mechanism was demonstrated by Gold et al (1972B) who challenged one lung in sensitized mongrel dogs with homologous antigen. Challenge increased airway resistance in both lungs, the increase was reversed by ipsilateral vagal blockade. They concluded that aerosol challenge activated pulmonary receptors resulting in a reflex bronchoconstriction mediated via the vagus nerve. Rubinfeld et al (1978) showed that ventilation-perfusion mismatch, characteristic of human asthma, also occurred in this dog model.

The reflex nature of antigen-induced bronchoconstriction in the sensitized mongrel dogs does not go unchallenged, however, as Krell et al (1976) found that large intravenous doses of atropine did not result in significant reductions in the response to Ascaris antigen, although in some animals the increase in pulmonary resistance was attenuated. The variable result could reflect differences in antigen preparation, reactivity of individual dogs and experimental conditions. The Basenji-Greyhound, sensitized to Ascaris antigen, is distinguished from the mongrel dog by exaggerated bronchoconstriction in response to nonspecific stimuli such as citric acid and methacholine (Hirshman et al, 1980). In support of the findings of Krell et al (1976), Hirshman et

al (1981) reported that in the Basenji-Greyhound the major component of antigen-induced bronchoconstriction is not cholinergically mediated as atropine pretreatment did not protect the dogs from antigen-induced bronchoconstriction.

In summary, Ascaris aerosol challenge of the sensitized dog results in increased pulmonary resistance and decreased dynamic compliance and PaO_2, in addition to ventilation-perfusion mismatch. These changes suggest generalized bronchoconstriction with impairment of gas exchange. The pathophysiology of the derangement in pulmonary function are presently in dispute as some studies seem to clearly indicate the importance of vagal reflex bronchoconstriction while others refute the major involvement of this reflex.

The pulmonary response of the sensitized guinea pig to antigen challenge has been studied by a number of investigators (Ratner et al, 1927, Stein et al, 1961, Mills et al, 1970, Richerson et al, 1972, Popa et al, 1974, Drazen et al, 1975, Roska et al, 1977, Pare et al, 1979). Even though method of sensitization and challenge and therefore immunologic response varied between investigators (Richerson, 1972), in all studies pulmonary resistance increases and dynamic compliance decreases. Maximum changes occurred between 2 and 10 minutes after challenge with resolution occurring over 30 minutes. However, pulmonary resistance returns to normal before dynamic compliance recovers (Drazen et al, 1975). This suggests that, like in human asthma, central airway recovery preceeds return to normal caliber in small airways.

The role of vagal mechanisms in antigen induced bronchoconstriction in the guinea pig was studied by Mills et al (1970) and Drazen et al (1975). Mills et al reported that vagotomy reduced by 75% the

increased resistance and halved the decreased compliance due to antigen challenge and concluded that vagal mechanisms played an important role in antigen induced bronchoconstriction in the guinea pig. Drazen et al (1975) found that atropine pretreatment prevented the decrease in pulmonary resistance but did not hinder the fall in dynamic compliance. This suggests that in the guinea pig, alterations in central airway tone resulting from antigen exposure are mediated predominantly by secondary cholinergic mechanisms while peripheral airway effects are mainly non-cholinergic.

The pulmonary response to antigen challenge has also been studied in rabbits (Karcsewski et al, 1968, Halonen et al, 1976). Following challenge, dynamic compliance is reduced and pulmonary resistance is increased. Vagotomy decreased pulmonary resistance without changing dynamic compliance suggesting that, as in guinea pigs, cholinergic mechanisms play a role in central airway response, but is unimportant in small airway narrowing.

In summary, the pulmonary response to antigen challenge in the small laboratory mammals is characterized by generalized bronchoconstriction occurring immediately following challenge. The majority of evidence suggests that central airways recover before peripheral airways dilate and that central airway narrowing but not peripheral airway constriction is vagally mediated.

In the sensitized Rhesus monkey and sheep, antigen challenge also causes a decrease in dynamic compliance and increase in pulmonary resistance (Pare et al, 1976, Wanner et al, 1979). However, in these species, the role of vagal mechanisms in bronchoconstriction has not been studied to date.

As mentioned above, functional residual capacity and residual volume increase in acute attacks of asthma in persons (Hurtado et al, 1934). Following antigen challenge of the sensitized sheep and some Basenji-Greyhounds, functional residual capacity also increases, while in other Basenji-Greyhounds, the mongrel dog and monkey, challenge does not increase functional residual capacity (Dain et al, 1975, Pare et al, 1976, Wanner et al, 1979, Hirshman et al, 1981). The effect of challenge on lung volumes has not been studied in rabbits, but in guinea pigs, challenge results in an increased minimum volume in vitro, suggesting that residual volume and functional residual capacity may also be increased in vivo. Difference in lung volume changes following challenge between the mongrel dog and some Basenji-Greyhounds may be explained by the difference in magnitude of bronchoconstriction. Following antigen challenge of the Basenji-Greyhounds, pulmonary resistance increases 15-fold and dynamic compliance decreases by 73%, while in the mongrel dog pulmonary resistance increases only 3-fold and dynamic compliance decreases by 23.7% (Gold et al, 1972, Hirshman et al, 1981). These data suggest a more severe airway response in the Basenji-Greyhound model, which may result in gas trapping in the tidal volume range in some individuals. However, functional residual capacity does not increase in other challenged Basenji-Greyhounds with pulmonary mechanics changes as severe as seen in Basenji-Greyhounds that show significant increases in lung volumes (Hirshman et al, 1981). Thus within the Basenji-Greyhound model, the severity of bronchoconstriction does not correlate with changes in lung volumes. In monkey's, Pare et al (1976) reported similar findings. Following challenge, pulmonary resistance increased 7-fold while dynamic compliance decreased 82%. In

spite of this severe response, no changes in functional residual capacity were observed. It appears that the monkey is similar to the mongrel dog in its ability to ventilate peripheral lung units in spite of severe bronchoconstriction. In contrast, Wanner et al (1979) showed that in sheep, antigen induced decrease in pulmonary conductance of only 33% caused a significant increase in functional residual capacity, presumably due to airway closure. Species difference in their ability to maintain ventilation distal to airway obstruction in the face of severe bronchoconstriction may be related to degree of collateral ventilation. The mongrel dog has low resistance collateral channels while collateral resistance is high in persons and in species with lobulated lungs such as sheep (Van Allen et al, 1931). Since the pulmonary anatomy of the monkey lung is similar to dog lung, collateral ventilation is likely to offer a low resistance pathway in this species (McLaughlin et al, 1961). Therefore it appears that low resistance ventilation of obstructed lung units through collateral channels may prevent air trapping in the tidal volume range. If this is true, it is not apparent why, after antigen challenge, functional residual capacity increases in some Basenji-Greyhounds and not in others.

Naturally occurring and experimental lung diseases of the horse

Since the horse is an unusual laboratory animal, its use in these studies needs to be justified. The horse is the only domestic animal that commonly suffers from recurrent airway obstruction, clinically similar to asthma in man (Lowell, 1964, Thurlbeck et al, 1964) making it unique as an animal model for this disease. In addition, since the horse has a substantial financial and social value, study of the

condition itself is also of importance. The disease syndrome, characterized by recurrent airway obstruction, is commonly called heaves, chronic obsructive pulmonary disease or equine emphysema, but destructive emphysema is not a feature of the disease (Thurlbeck et al, 1964). The cause of heaves is not known and the etiology may be multifactoral (Gerber, 1973). Clinical signs may vary depending upon chronicity but typically include expiratory and inspiratory dyspnea, diffuse wheezing, increased sputum production, and reduced exercise tolerance (Gillespie et al, 1969, McPherson et al, 1978). Usually signs are intermittent but in advanced cases the animal may be continuously dyspneic. Signs frequently begin after a viral respiratory infection (Platt, 1972). Subsequently animals exhibit periods of severe airway obstruction following exposure to organic dust in stables and clinical signs abate when animals are at pasture (Breeze, 1979). The onset of signs can occur acutely following exposure to dust but more typically signs occur 4 to 8 hours after exposure. Chronically affected animals typically have diffuse bronchiolitis with goblet cell metaplasia of the bronchioles, excessive mucus in the small airways and acinar overinflation (Thurlbeck et al, 1964). Although centrilobular emphysema and alveolitis have been described, they are not a consistent finding (Gillespie et al, 1966).

Physiologic investigations in heaves have not been correlated with pathologic lesions so that multiple physiologic conditions may have been studied. Decreased dynamic compliance, increased pulmonary resistance, prolonged nitrogen washout, hypoxia and decreased maximal expiratory flows indicate diffuse small and large airway obstruction (Sporri, 1964, Gillespie et al, 1966, Leith et al, 1971, Muylle et al, 1973, Willoughby

et al, 1979). Gas dilution functional residual capacity is not increased but thoracic gas volume measured plethysmographically increases suggesting extensive gas trapping (Leith et al, 1971). This is confirmed at necropsy as lungs at minimum volume are hyperinflated. Similarities of intermittent heaves to human asthma include natural occurrence of the disease, chronicity of the condition with intermittent exacerbations and multifactoral etiology. In addition, pathologic lesions and tests of lung function are also similar.

Since the naturally occurring disease condition may have various etiologies and clinical manifestations and functional lesions may be diverse, I studied experimentally induced lung disease in normal ponies. Two models were studied. The first model of airway obstruction is an allergen induced bronchoconstriction caused by challenging sensitized horses with aerosol ovalbumin (Mansman, 1973). Following aerosol challenge, dyspnea develops gradually and peaks at about 4 hours. Animals appear clinically normal at 24 hours. Data indicate that this reaction is a type III Arthus hypersensitivity like farmer's lung syndrome and some forms of asthma in man (Dickie et al, 1958). This is contrast to other existing animal models in which reactions are immediate and of short duration. The mechanism of bronchoconstriction induced by this new model is not known and either one or a combination of the mechanisms offered above could play a role in the pathogenesis.

In the second model, chronic small airway disease is created by the oral administration of 3-methylindole (3MI) (Breeze et al, 1978A). Clinical signs of dyspnea appear at about 24 hours, peak in 6 to 12 days and animals are clinically normal in about 30 days (Breeze et al, 1978A). 3-methylindole is a metabolite of L-tryptophan and is a cause

of atypical interstitial pneumonia in cattle, grazing on pasture rich in L-tryptophan (Carlson et al, 1975). Pulmonary disease has been experimentally reproduced in cattle, sheep, goats and horses by the oral administration of 3MI (Atkinson et al, 1977, Bradley et al, 1978, Breeze et al, 1978B). A single dose of 3MI has a half-life of about 30 minutes, most being excreted in the urine as oxendole derivatives. 3-methylindole does not accumulate in the tissues and is not present in the urine (Breeze, 1978B). The mixed function oxidase system, which is the main metabolic pathway of xenobiotics, appears to be involved in metabolism of 3MI and is an essential factor in the development of pneumotoxicosis (Hammond et al, 1979). Goats, pretreated with piperonyl butoxide (an inhibitor of the MFO system) do not develop clinical signs or pulmonary lesions when given an intravenous infusion of 3MI, whereas animals pretreated with phenobarbital (an inducer of the MFO system) develop more severe clinical signs and pulmonary lesions (Bray et al, 1979). Lesions are those of an alveolitis and bronchiolitis, mainly involving the bronchiolar epithelium (Breeze, 1978A). This disease model is not allergic in nature, and was studied to provide a comparison between the role of vagal mechanisms in the pathogenesis of allergic and toxicologic lung diseases.

Purpose of the studies

At the outset of this investigation we were concerned about the validity of pleural pressure measurements in the horse. Since transpulmonary pressure (the pressure difference between airway opening pressure and pleural pressure) is an essential measurement in pulmonary function studies, this question needed to be resolved before any further

study could be undertaken. There was no standard technique for measuring pleural pressure in the horse but commonly used methods employed esophageal balloons or esophageal balloons made from condoms and direct puncture of the pleural space at various sites (Denac-Sikiric, 1970, Sasse, 1971, Sorenson et al, 1980). In persons and dogs, esophageal pressure is commonly used as a measure of intrapleural pressure (Mead et al, 1955, Cherniack et al, 1955, Milic-Emili et al, 1964). Although esophageal pressure may not always reflect absolute pleural pressure in these species, changes in esophageal pressure during breathing are similar to changes in local pleural pressure (Daly et al, 1963). In persons an esophageal pressure measuring technique has been standardized. Use is made of a 10 cm long esophageal balloon containing 0.5 ml of air, placed in the caudal portion of the thoracic part of the esophagus so as to minimize artifacts caused by heart beat, changes in posture, and pressure from mediastinal content (Milic-Emili et al, 1964).

In all mammalian species studied, there is a gradient of pleural pressure from the dorsal to the ventral parts of the thorax (Krueger et al, 1961, Proctor et al, 1968, Fahri et al, 1969, Hoppin et al, 1969, Hogg et al, 1969). In addition, regional changes in pleural pressure during breathing can be variable (Rousson et al, 1976, Engel et al, 1977). If the latter is true in the horse, pleural pressure may vary with the site of measurement. Thus, the purpose of the first study, described in Chapter 1, was to compare intrapleural pressure measured at 3 sites in the thorax, with esophageal pressure at different points in the thoracic part of the esophagus, using 2 commonly used balloons.

In order to study the role of vagal mechanisms in our disease

models, I wanted to be able to reversibly block the vagus nerves in conscious chronic animals. As reviewed by Franz et al (1968) mammalian nerve conduction can be inhibited by cold block. When temperature of a nerve decreases, the maximum transmissible frequency of impulses decreases so that for example in a myelinated nerve with a conduction velocity of 40 meters second^{-1}, a drop in temperature from 20 to 10°C causes a decrease in maximum transmissible frequency from 240 to 40 impulses second^{-1}. When the vagus nerve is cooled to 7°C, activity in myelinated fibers is almost completely blocked (Franz et al, 1968). However, low frequency activity in nonmyelinated fibers will continue to be conducted until a temperature of 4°C is reached (Paintal, 1971). Cooling of a portion of the vagus nerves is facilitated by the creation of cervical vagal loops. Cooling of surgically prepared vagal loops is a commonly used technique for vagal blockade in dogs (Phillipson et al, 1975, Snapper et al, 1979). The purpose of the study presented in chapter 2 was to describe the adaptation and use of this technique in the standing conscious pony.

Although pulmonary function tests have been used to evaluate horses with clinically normal lungs, few comprehensive studies of equine respiratory function have been made and the range of reported values is large (Mauderly, 1974, Orr et al 1975, Willoughby and McDonell, 1979). This may be due to differences in techniques used by the various investigators or because of real variation in values. Information about the repeatability of pulmonary function tests in normal horses was therefore necessary before models of lung disease could be studied. In addition, since I was interested in studying vagal mechanisms in disease, the role of the vagus nerve in control of pulmonary function in

healthy animals needed first be established. This information was not available for the equid. Therefore, the purpose of the study reported in chapter 3 was to assess the repeatability of pulmonary function measurements within a day, and over a 6-month period, to determine the effect of changes in lung volume on total respiratory resistance, to evaluate the effect of respiratory frequency on dynamic compliance and to study the effect of vagal blockade on pulmonary mechanics, lung volumes and gas exchange.

3-methylindole induced pulmonary toxicosis in the horse is unique because the horse is the only species studied so far in which oral or intravenous administration of 3MI produces a pure small airway obstruction (Breeze, 1978). The study of this disease model was of interest because clinical signs of the disease are indistinguishable from the naturally occurring asthma-like syndrome in the horse and because it provided a comparison between the importance of vagal mechanisms in the pathogenesis of an allergic disease model (Chapter 5) and a pneumotoxicosis with no allergic etiology. Thus, in chapter 4 I report changes in pulmonary function in the early stages of 3MI induced pulmonary toxicosis, correlate functional changes with pathologic lesions and determine the role of vagal mechanisms in the pathogenesis of disease.

Chapter 5 represents an in-depth study of the role of vagal mechanisms in ovalbumin induced allergic lung disease in the sensitized horse. In awake sensitized ponies I studied the effect of aerosol ovalbumin challenge on ventilation, pulmonary mechanics, lung volumes and gas exchange over a five-hour period and before and after vagal blockade. I subsequently challenged one lung in a second group of sensitized ponies and measured respiratory rate and right and left lung

resistance (R_{totR} and R_{totL}) during the same time period and before and after both ipsilateral and bilateral vagal blockade. I reasoned that if vagal reflexes, originating in the challenged lungs or a challenge induced increase in efferent parasympathetic bronchomotor activity were responsible for airway narrowing in this disease model, unilateral aerosol antigen challenge would result in airway narrowing in both lungs, abolished by either unilateral or bilateral vagal blockade. If aerosol antigen challenge increased the sensitivity of airway smooth muscle to normal vagal tone or if a decreased baseline airway caliber was important, left unilateral challenge would result in increase in R_{totL} only, abolished by either unilateral or bilateral vagal blockade, while if local mechanisms were important in airway caliber changes, unilateral challenge would only cause an increase in R_{totL}, unaffectd by vagotomy.

Since pilot studies suggested that aerosol challenge following both systemic and local sensitization of the lung results in more severe dyspnea of rapid onset, using both unilateral and bilateral challenge protocols I investigated the pulmonary response to aerosol challenge in both systemically and locally sensitized ponies and studied the role of local and vagal mechanisms in this response. In addition, I correlated functional changes with pathologic lesions as presently no information is available to document that changes in pulmonary function values have value in predicting location and relative severity of pathologic lesions in the equine lung.

CHAPTER 1

Esophageal and Intrapleural Pressure in The Healthy Conscious Pony

Introduction

Dynamic compliance and pulmonary resistance are measured as lung function tests in horses. To determine these values, measurements must be made of transpulmonary pressure, i.e., the pressure gradient between the airway opening and the pleural cavity. There is no standard technique for measuring pleural pressure. Commonly used methods include using esophageal balloons or esophageal balloons made from condoms and direct puncture of the pleural space.[1-10] Reported values for dynamic compliance vary, and we sought to determine whether the variation was partly due to different techniques for measuring pleural pressure.

In persons and dogs, esophageal pressure is commonly used as a measure of intrapleural pressure.[11-21] Although esophageal pressure may not always reflect absolute pleural pressure, changes in esophageal pressure during breathing are similar to changes in local pleural pressure.[11,12,14,16] In persons, an esophageal pressure measuring technique has been standardized; use is made of a 10-cm long esophageal balloon containing 0.5 ml of air placed in the caudal portion of the thoracic part of the esophagus so as to minimize artifacts caused by heart beat, changes in posture, and pressure from mediastinal contents.[15,20]

In all mammalian species studied, there is a gradient of pleural pressure from the dorsal to the ventral parts of the thorax.[22-27] In addition, regional changes in pleural pressure during breathing can be variable.[28-30,a,b] If the latter is true in the horse, variability in

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[a] Kelly S, Roussos CS, Engel LA: Gravity independent sequential emptying from topographical lung regions. Clin Res. 23:645A, 1975.

[b] Roussos CS, Genest J, Cosco MJ, et al: Rib cage vs abdominal breathing and ventilation distribution. Clin Res. 23:648A, 1975.

reported values of dynamic compliance may be related to the site at which pleural pressure is measured.

In the literature, there are no statistical comparisons of pleural and esophageal pressure in horses. The purpose of the present study was to compare intrapleural pressure (measured at 3 sites in the thorax) with esophageal pressure at different points in the thoracic part of the esophagus, using 2 commonly used balloons.

Materials and Methods

Six grade ponies, between 2 and 10 years old and weighing 160 to 180 kg each, were tranquilized with xylazine[c] to effect and were restrained in stocks. Using local anesthesia, a tracheostomy was performed and a 20-mm diameter endotracheal tube was introduced into the trachea. A Fleisch pneumotachograph (No. 4)[d] and associated pressure transducer[e] were attached to the endotracheal tube. The pneumotachograph transducer system produced a signal proportional to flow which was electronically integrated to give tidal volume. This system was calibrated by forcing a known volume of air through the pneumotachograph after each experiment.

Pleural pressure was recorded through three 6.5-cm blunt tipped 12 gauge needles, with 2 side holes near the tip. The needles were attached with 60-cm lengths of polyethylene tubing (ID = 1.67 mm, OD = 2.42 mm) to 3 pressure transducers.[f] The transducers were taped to the thoracic wall, using elastic tape. The 1st needle was introduced into

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[c] Rompum, Haver Hockhart, Shawnee Mission, Kan.
[d] Dynasciences Bluebell, PA.
[e] Model PM5, Statham Instruments, Hato Rey, Puerto Rico.
[f] Model P23D6 Statham Instruments, Hato Rey, Puerto Rico.

the pleural cavity at a slight angle downwards through the right 10th intercostal space at the level of the point of the shoulder. A distinctive pop was felt when the needle penetrated the parietal pleura. The 2nd and the 3rd needles were introduced 10 cm and 20 cm, respectively, above the 1st needle.

Two types of esophageal balloons were used. The first balloon as recommended by Milic-Emili et al[15] for use in persons was made of rubber and had the following dimensions: length 10 cm, perimeter 3.5 cm, wall thickness 0.06 mm. The 2nd balloon, as described by Gillespie et al[2] and Willoughby and McDonell,[3] was made from a condom[g] and was 15 cm long with a perimeter of 10 cm. Both balloons were sealed over the end of polyethylene catheters (ID =3 mm, OD = 4.4 mm, length = 140 cm) which had a number of spirally arranged holes in the part covered by the balloons.

Distances from the nares to the caudal, middle, and cranial portions of the thoracic part of the esophagus were visually approximated and marked on the esophageal balloon catheter with indelible ink. The same distances were used on all subjects due to the similarity in size. Esophageal balloon catheters were made rigid by introduction of a length of 18 gauge steel wire and passed via the nares into the cranial portion of the esophagus. The wire was removed and the balloon was attached to a pressure transducer[h] which was taped to the forelock. Balloon volume was adjusted to contain 0.5 ml of air in the esophageal balloon or 3.5 ml in the condom. Esophageal pressure, 3 pleural pressures, and tidal

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[g] Trojan-enz Youngs Rubber Co., Trenton, NJ.
[h] Model PM131TC Statham Instruments, Hato Rey, Puerto Rico.

volume were amplified and recorded on a 6-channel recorder.[i] Three pleural pressures and tidal volume were recorded continuously. Esophageal pressure was recorded for at least 5 breaths at each esophageal location. The sequence of introduction of the esophageal balloon and condom was randomized. At each measuring site, dynamic compliance was calculated during at least 4 breaths from tidal volume and the change in the esophageal or pleural pressure between the start and end of inspiration. Results were analyzed with a 2-way analysis of variance and Tukey's W procedure at the 0.05 level of significance.[31]

To avoid phase differences between various measuring devices, a check of frequency response was made. An alternating pressure was generated in a closed flask by means by a syringe. The interior of the flask was connected with a 1 cm long (ID = 0.5 cm) tube to a differen tial pressure transducer.[h] This recording system was assumed to measure the true pressure fluctuations within the container. The pleural pressure needle or the esophageal balloons were introduced into the flask through a side arm and connected to the opposite side of the pressure transducer with the same tubing used during the experiments. Using pressure variations up to 30 cm of water and a frequency of 5 Hz, a flat response was recorded.

The frequency response of the pleural needles and catheters was matched to that of the esophageal balloons by attaching these devices to opposite sides of a differential pressure transducer and exposing them to a quasisinusoidal oscillating pressure. The frequency response of both ports of the pneumotachograph were similarly matched. Finally,

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[h] Model PM131TC Statham Instruments, Hato Rey, Puerto Rico.
[i] Model KA, Soltec Corp., Sun Valley, Calif.

the response of the pneumotachograph transducer system and the pleural pressure and esophageal pressure transducer systems were checked by comparing pressure recorded with esophageal or pleural catheters and transducers against pressure recorded with the pneumotachograph transducer on an XY plotter[j] while exposing all devices to the same oscillating pressure source. All frequency responses were checked up to 5 Hz and were flat.

Results

A biphasic expiratory pattern was observed in 5 of the 6 horses. A passive exhalation was followed by a pause and an abdominal excursion immediately preceding the next inhalation (Fig 1-1). The pleural pressure at the plateau which occurred just before the abdominal effort was relatively constant from breath to breath and seemed to correspond with the end of a passive exhalation, whereas pressures at the end of the abdominal effort were highly variable. The pressure at the expiratory plateau was therefore recorded as pressure at the equilibrium position of the respiratory system. This pressure increased from the dorsal to the ventral thoracic positions (Fig 1-1 and 1-2). Pressures in the middle and caudal portions of the thoracic part of the esophagus were similar, and not significantly different from pressures measured at the middle and ventral thoracic positions (Fig 1-2 and 1-3). Pressures in the cranial portion of the thoracic part of the esophagus were significantly higher than pressures at the dorsal and middle thoracic positions and dorsal thoracic position pressures were significantly

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[j] Model VR6, E for M, White Plains, NY.

lower than esophageal pressures. Pressures at the expiratory plateau as measured by the 2 balloon types were not significantly different.

Changes in pleural pressure during respiration were variable from breath to breath. To compare the esophageal pressure waves which were not recorded simultaneously, a pressure amplitude that occurred in all middle thoracic position tracings for each animal was selected as a standard with which the other pressure changes could be compared. Mean selected pressure changes are reported in Table 1-1. Variables are listed in order of magnitude with the lowest value 1st and the highest value last. Pressure changes underscored by the same line do not differ significantly. These data can be interpreted to mean that pressure changes in the cranial portion of the thoracic part of the esophagus were the least, pressure changes in the middle and ventral thoracic positions the greatest, and the pressure changes in the dorsal thoracic position and middle and caudal portions of the thoracic part of the esophagus were intermediate.

Cardiogenic pressure oscillations were obvious in the tracings from the cranial portion of the thoracic part of the esophagus and masked pressure changes of respiratory origin. These artifacts were not present in the middle and caudal portions of the thoracic part of the esophagus. Large positive deflections corresponding to swallowing were most frequent in the cranial portion of the thoracic part of the esophagus but were also present in the middle and caudal portions of the thoracic part of the esophagus. There was no significant difference between the 2 balloon types with respect to pressures, amplitudes, or artifacts.

Air (10 ml) was introduced through the needles into the pleural

Figure 1-1 (a) Pleural pressures and tidal volume during 1 breath. Ventral, middle, and dorsal refer to the position of the pleural needles in the thoracic wall. (b) A comparison of pressures of the middle part of the thoracic part of the esophagus and dorsal part of the thorax during 1 breath. On the volume traching, inspiration is up. Note the mid-expiratory pause during exhalation.

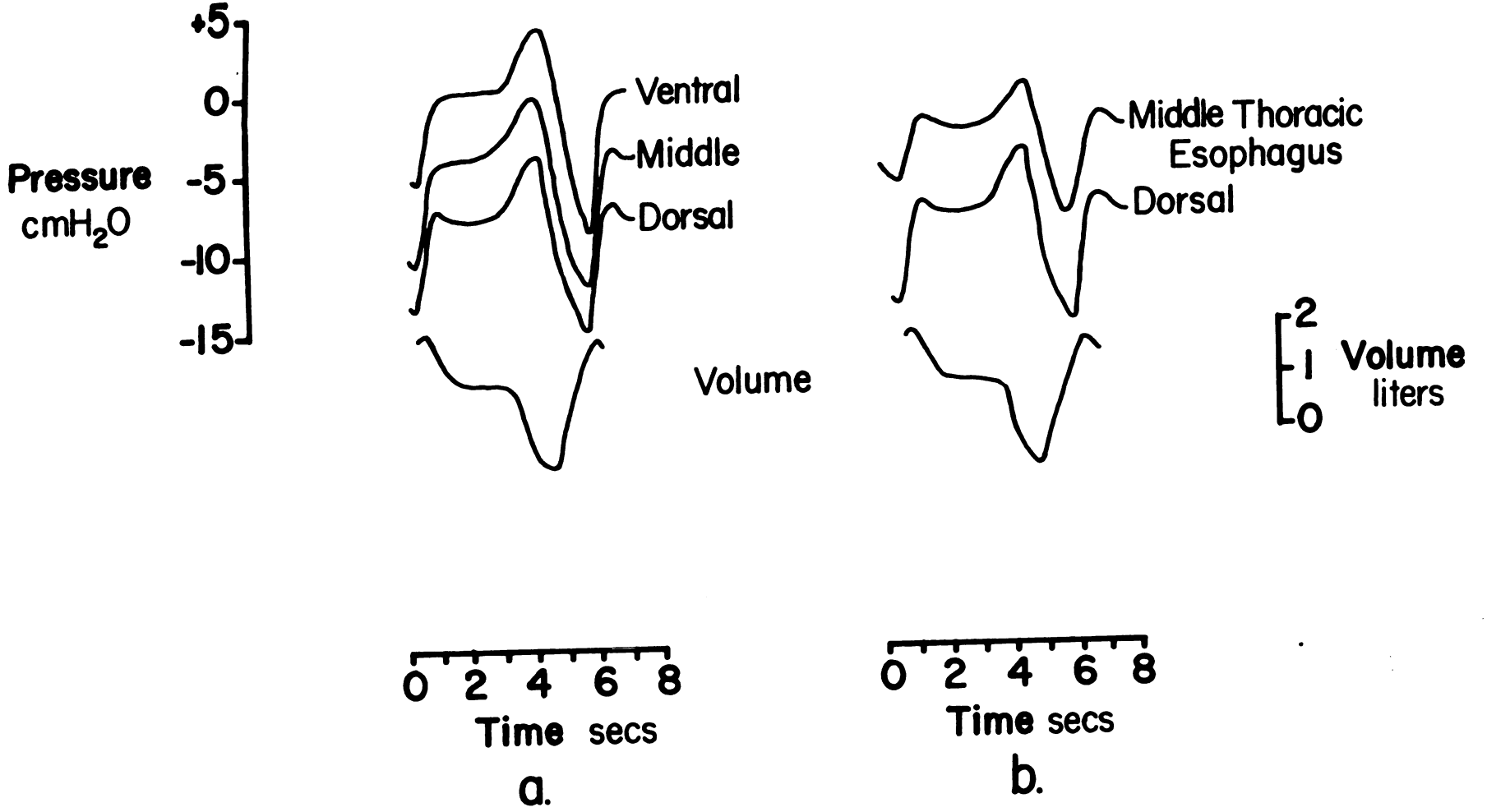
+5
0
-5
-10
-15
Pressure
cmH2O
Ventral
Middle
Dorsal
Volume
0 2 4 6 8
Time secs
a.
Middle Thoracic
Esophagus
Dorsal
2
1
0
Volume
liters
0 2 4 6 8
Time secs
b.

Figure 1-1

Figure 1-2 Pleural pressure ($\bar{x} \pm$ SEM) measured during the mid-expiratory volume plateau in the ventral, middle, and dorsal thoracic wall. Distance = height of middle and dorsal thoracic pleural cannulae above the ventral thoracic cannulae.

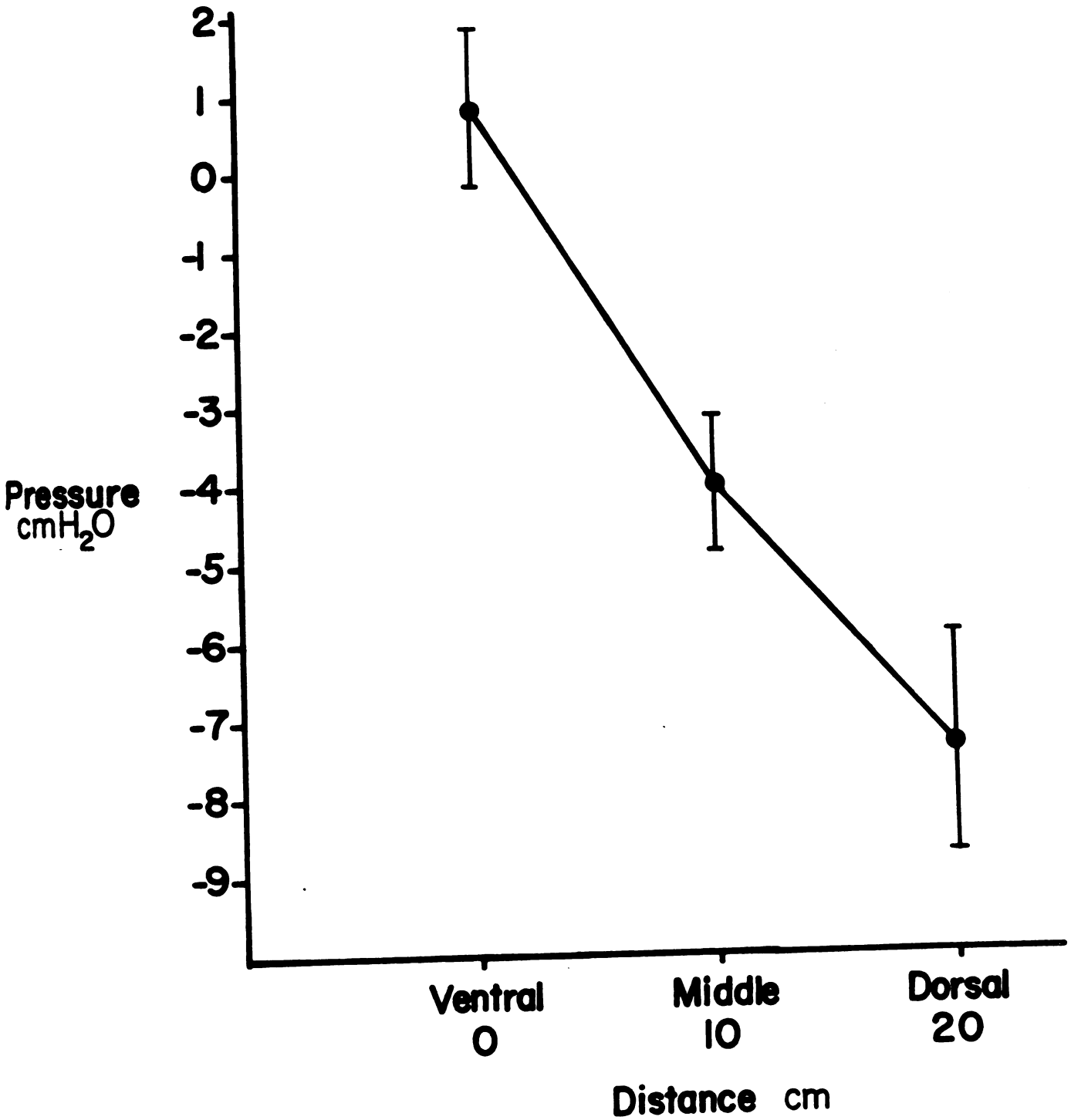
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
Pressure
cmH2O
Ventral
0
Middle
10
Dorsal
20
Distance cm

Figure 1-2

Figure 1-3 Esophageal pressure ($\bar{x} \pm$ SEM) measured during the mid-expiratory volume plateau in the cranial, middle, and caudal portions of the thoracic part of the esophagus. Distance = distance from the tip of the esophageal balloon to the external nares.

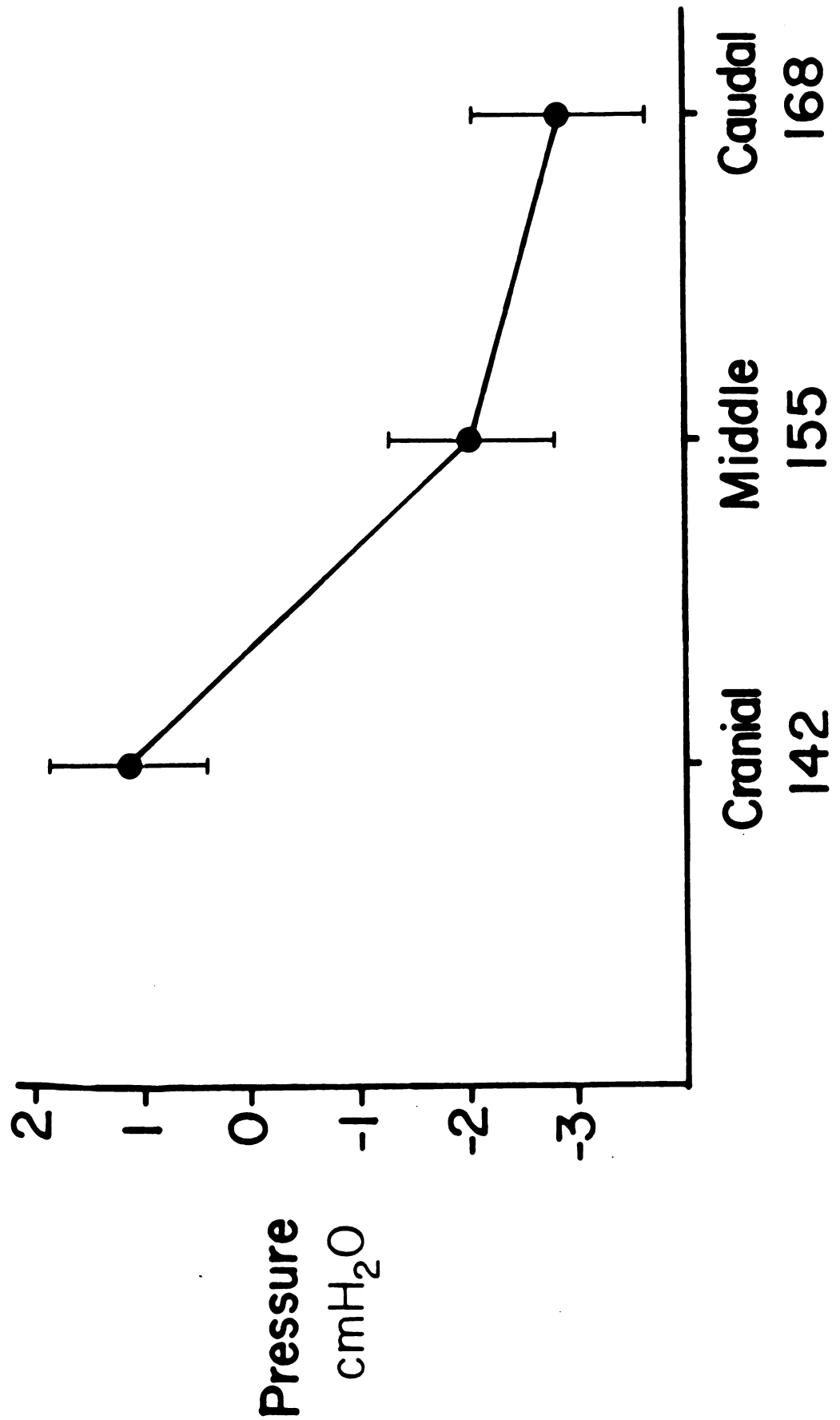
Pressure
cmH2O
2
1
0
-1
-2
-3
Cranial
142
Middle
155
Caudal
168
Distance cm

Figure 1-3

TABLE 1-1 - Comparison of Mean Selected Pressure Changes (ΔP) as a Function of Measuring Site

	Cranial portion of the thoracic part of the esophagus		Middle portion of the thoracic part of the esophagus (condom)	Caudal portion of the thoracic part of esophagus		Middle portion of the thoracic part of the esophagus (balloon)	Thoracic wall positions		
	Balloon	Condom		Condom	Balloon		Dorsal	Ventral	Middle
P cm of H_2O	1.93	2.40	6.27	6.97	7.13	7.37	7.55	9.7	11.55

Underscoring shows result of multiple comparisons of means, using Tukeys W prodedure. Underscored values are not statistically different.

TABLE 1-2 - Dynamic Compliance (C_{dyn}) Calculated from Change in Pleural Pressure Measured at Different Sites

	Cranial portion of the thoracic part of the esophagus		Middle portion of the thoracic part of the esophagus		Caudal portion of the thoracic part of the esophagus		Thoracic wall positions		
	Balloon	Condom	Balloon	Condom	Balloon	Condom	Dorsal	Ventral	Middle
C_{dyn} L/cm of H_2O $\bar{X} \pm$ SEM	NC	NC	0.673 ±0.082	0.571 ±0.097	0.429 ±0.050	0.606 ±0.098	0.502 ±0.042	0.322 ±0.046	0.342 ±0.063

NC = not calculated

space at the end of each experiment. A change in amplitude configuration or resting expiratory pressure was not observed.

Dynamic compliance was calculated from each pressure tracing except the cranial portion of the thoracic part of the esophagus, where artifacts and small deflections made measurements of change in pressure difficult. Dynamic compliance calculated from the middle and ventral thoracic position tracings were generally lower than compliance measured from the thoracic part of the esophagus and from the dorsal thoracic position tracings (Table 1-2).

Discussion

The results of this study indicate that pleural and esophageal pressure changes during breathing vary with the site and technique of measurements. The smallest pressure amplitudes are measured in the cranial portion of the thoracic part of the esophagus and may be a result of dampening of pressure waves by mediastinal contents in this narrow part of the thorax. Pressure changes measured in the middle and caudal portions of the thoracic part of the esophagus do not differ from those measured in the dorsal thoracic position. Necropsy examination of other ponies showed that the middle and caudal portions of the thoracic part of the esophagus are in the same horizontal plane as the dorsal pleural pressure needle (about 20 cm above the point of the shoulder). It seems therefore that changes in esophageal pressure reflect local changes in pleural pressure in the standing pony. In persons, similar conclusions were reported by Milic-Emili et al[15] and Fry et al.[16]

The changes in pleural pressure during breathing measured in the

middle and ventral thoracic positions tended to be greater than those measured in the dorsal thoracic position and in both middle and caudal portions of the thoracic part of the esophagus. Regional differences in pleural pressure amplitude during breathing have been reported in other species and have been attributed to selective use of different respiratory muscles.[28-30] Another explanation for these variations is based on differences in mechanical time constants between pulmonary units.[28,29] It is probable that time constant inequality exists within the normal lungs and thus different lung zones might fill asynchronously.[28] Blake et al[29] suggested that the pleural pressure over lagging lung regions may become more negative than that over leading lung units because of the resistance of the thoracic wall to deformation.

Alternatively, regional pleural pressure differences may result from local deformation of the pleural surface caused by the introduction of a measuring device.[32] McMahon et al[33] evaluated several types of measuring devices and found that the more local a deformity, the greater the error. They recommended the use of a flat disk-like device with a central hollowed depression (a Starling resistor).[23] Implantation of such a device or any other flat device necessitates open thoracic surgical technique and is therefore impractical for routine use in the standing conscious horse during clinical evaluation of pulmonary function.

Because of the local variations in pleural and esophageal pressure changes during breathing, dynamic compliance tends to vary with the site of pleural pressure measurement. Most investigators use the middle thoracic position when measuring pleural pressure in the horse

(Table 1-3). Others measure esophageal pressure in the middle portion of the thoracic part of the esophagus. Generally, dynamic compliance values determined from esophageal pressures are greater than those determined from direct pleural pressure measurements. However, Willoughby and McDonell[3] and Gillespie et al[2] stated there were no differences between esophageal and pleural pressure measurements, although no comparative data were presented.

Dynamic compliance in animals of different body weights can be predicted by:

$$C_{dyn} = 0.0021m^{1.08} L/cm \text{ of } H_2O$$

where C_{dyn} = dynamic compliance, m = body weight in kg.[34]

Using this formula, a dynamic compliance of 0.504 to 0.573 L/cm of H_2O can be predicted for the horses used in this experiment and 1.73 L/cm of H_2O for a 500-kg horse. When pressure changes in the middle portion of the thoracic part of the esophagus and in the dorsal thoracic position were used in compliance calculations, dynamic compliance was similar to the predicted value ($\bar{x}$ = 0.556 L/cm of H_2O). Dynamic compliance values calculated, using pleural pressures recorded from the middle and ventral thoracic positions, were lower than predicted ($\bar{x}$ = 0.332 L/cm of H_2O). This indicates that variability in reported values for dynamic compliance in the horse may be partially attributed to variations in measurement techniques.

In persons, functional residual capacity is the volume of gas in the lungs at the end of a passive exhalation and corresponds to the midposition of the respiratory system.[35] Because of the active expiratory effort, end-expiratory volume in the horse may not correlate with

the midposition where lung recoil is opposed by outward passive recoil of the thoracic wall.[18] In our recordings in which the upper airway was bypassed, there was frequently a pause midway through exhalation. This pause which appeared to correspond to the end of a passive exhalation and preceded an active expiratory effort probably occurred at the midposition. Pleural pressure was relatively constant at the mid-expiratory pause, whereas end-expiratory pressure was variable. For this reason, and because the mid-expiratory pause offered the longest period of zero airflow, we measured the mid-expiratory pleural pressure in order to estimate pleural pressure gradients.

Although biphasic expiratory flow has been reported in horses,[1,7,36] a mid-expiratory cessation of flow has not been described. In intact horses, it is probable that a variable upper airway resistance modulates expiratory flow rates so that flow continues throughout exhalation. Presumably, inhalation occurs as a result of passive recoil of the abdomen and active inhalation. These 2 actions must be concurrent, as we never observed a mid-inspiratory change in air flow rate.

A pressure gradient of 0.330 cm of water/cm of descent occurred in the dorsal half of the thorax and 0.484 cm of water/cm of descent in the ventral half of the thorax. In other species, gradients between 0.2 and 1 cm of water/cm of descent are reported and are thought to be due to gravitational effects on the lung, a structure of varying density.[22-27] However, Hogg and Nepszy[27] showed that pleural pressure gradients do not alter with changing lung volumes and thus lung density, and Banchero et al[21] and Rutishauser et al[24] concluded that the average density of all thoracic structures were responsible for pleural pressure gradients. This latter hypothesis might be used to explain

Figure 1-4 Expiratory limb of a quasi-static lung pressure volume curve (from reference 8). Pressure = transpulmonary pressure. Mean pressures measured during the mid-expiratory plateau at the ventral, middle, and dorsal thoracic positions are marked on the curve to show the possible regional variations in lung inflation.

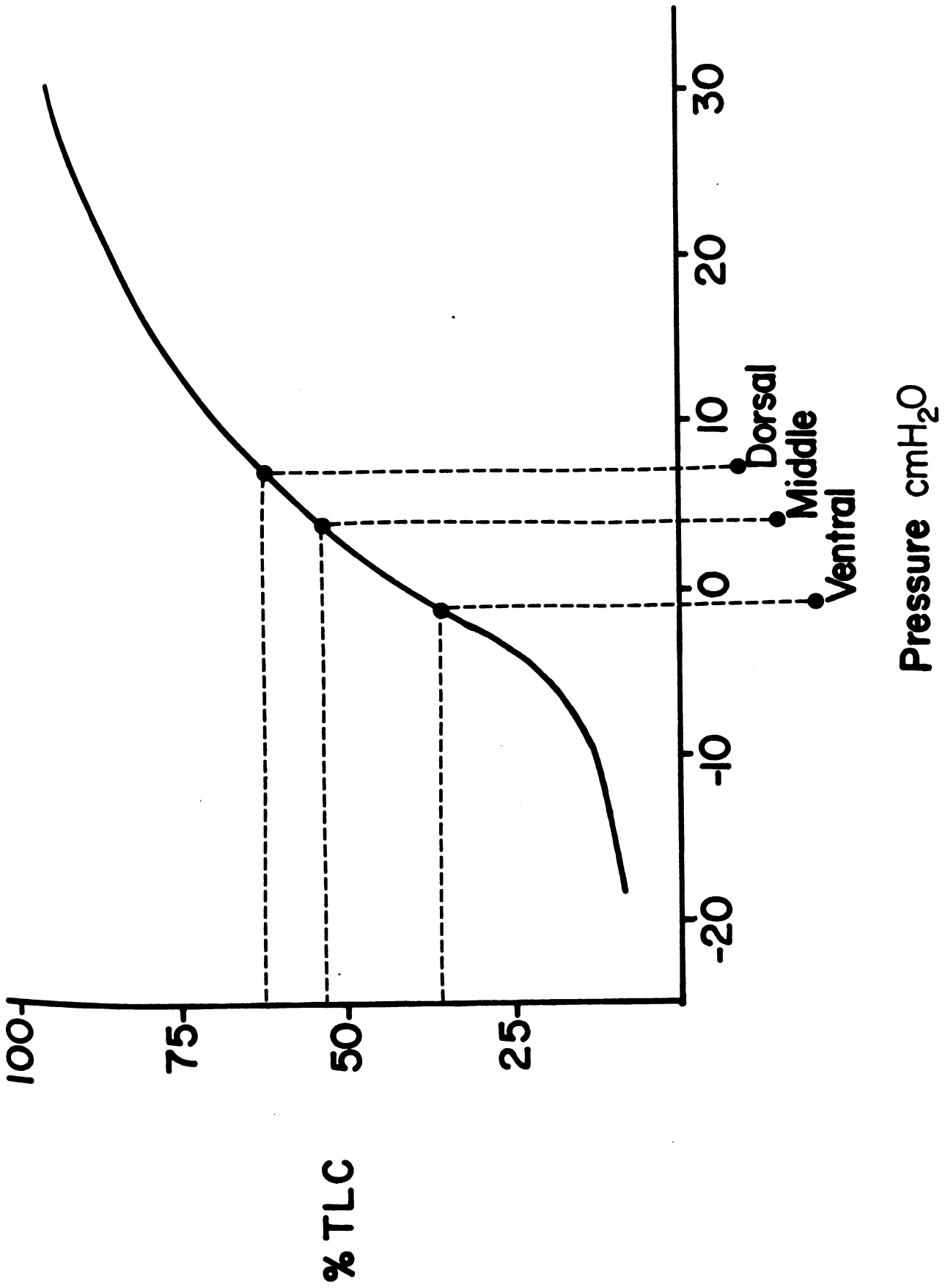
100
75
50
25
% TLC
30
20
10
0
-10
-20
Dorsal
Middle
Ventral
Pressure cmH2O

Figure 1-4

TABLE 1-3 - Comparison of Measuring Technique Used and Dynamic Compliance (C_{dyn}) Value Obtained in Normal Standing Horses

Body weight in kilograms (X ± SEM)	No. of horses	Measurement technique	C_{dyn} L/cm of H_2O	Description of site	Reference
486 ± 3.5	15	Esophageal pressure and direct pleural measurement	5.1 ± 5.1	Dorsal third of thorax on the 7th intercostal space	2
486 ± 3.5	19	Esophageal balloon	6.13 ± 0.03	Caudal to base of heart	1
450	10	Direct pleural measurement	2.12 ± 1.01	16th Intercostal space 10 in below the horizontal plane of lumbar transverse processes	5
509 ± 14.5	24	Direct pleural	2.3 ± 0.29	10th Intercostal space on a line between tuber coxae and the shoulder	6
554 ± 46.6	12	Direct pleural measurement	172 ± 0.75	Site not reported	4
485 ± 19.5	6	Direct pleural measurement and esophageal balloon	2.27 ± 0.47	14th Intercostal space on a line between the elbow and the tuber coxae. Middle portion of the thoracic part of the esophagus	3

larger pleural pressure gradient in the ventral thoracic area as the average density of structures is greater in that region than the dorsal region of the thorax. Alternatively, it can be postulated that the density of the ventral lung regions is greater than that of dorsal regions because of greater perfusion.

Pressures at the expiratory plateau were greatest in the cranial portion of the esophagus. Petit and Milic-Emili[19] demonstrated a high resting end-expiratory intraesophageal pressure in the cranial portion of the esophagus in persons and postulated that this high pressure was due to compression by mediastinal contents. The same mechanism may play a role in the horse.

Since the middle and caudal portions of the thoracic part of the esophagus and the dorsal pleural pressure measurement site are located about 20 cm above the point of the shoulder, pressures at the sites during the expiratory plateau were expected to be similar. However, esophageal pressures at the expiratory plateau were significantly higher than pressures in the dorsal thoracic position, probably as a result of the influence of elastic properties of the esophageal wall.

In Figure 1-4, we have plotted the mid-expiratory pleural pressure recorded from 3 thoracic positions on an equine lung pressure volume curve.[8] In other species, pressure volume relationships do not vary throughout the lung and we assume this is also true in the horse. The greater distending pressure in the dorsal region of the thorax indicates that alveoli are more inflated than those in the ventral thorax, where distending pressures are less. When a similar change in pleural pressure is applied to each region, the dorsal alveoli, which are operating on the upper portion of the pressure volume curve, inflate

less than the ventral alveoli, which are operating on a steeper portion of the curve. Our data further indicate that changes in pleural pressure may actually be greater in the ventral and middle than the dorsal thoracic position, further contributing to the preferential distribution of ventilation to the ventral portions of the lung during lung inflation.

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CHAPTER 2

Technique for Reversible Vagal Blockade in the Standing Conscious Pony

Introduction

Since the vagus nerve is involved in the control of the pulmonary, gastrointestinal and cardiovascular systems, investigation of the function of these organs is facilitated by repeated and reversible vagal blockade in the conscious chronic animal. Cooling of surgically prepared vagal loops is a commonly used technique for vagal blockade in dogs.[1,2,3] This paper describes the adaptation and use of this technique in the standing conscious pony.

Surgical preparation

Five ponies weighing between 125 and 272 kg were used in this experiment. Under general anesthesia, animals were positioned in lateral recumbency and prepared for aseptic surgery. A 20 cm skin incision was made parallel and just dorsal to the jugular vein in the middle third of the neck. The carotid artery, recurrent laryngeal nerve and vagosympathetic trunk were exposed by sharp dissection through the platysmus and by blunt dissection through the brachiocephalicus and omohyoideus muscles. The carotid artery and the vagus nerve were dissected free from the surrounding tissues for the length of the incision, taking care not to disturb the recurrent laryngeal nerve and sympathetic trunk. The carotid artery and vagus nerve were simultaneously elevated and the brachiocephalicus muscle was apposed beneath these two structures over a length of 5 cm. A 5 cm long skin incision was made parallel and 2.5 cm dorsal to the first incision, and the skin between the two incisions was dissected free from the subcuticular tissue. This section of skin was used to form a loop around the exposed section of the vagus nerve using a simple interrupted suture pattern. The remaining skin defect was

closed over the carotid artery with a tension suture pattern.

In the preparation of four loops, a longitudinal, 10 cm long, tension relieving incision was made on the ventral midline of the trachea. This wound was left open to heal by second intention. Six skin loops were prepared without making use of the ventral midline release incision. In five of these, the skin wound dehisced underneath the loop because of excessive tension and seroma formation. These wounds were also left open to heal by second intention. During healing, vaseline impregnated gauze was used to isolate the skin loop from the underlying wound. The four vagal loops, prepared using the release incision, healed by first intention.

At least two weeks were allowed before the procedure was repeated on the contralateral side. Fig. 2-1 and 2-2 show a vagal loop 14 days and 90 days postoperatively.

A laryngoscopic examination was performed immediately after each surgical procedure to assess laryngeal function. In seven cases, examination revealed a mild ipsilateral laryngeal hemiplegia. This condition resolved spontaneously within two weeks in all affected ponies.

Experimental Methods

At least 30 days were allowed for healing of the surgical wounds. Animals were restrained in stocks and sedated with xylazine[a] to effect. Under local anesthesia, a tracheostomy was performed. A cuffed endotracheal tube was inserted and a pneumotachograph[b] and differential

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[a] Rompun, Cutter Laboratories Inc, Shawnee Mission, Kan.
[b] Fleisch #4, Dynasciences, Blue Bell, PA

Figure 2-1 A left cervical vagal loop, 14 days after the surgical procedure. Notice the release incision, healing by 2nd intention.

Figure 2-1

Figure 2-2 Right cervical vagal loop, 90 days after the surgical procedure.

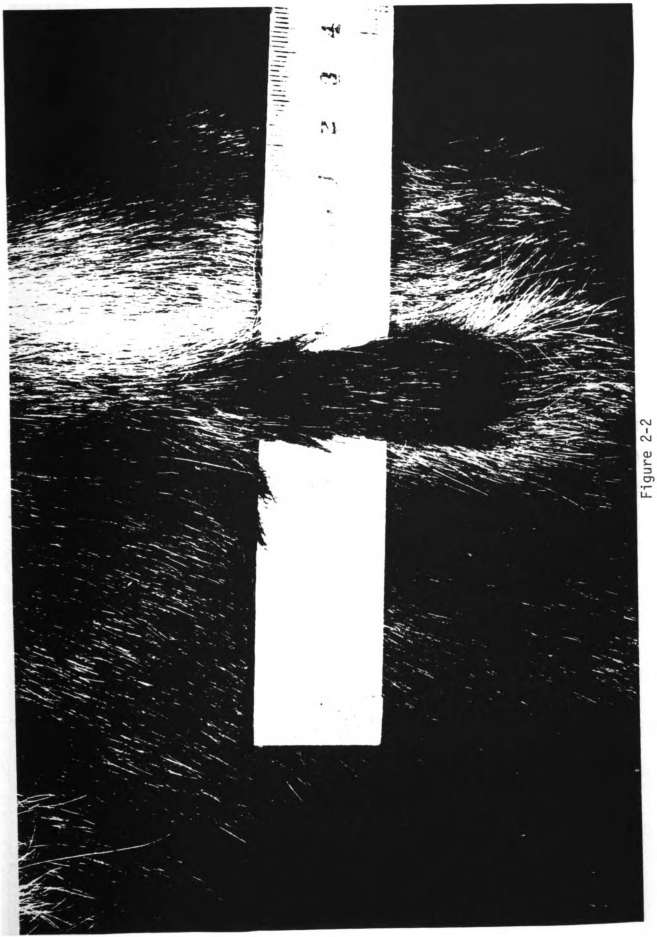

Figure 2-2

Figure 2-3 Copper cooling coil used to refrigerate the vagal loops. Coils were made of 2 parts and shaped to fit the vagal loops.

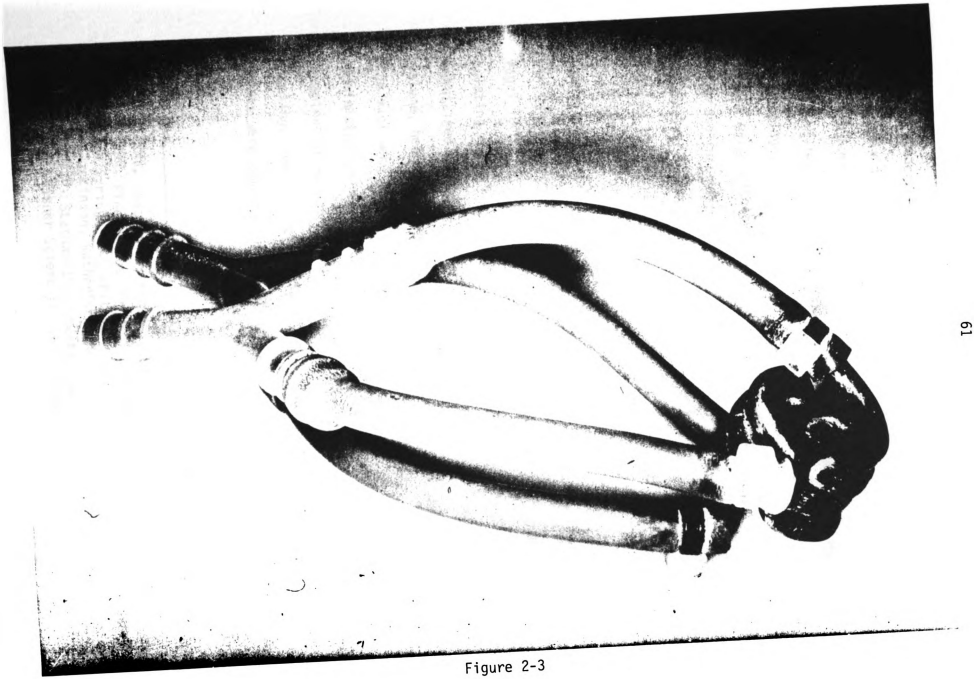

Figure 2-3

pressure transducer[c] were attached to measure flow. The flow signal was electronically integrated to give tidal volume.[d] After each experiment, the pneumotachograph was calibrated by forcing air at known flow rates through the instrument. The volume signal was calibrated using a three liter syringe.[e]

A 20 gauge catheter[f] was inserted into one exteriorized carotid artery and connected to a pressure transducer[g] placed at the level of the shoulder. Respiratory rate, tidal volume, heart rate and mean systemic blood pressure were recorded on light sensitive paper.

After control measurements were taken, cooling coils were wrapped around both vagal loops. Cooling coils were made from copper piping (I.D. 4 mm, O.D. 5 mm) and consisted of two parts shaped to fit the vagal loop (Fig. 2). The coils were attached via tubing to a circulating cooler[h] containing methanol. The temperature of this fluid was maintained at -2°C ($\pm$ .2°C). Measurements of respiratory rate, tidal volume, heart rate and mean systemic blood pressure were repeated while the vagi were cooled, five minutes after removal of the coils and after administration of 0.04 mg/kg Atropine I/V. In addition, a laryngoscopic examination was performed on three ponies before, during and after vagal cooling. Results were analyzed using two-way analysis of variance. Means were compared by the Student Newman Keul's test.[4]

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[c] Model PM5, Statham Instruments, Hato-Rey, PR
[d] Model VR6, Electronics for Medicine, White Plains, NJ
[e] Hamilton Syringe Co., Whittier, CA
[f] Becton-Dickinson, Rutherford, NJ
[g] Model P23DB, Statham Instruments, Hato-Rey, PR
[h] Model 90, Fisher Scientific Co., Livonia, MI

Results

Cooling of the vagus nerve increased tidal volume, heart rate and mean systemic blood pressure and decreased respiratory rate (Fig 2-4). Control values were not significantly different before and after vagal cooling. Atropine administration did not alter the respiratory parameters but increased heart rate and mean systemic pressure to levels similar to those measured during vagal cooling (Fig 2-4). Laryngoscopic examination revealed complete bilateral laryngeal paresis during vagal blockade and normal laryngeal mobility during the control periods.

Discussion

The technique we have described resulted in the incorporation of a functional vagus nerve in a skin loop. The principal problem encountered was a tendency for skin wounds to dehisce beneath the vagal loop because of excessive tension. The tension relieving incision on the ventral midline of the neck successfully alleviated this problem whenever it was used. The functional integrity of the vagus nerve was indicated by the changes in respiratory rate, tidal volume, heart rate and blood pressure following vagal cooling and the return of these parameters to control levels when the vagi were warmed.

Great care was taken not to incorporate the recurrent laryngeal nerve into the skin loop nor to excessively traumatize this nerve during separation from the vagus nerve. Although several ponies exhibited transient laryngeal hemiplegia, all returned to normal. Because of this transient hemiplegia, bilateral loops were never simultaneously created.

The sympathetic trunk was also isolated from the vagus during surgery. Its functional integrity was indicated by the absence of

Figure 2-4 Respiratory rate (RR), tidal volume (VT), heart rate (HR), and systemic blood pressure (P_{syst}) during a base-line period, after vagal cooling, during a 2nd base-line period, and after IV administration of 0.04 mg of atropine/kg of body weight ($\bar{x} \pm$ SEM). *Indicates significant difference from control value.

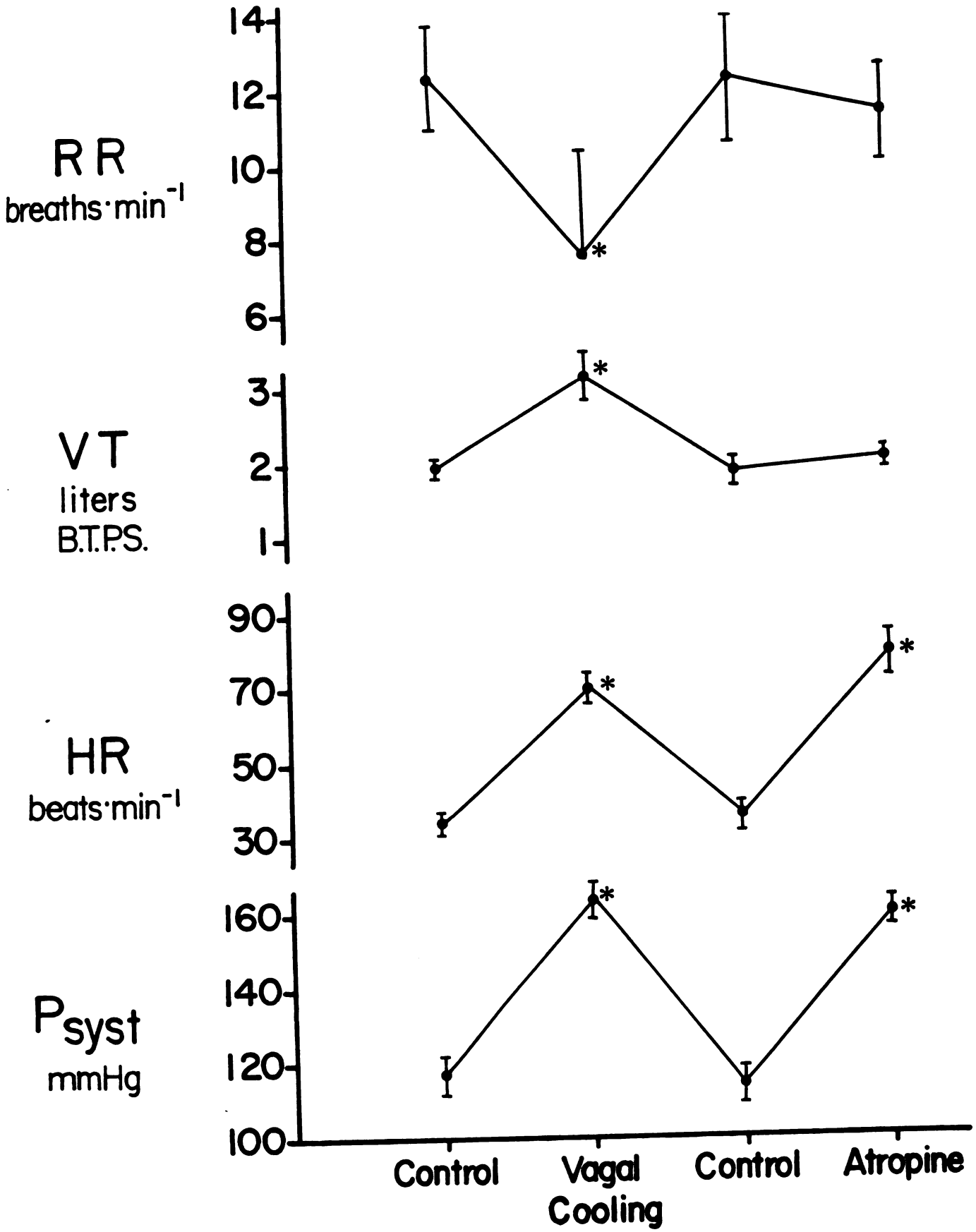
RR
breaths·min⁻¹
14
12
10
8
6
*
VT
liters
B.T.P.S.
3
2
1
*
HR
beats·min⁻¹
90
70
50
30
*
*
Psyst
mmHg
160
140
120
100
*
*
Control
Vagal
Cooling
Control
Atropine

Figure 2-4

Horner's syndrome following surgery or during blockade. Horner's syndrome is usually observed in horses following damage of the sympathetic trunk.[5,6]

Results of this experiment indicate that cooling of vagal loops caused reversible blockade of both afferent and efferent vagal nerve fibers in the standing conscious pony. The increased tidal volume and decreased respiratory rate observed after vagal cooling are also reported in dogs after vagotomy and are attributed to stretch receptor fiber blockade and subsequent interruption of the Hering-Breuer reflex.[2,7,8] In addition, ponies exhibited increased heart rate and mean systemic blood pressure after vagal blockade. Similar changes in dogs are attributed to blockade of cardiac efferent preganglionic parasympathetic fibers.[7,8,9,10] The increased heart rate is thought to increase cardiac output and therefore increase mean systemic pressure.[9] Since heart rate and systemic pressure were not different during vagal cooling and following atropine, it appears that efferent cardiac preganglionic parasympathetic fiber blockade was complete during vagal cooling.

Laryngoscopy of three ponies during vagal cooling revealed complete bilateral laryngeal paresis. Since the recurrent laryngeal nerves were not included in the skin loops, the laryngeal paresis probably resulted from blockade of vagal fibers that subsequently form the recurrent laryngeal nerve.[11] In two ponies, the endotracheal tube was removed during vagal blockade and the tracheostomy opening occluded. Animals breathed normally until a deep breath was taken. At that time, the larynx collapsed and animals became extremely dyspneic until the endotracheal tube was reinserted. A tracheostomy is therefore essential to insure a patent airway when blocking both vagi simultaneously.

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CHAPTER 3

Pulmonary Function Tests in Standing Ponies: Reproducibility and Effect of Vagal Blockade

Introduction

Although pulmonary function tests have been used to evaluate horses with clinically normal lungs and those with chronic lung disease, few comprehensive studies of equine respiratory function are presently available and the range of reported normal values is large.[1-6] This may be due to differences in techniques used by the various investigators or because of real variation in values. Information about the repeatability of pulmonary function tests in individual horses and groups of horses is therefore necessary to resolve this question.

Clinical evidence suggests that the parasympathetic nervous system plays a role in the pathogenesis of chronic obstructive pulmonary disease, as many cases respond to atropine administration.[7] Similarly vagal mechanisms play a role in pathogenesis of allergic lung disease in other species.[8-10] In order to study vagal mechanisms in disease, the role of the vagus nerve in control of pulmonary function in healthy animals must first be established. Presently this information is not available for the equid.

The purpose of this investigation was to assess the repeatability of pulmonary function measurements within a day and over a six-month period, to determine the effect of changes in lung volume on total respiratory resistance, to evaluate the effect of respiratory frequency on dynamic compliance, and to study the effect of vagal blockade on pulmonary mechanics, lung volumes and gas exchange.

Materials and Methods

Five ponies between two and ten years of age ($\bar{x}$ = 6.6 years) weighing 199 $\pm$ 27.0 kg ($\bar{x} \pm$ SEM) with bilateral cervical vagal loops and

exteriorized carotid arteries were used in the experiments.[11] Prior to use, animals had been on pasture for at least two months and all were vaccinated for the common viral respiratory diseases. Animals were regularly examined to detect any signs of respiratory disease.

Pulmonary Function Measurements

Ponies were tranquilized with xylazine[a] (0.5 mg/kg) and restrained in stocks. A 20 mm diameter cuffed endotracheal tube was introduced into the trachea via a tracheostoma. A Fleisch pneumotachograph (no4)[b] and associated pressure transducer[c] were attached to the endotracheal tube. The pneumotachograph transducer system produced a signal proportional to flow which was electronically integrated to give tidal volume. After each experiment, this system was calibrated by forcing known volumes and flows of air through the pneumotachograph using a three liter calibrated syringe[d] and a rotameter flow meter.[e]

An esophageal balloon (length 10 cm, perimeter 3.5 cm, wall thickness 0.06 cm) was sealed over the end of a polyethylene catheter (ID = 3 mm, O.D. = 4.4 mm, length 140 cm) which had a number of spirally arranged holes in the part covered by the balloon. The distance from the nares to the middle portion of the thoracic esophagus was visually approximated and marked on the esophageal balloon catheter with indelible ink. The esophageal balloon catheter was made rigid by introducing a length of 18 gauge steel wire and passed via the nares into the

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[a] Rompun, Haver Lockhart, Shawnee, Mission, KS
[b] Dynasciences, Blue Bell, PA
[c] Model PM5, Statham Instruments, Hato Rey, PR
[d] 3 liter Super Syringe, Warren E. Collins Inc, Braintree, MA
[e] Model 10A3500, Fisher & Porter Co, Warminster, PN

middle portion of the thoracic esophagus. The wire was removed and the balloon attached to a pressure transducer[f] which was taped to the forelock. The opposite side of the differential pressure transducer was attached to an identical balloon catheter system, with the balloon located just inside the distal end of the endotracheal tube to measure airway opening pressure (P_{ao}). Transpulmonary pressure (P_{tp}) was defined as the pressure difference between the airway opening pressure (P_{ao}) and esophageal pressure (P_{es}). Balloon volumes were adjusted to contain 0.5 ml of air. Transpulmonary pressure, tidal volume (V_T) and flow were recorded on light sensitive paper.[g] From these traces, dynamic compliance (C_{dyn}), respiratory rate (RR) and minute ventilation ($\dot{V}_{min}$) were calculated.[12]

A pressure cycled ventilator[h] was attached to the endotracheal tube via the pneumotachograph. Animals were force ventilated to 20 cm H_2O P_{tp} for two breaths to insure constant lung volume history prior to recording quasistatic pressure volume curves. Quasistatic pressure volume curves of lung and chest wall were generated by inflating the respiratory system to P_{tp} = 20 cm H_2O and allowing it to deflate slowly to functional residual capacity (FRC). To minimize flow resistive forces, rate of deflation was slowed by a retard valve on the expiratory line of the ventilator. Lung and thoracic cage pressure volume curves were recorded by plotting P_{tp} and P_{es} respectively against lung volume on an x-y plotter[i] during at least two quasistatic pressure volume

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[f] Model PM 131 TC, Statham Instruments, Hato Rey, PR
[g] Model VR6, Electronics for Medicine, White Plains, NY
[h] Mark 9, Bird Co, Palm Springs, CA
[i] Model XY575, Esterline Angus Co, Indianapolis, IN

maneuvers. Using a digital computer[j] the deflation limb of the lung pressure volume curves was empirically described as a single rising exponential.[13]

$$V = V_{max} (1-e^{-\alpha P_{tp}}) \quad (1)$$

where V = lung volume at a given transpulmonary pressure (P_{tp}), V_{max} is the lung volume at which the slope of the curve is zero (i.e., at infinite P_{tp}) and α defines the rate of rise of the curve from FRC to the V_{max}. Quasistatic compliance (C_{stat}) was calculated from the first derivative of equation #1 at P_{tp} = 3 cm H_2O.

Subsequently, animals were force ventilated four times up to a transpulmonary pressure of 20 cm H_2O to create a period of apnea, lasting between 10 and 30 sec. During this period of apnea, an oscillation system consisting of a sine wave generator,[k] an amplifier and a 12" speaker in box (Fig 3-1), was attached to the endotracheal tube via the pneumotachograph. Pressure and flow were recorded on an oscilloscope as sinusoidal flow oscillations were applied to the lung via the endotracheal tube. Oscillation frequency was modulated until the pressure flow loop closed, usually between 5 and 10 Hz. The closed pressure flow loop was recorded on light sensitive paper and total respiratory resistance was calculated as the slope of the line. At least two recordings were made for each measurement.

In order to prevent phase differences between pressure and flows, frequency responses of catheter systems were carefully evaluated as previously described.[14] In addition, the airway opening pressure signal and flow signal used to measure oscillatory resistance were evaluated up to

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[j] Model PDP11, Digital Equipment Co, Maynard, MA
[k] Model 200, Continental Specialties Co, New Haven, CT

Figure 3-1 Forced oscillation system used to measure total respiratory resistance.

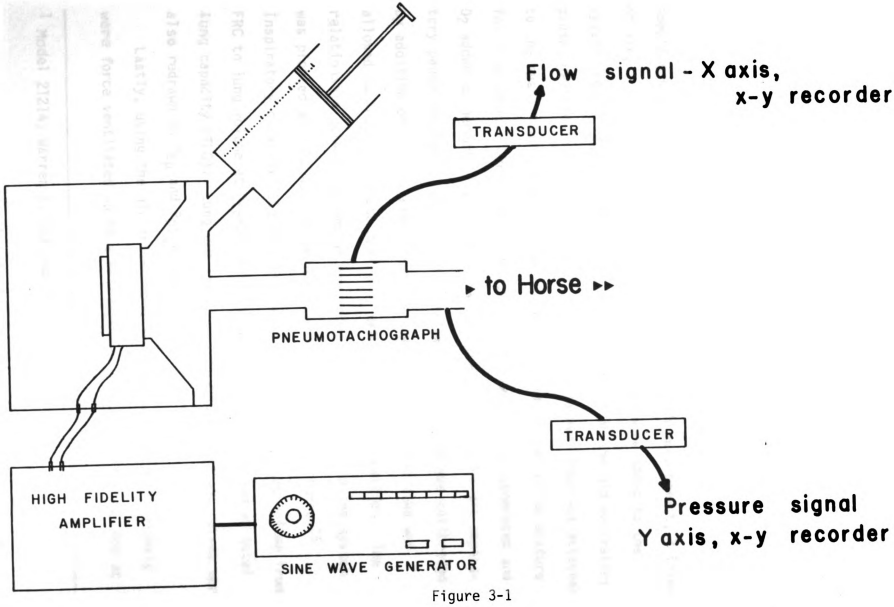
Flow signal - X axis,
x-y recorder
TRANSDUCER
to Horse
PNEUMOTACHOGRAPH
TRANSDUCER
Pressure signal
Y axis, x-y recorder
HIGH FIDELITY
AMPLIFIER
SINE WAVE GENERATOR

Figure 3-1

a frequency of 20 Hz, and up to this frequency, these signals were in phase.

Functional residual capacity (FRC) was measured using a closed system helium equilibration technique. The system consisted of a 120 liter Tissot spirometer, a sodalime CO_2 absorber and an in-line fan used to ensure gas mixing (Fig 3-2). Gas was sampled continuously through a helium analyzer and returned to the system. Percent helium in the sample gas was recorded continuously.[1] Before each measurement, 3 liters of air, 3 liters of helium and 3 liters of oxygen were added to the system, resulting in 10.5% He in the spirometer. At the mid expiratory pause, defined in a previous paper,[14] the endotracheal tube was attached to the system and the animal was allowed to breathe the helium mixture for 10 minutes. During this period, CO_2 was absorbed as generated and O_2 added as needed to keep the volume of the system at the mid expiratory pause constant. After each experiment, the system was calibrated by addition of air in three liter increments. Sufficient time was allowed to insure complete mixing of gases after each addition. The relationship between volume of air added and percent He in the system was plotted and FRC was determined from this calibration curve.[15] Inspiratory capacity (IC) was defined as the change in lung volume from FRC to lung volume at P_{tp}=30 cm H_2O and FRC + IC was defined as total lung capacity (TLC). Lung and thoracic cage pressure-volume curves were also redrawn as P_{tp} and P_{pl} versus %TLC.

Lastly, using the air driven pressure cycled ventilator, animals were force ventilated up to a transpulmonary pressure of 10 cm H_2O at

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[1] Model 21214, Warren E. Collins Inc, Braintree, MA

Figure 3-2 Helium dilution system, used to measure functional residual capacity.

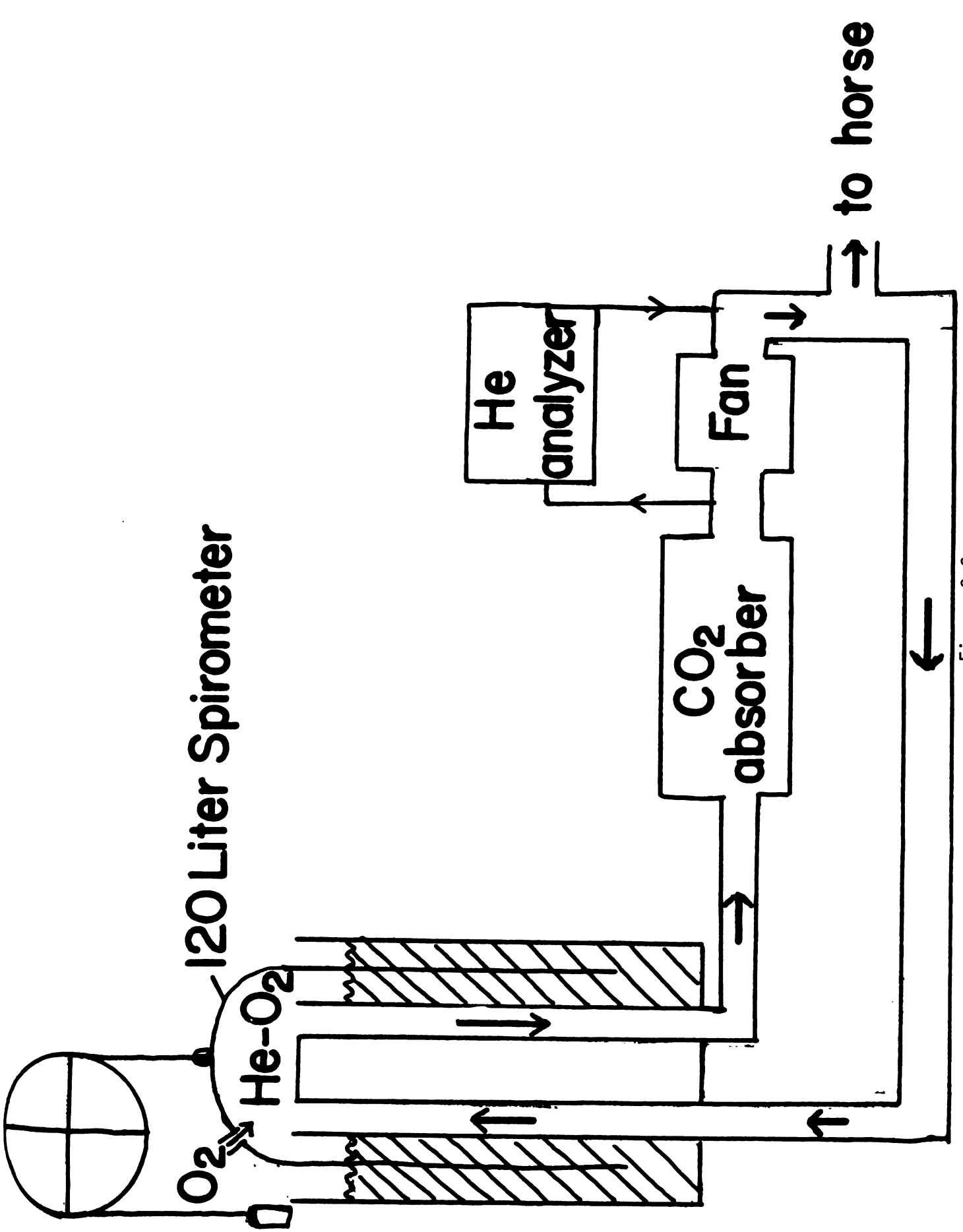
120 Liter Spirometer
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Figure 3-2

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frequencies ranging from 15 to 60 min^{-1}. Transpulmonary pressure, tidal volume and flow were recorded, and dynamic compliance was calculated over this range of frequencies.

Reproducibility of Measurements

Arterial blood gas tensions, pulmonary mechanics and lung volumes were measured in 4 animals every hour for 6 hours and in 5 animals 4 times at 2 monthly intervals to assess the short and long-term reproducibility of pulmonary function measurements. On one occasion we did not record quasistatic pressure volume curves.

Vagal Blockade

After baseline values of arterial blood gas tensions, pulmonary mechanics and lung volumes were determined in 5 animals, the cervical vagus nerves were blocked by circulating coolant[m] at a temperature of -2°C through copper cooling coils wrapped around both loops. In an earlier study on the same ponies we established criteria for bilateral cervical vagal blockade: tachycardia, slow deep breathing and paresis of the cricoarytenoideus dorsalis m., as determined through the endoscope by failure of the arytenoid cartilages to abduct during inhalation.[11] Measurements were repeated while the vagi were blocked and 10 minutes after removal of the coils, when heart rate, respiratory rate, tidal volume and activity of the cricoarytenoideus dorsalis muscle had returned to baseline values. Results were analyzed using two-way analysis of variance in a randomized complete block design.

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[m] Model 90, Fisher Scientific Co, Niles, IL

Significance was set at $P < 0.05$.[16]

The effect of changes in lung volume on total respiratory system resistance was studied before and after vagal blockade. After total respiratory system resistance was determined at FRC, 8 liters of air was added to the lungs in 2 liter increments. Pressure-flow relationships were recorded at the resonant frequency of the respiratory system after each addition of air. The effect of vagal blockage and changing lung volumes on R_{tot} was analyzed using a 2 x 5 factorial analysis.[16]

Results

Tables 3-1 and 3-2 show the mean and standard deviation of pulmonary function measurements in individual ponies. Table 3-1 shows the values obtained within a day, while Table 3-2 depicts values obtained over a period of several months. Examination of the coefficients of variability shows that variability in blood gas tensions and total lung capacity was small over both the short and long term. In contrast the variability in respiratory resistance and FRC was small over the short term but large over the long term. Tidal volume, minute ventilation, respiratory rate, dynamic and quasistatic compliance showed considerable variability over both the short and the long term.

Table 3-3 shows mean values of pulmonary function variables from 5 ponies measured at two month intervals. There was no significant change in any measured or calculated variable over the six month period. (table 3-2)

Composite expiratory limbs of the lung and thoracic cage pressure-volume curves are shown in figure 3-3. The broken line indicates the fit of the lung pressure-volume data to a single rising exponential

Figure 3-3 Composite expiratory limbs of thoracic cage (T) and lung (L) pressure-volume curves. The dotted line is the best fit to a single rising exponential. Functional residual capacity ($\bar{x} \pm$ SEM) measured by a helium dilution technique is also shown. TLC = total lung capacity; FRC = functional residual capacity; P_{tp} = transpulmonary pressure; P_{pl} = pleural pressure.

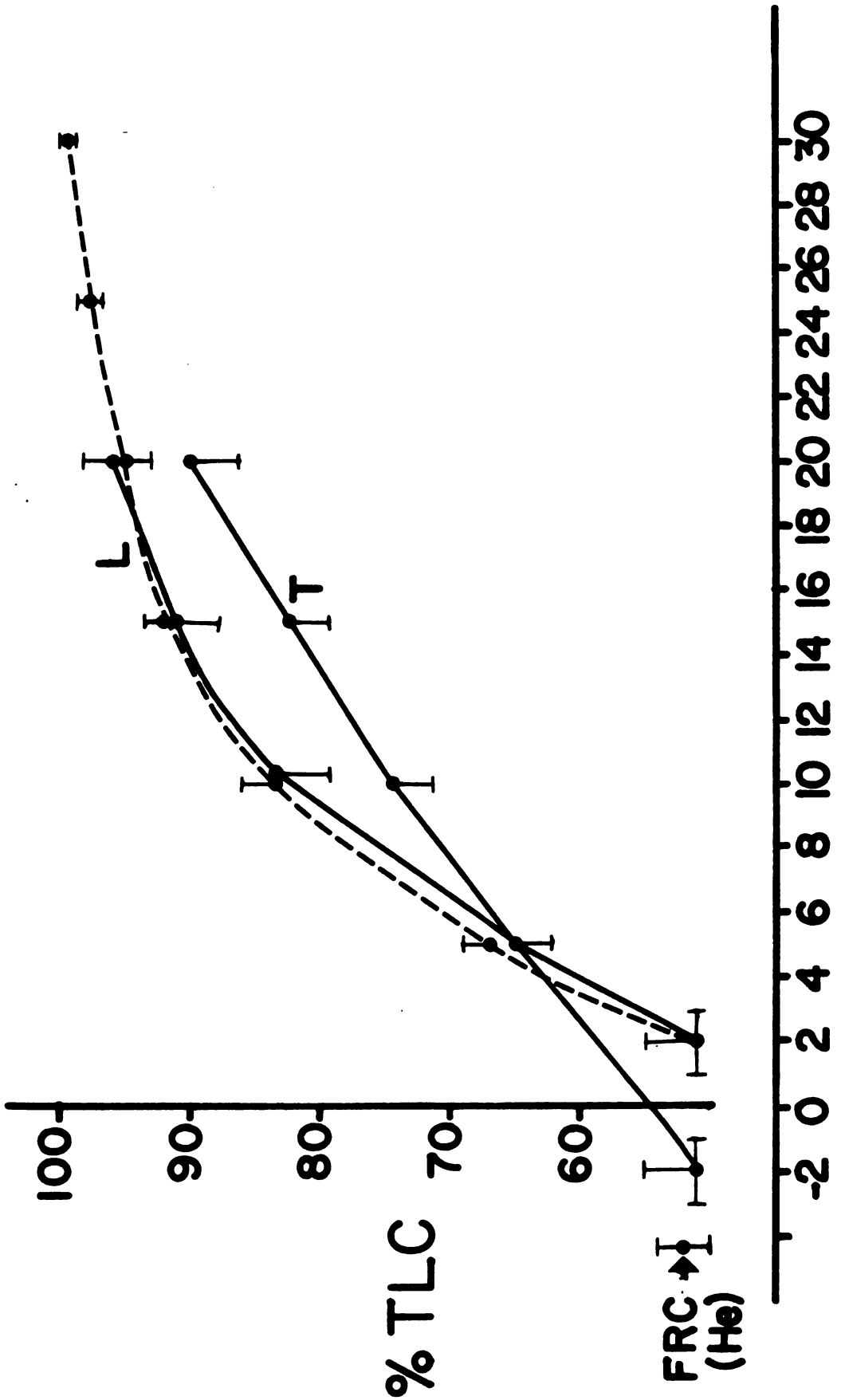
100
90
80
70
60
% TLC
FRC (He)
L
T
-2 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Ptp or Ppl
cmH2O

Figure 3-3

Figure 3-4 Total respiratory system resistance (R_{tot}) measured at increasing lung volumes, before and after vagal blockade. VB = vagal blockade; TLC = total lung capacity.

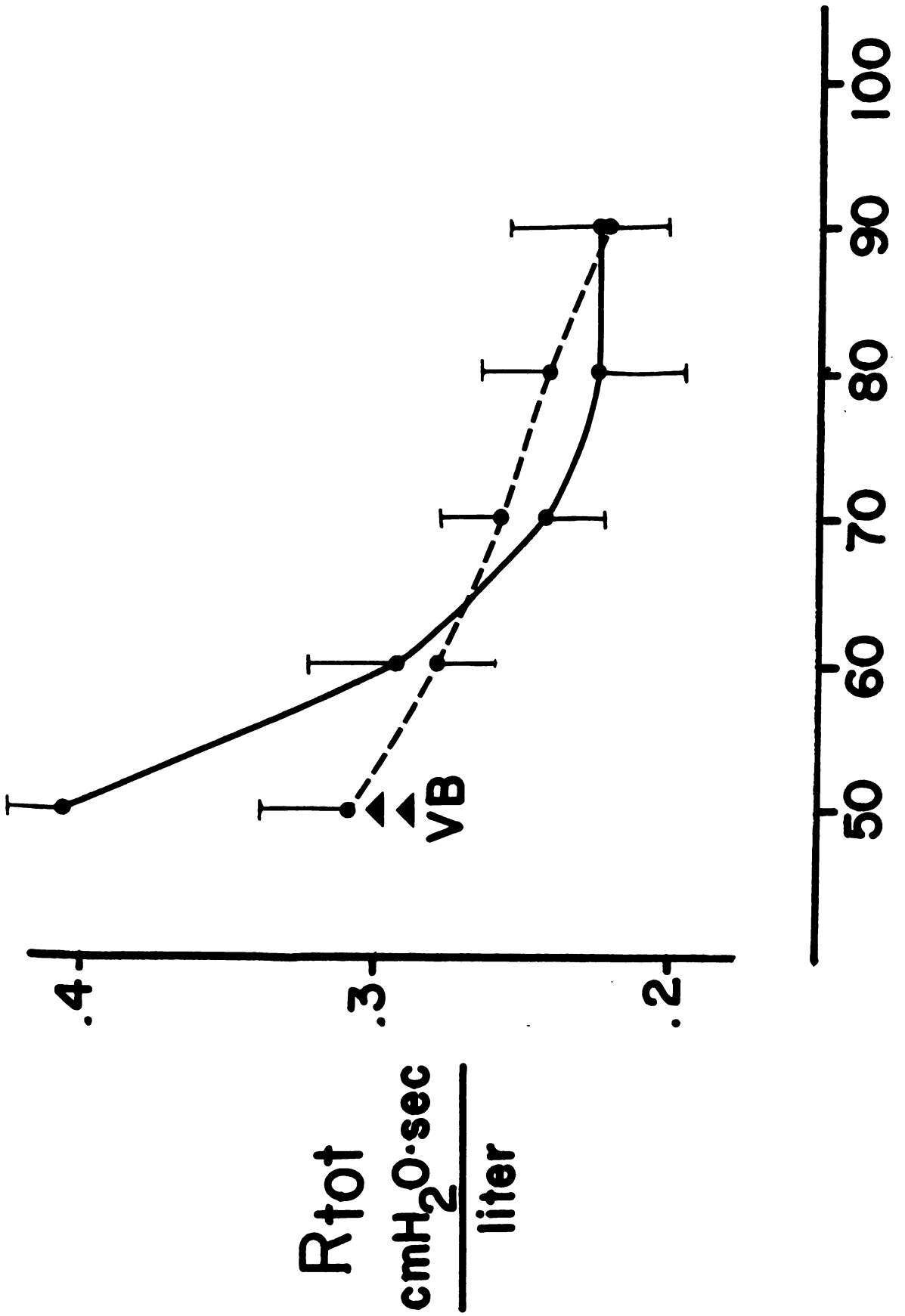

Figure 3-4

Table 3-1 Pulmonary function values (mean and standard deviation) derived from 6 studies repeated at hourly intervals.

Measurement	Pony #1		Pony #2		Pony #3		Pony #4		
	$\bar{x}$	Sx	$\bar{x}$	Sx	$\bar{x}$	Sx	$\bar{x}$	Sx	CV
Body Weight (kg)	143.0		181.8		204.5		250.0		
PaO_2 (Torr)	82.9	1.66	83.1	8.4	85.8	6.2	82.9	8.1	7.3
$PaCO_2$ (Torr)	41.2	0.62	38.0	2.8	41.6	1.5	42.1	4.5	5.6
RR (min^{-1})	14.6	2.6	12.7	1.4	13.7	1.3	16.5	2.0	12.5
V_T (L btps)	1.50	0.23	2.5	0.17	2.3	0.22	1.8	0.25	11.2
$\dot{V}_{min}$ (L min^{-1})	22.0	4.7	31.6	3.8	31.6	3.2	29.6	1.5	12.1
R_{tot} (cm H_2O sec L^{-1})	0.397	0.059	0.430	0.029	0.359	0.013	0.428	0.039	8.6
C_{dyn} (L cm H_2O^{-1})	0.568	0.092	0.754	0.177	0.894	0.100	0.901	0.135	16.4
C_{stat} (L cm H_2O^{-1})	0.922	0.302	1.335	0.289	1.210	0.213	1.896	0.240	21.5
FRC (L btps)	7.3	0.62	8.6	0.57	12.6	0.4	8.6	0.42	5.8
TLC (L btps)	14.9	2.4	19.2	1.59	26.3	1.3	19.9	1.3	8.9
FRC/TLC	0.49	0.06	0.45	0.05	0.48	0.02	0.43	0.03	8.2

RR = respiratory rate; V_T = tidal volume; V_{min} = minute ventilation
R_{tot} = total respiratory system resistance; C_{dyn} = dynamic compliance
C_{stat} = quasistatic compliance; FRC = functional residual capacity
TLC = total lung capacity; CV = coefficient of variation

Table 3-2 Pulmonary function values (mean and standard deviation) derived from at least 3 studies conducted at two-month intervals.

Measurement	Pony #1		Pony #2		Pony #3		Pony #4		Pony #5		
	$\bar{x}$	Sx	$\bar{x}$	Sx	$\bar{x}$	Sx	$\bar{x}$	Sx	$\bar{x}$	Sx	CV
Body Weight (kg)	136.0	17.0	168.1	11.7	204.0	0.0	247.0	14.5	272.0	45.0	4.2
PaO_2 (Torr)	86.8	6.3	84.2	15.6	85.1	7.3	85.9	7.3	87.1	2.3	9.2
$PaCO_2$ (Torr)	41.0	1.7	37.5	1.0	41.3	1.4	42.1	2.1	40.7	1.0	3.4
RR (min^{-1})	18.0	1.2	11.2	3.8	10.5	2.3	12.7	2.2	10.7	1.5	19.3
V_T (L btps)	1.6	0.18	1.9	0.22	2.0	0.37	2.1	0.51	2.2	0.99	24.0
$\dot{V}_{min}$ (L min^{-1})	28.7	2.7	21.1	6.8	21.0	6.9	25.7	5.9	24.0	11.5	29.1
R_{tot} (cm H_2O sec L^{-1})	0.456	0.155	0.480	0.087	0.329	0.084	0.503	0.119	0.474	0.080	23.8
C_{dyn} (L cm H_2O^{-1})	0.793	0.261	0.673	0.236	0.783	0.292	0.756	0.279	0.864	0.455	39.0
C_{stat} (L cm H_2O^{-1})	1.008	0.261	1.059	0.330	1.413	0.424	1.666	0.584	1.390	0.261	28.0
FRC (L btps)	9.1	1.6	10.7	2.5	11.3	2.7	10.0	2.2	11.0	2.3	21.6
TLC (L btps)	16.3	1.9	18.8	3.4	25.5	3.0	20.0	3.2	21.0	3.8	13.2
FRC/TLC	0.55	0.08	0.56	0.05	0.44	0.06	0.50	0.07	0.52	0.03	11.0

RR = respiratory rate; V_T = tidal volume; V_{min} = minute ventilation
R_{tot} = total respiratory system resistance; C_{dyn} = dynamic compliance
C_{stat} = quasistatic compliance; FRC = functional residual capacity
TLC = total lung capacity; CV = coefficient of variation

Table 3-3 Pulmonary function values ($\bar{x} \pm$ SEM) derived from 4 studies conducted five ponies, conducted at two-month intervals.

Measurement	Time 1	Time 2	Time 3	Time 4
	$\bar{x} \pm$ SEM	$\bar{x} \pm$ SEM	$\bar{x} \pm$ SEM	$\bar{x} \pm$ SEM
Body Weight (kg)	199 ± 27.0	205 ± 25.0	208 ± 24.0	210 ± 23.3
PaO_2 (Torr)	84.1 ± 2.7	86.3 ± 6.0	85.1 ± 2.1	88.0 ± 4.2
$PaCO_2$ (Torr)	40.0 ± 1.0	40.9 ± 0.6	40.8 ± 1.4	40.5 ± 0.7
RR (min^{-1})	11.6 ± 1.6	12.4 ± 1.9	12.6 ± 2.0	14.7 ± 1.0
V_T (L btps)	2.0 ± 0.21	1.83 ± 0.10	1.98 ± 0.36	2.0 ± 0.20
$\dot{V}_{min}$ (L min^{-1})	22.3 ± 3.0	22.2 ± 2.7	24.0 ± 4.3	28.9 ± 1.1
R_{tot} (cm H_2O sec L^{-1})	0.496 ± 0.057	0.345 ± 0.092	0.496 ± 0.054	0.458 ± 0.035
C_{dyn} (L cm H_2O^{-1})	0.670 ± 0.103	0.788 ± 0.181	0.817 ± 0.097	0.808 ± 0.135
C_{stat} (L cm H_2O^{-1})	ND	1.429 ± 0.259	1.249 ± 0.171	1.244 ± 0.138
FRC (L btps)	10.7 ± 0.89	9.3 ± 0.74	11.8 ± 0.9	9.9 ± 1.28
TLC (L btps)	ND	20.8 ± 2.0	21.1 ± 1.85	19.1 ± 1.9
FRC/TLC	ND	0.45 (0.03)	0.55 (0.03)	0.52 (0.03)

RR = respiratory rate; V_T = tidal volume; $\dot{V}_{min}$ = minute ventilation
R_{tot} = total respiratory system resistance; C_{dyn} = dynamic compliance
C_{stat} = quasistatic compliance; FRC = functional residual capacity
TLC = total lung capacity

defined by equation 1. Alpha averaged 0.1594 $\pm$ 0.0135 cm H_2O^{-1}. The volume at which thoracic cage and lung elastic recoil were equal and opposite was not significantly different from FRC measured by He equilibration.

Vagal blockade increased V_T and decreased RR. In addition respiratory resistance at functional residual capacity was decreased by vagal blockade from 0.496 $\pm$ 0.054 to 0.361 $\pm$ 0.03 cm H_2O sec liter^{-1} but arterial blood gas tensions, $\dot{V}_{min}$, C_{dyn}, C_{stat} FRC, TLC and lung and thoracic cage pressure-volume curves were unaffected. Baseline values of all variables were the same before and after vagal blockade.

Figure 3-4 shows the effect of vagal blockade and lung volume on R_{tot}. Resistance decreased significantly with increasing lung volume. Vagal blockade significantly decreased R_{tot} at FRC but was without effect at higher lung volumes.

Dynamic compliance did not change as respiratory frequency increased from 15-60 breaths/minute.

Discussion

This study has documented the daily and monthly variability of pulmonary function measurements in standing conscious ponies. Variability of TLC and arterial blood gas tensions was small over both the short and long-term measurement periods. This finding is not surprising. Total lung capacity is a fixed volume probably defined by the elastic limits of the lung. In the case of arterial blood gases, respiratory control mechanisms maintain these values within fairly tight limits to ensure adequate gas transport to and from the tissues.

The variability in C_{stat} was surprising since the elastic properties

of the lung would appear to be determined by lung structure. However, airway closure also affects the shape of the pressure-volume curve and may have been responsible for some of the variability in C_{stat} even though we attempted to eliminate this possibility by inflating the lung to P_{tp}=20 cm H_2O prior to recording the lung pressure-volume curve.

Variability of FRC, R_{tot}, C_{dyn}, $\dot{V}_{min}$, V_T, and RR was considerable over the long term. The variability in FRC may be the result of variations in posture, respiratory muscle tone and changes in abdominal filling caused by alterations in diet and fat deposition. As shown in Fig. 3-4, changes in lung volume result in changes in R_{tot} and it is possible that the variability in FRC was responsible in part for the variability in resistance.

Since C_{dyn} is determined by both lung elastic recoil (indicated by C_{stat}) and the resistance of airways (indicated by R_{tot}) and since both C_{stat} and R_{tot} were quite variable, the variability in C_{dyn} is not surprising.[17] Furthermore, calculation of C_{dyn} assumes inertial forces are negligible during tidal breathing.[18] This assumption may not be valid in the horse as there are rapid rates of change of flow particularly between inhalation and exhalation.

The variability in $\dot{V}_{min}$, V_T, and RR was not surprising since $\dot{V}_{min}$ is determined in part by metabolism and the possible combinations of RR and V_T for a given $\dot{V}_{min}$ are limitless.

With the exception of the variability in C_{dyn}, variability of our measurements was similar to that reported in conscious calves and dogs studied at daily and monthly intervals, respectively.[19,20] When data from five horses was grouped there was no significant change in any variable over the six-month study period. These data suggest that with

the exception of arterial blood gas tensions, the results of pulmonary function tests described in this paper are too variable to be useful in detecting individual horses with mild or moderate lung disease but may be useful in assessing the effects of treatments on lung function in a group of horses studied over a period of days or months.

We have previously reported a midexpiratory cessation of air flow in tracheostomized ponies.[14] This midexpiratory pause appears to occur at a relatively constant lung volume whereas lung volume at end expiration varies with the amount of abdominal expiratory effort. Leith[n] has suggested that the midexpiratory pause represents the equilibrium point of the respiratory system where lung and thoracic cage recoil are equal and opposite. Examination of lung and thoracic cage pressure-volume curves (Fig 3-3) shows that in our ponies, equilibrium volume was not significantly different from lung volume at the midexpiratory pause suggesting this volume is determined by passive relaxation of the respiratory system. However this conclusion must be tempered with caution because we did not ascertain that the respiratory muscles were relaxed although we did provide two deep breaths to induce apnea prior to recording pressure-volume curves.

Salazar et al showed empirically that the expiratory limb of the dog quasistatic pressure-volume curve can be described by a single rising exponential.[13] Our data suggest that this is also true in ponies. Alpha (the parameter describing the rate of rise of the expiratory limb of the pressure-volume curve) was similar to the value calculated from data obtained in anesthetized horses suspended upright.[21,22]

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[n] Leith DE, Personal communications, 1981

Static compliance calculated from the first derivative of the single rising exponential at P_{tp}=3 cm H_2O was also similar on a body weight basis to values reported in anesthetized upright horses but greater than values in anesthetized ponies in which there may have been considerable airway closure.[o,3,4]

The effects of vagal blockade in ponies are similar to effects in dogs.[23,24] Tidal volume increased and respiratory rate decreased probably as a result of blockade of vagal afferents from pulmonary receptors. Vagal blockade had no effect on lung and thoracic cage pressure-volume behavior. The primary effect of vagal blockade was a decrease in respiratory resistance at FRC but not at higher lung volumes. This interaction of parasympathetic tone and lung volume in determining resistance was also reported in dogs by Macklem et al who proposed the following explanation based on pressure diameter behavior of airways with and without bronchomotor tone.[25] Isolated bronchi lacking bronchomotor tone increase maximally in diameter with only small changes in transmural pressure (+ 3 cm H_2O) whereas intact bronchi with parasympathetic tone increase in diameter progressively as transmural pressure increases to 30 cm H_2O. In the ponies with vagal tone, resistance therefore decreases progressively with increasing lung volume. In contrast following vagal blockade airways are probably almost maximally dilated at FRC and increasing lung volume causes only a slight decrease in resistance.

Frequency dependence of lung compliance results when there is inequality of time constants in peripheral parallel units in the lung and

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o Leith DE, Gillespie JR: Respiratory mechanics of normal horses and one with chronic obstructive lung disease. Fed Proc 30:556, 1971.

for this reason is suggested as an indicator of peripheral airway obstruction.[26] The lack of frequency dependence in our normal ponies suggests equality of time constants and a lack of peripheral airway obstruction. However, Macklem et al calculated that a five-fold variation in time constant would cause only a 25% reduction in C_{dyn} at a respiratory frequency of 60 breaths/minute and a two-fold difference in time constants would not be detectable.[25] Thus considerable variability in peripheral time constants may exist in our normal ponies despite the lack of frequency dependent compliance. Even with these limitations frequency dependence of compliance is still one of the most sensitive tests of small airway obstruction in persons and may prove to be a valuable test in the detection of small airway disease in the horse.[26]

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CHAPTER 4

3-methylindole Induced Pulmonary Toxicosis in the Horse

Introduction

Horses commonly suffer from chronic obstructive pulmonary disease, the etiology of which is unknown but hypersensitivity to molds, viral and bacterial infections, and dietary factors have been incriminated.[1-5] Recently, 3-methylindole (3MI) has been suggested as a possible etiologic agent of the disease syndrome in horses.[6] This compound is a metabolite of the amino acid L-tryptophan and is found in the feces of mammals as well as in tobacco smoke.[7] Oral administration of 3MI to horses results in dyspnea, tachypnea and impaired gas exchange, most evident 7 days post treatment but to date there are only preliminary reports on the pathologic and physiologic changes induced by 3MI.[6]

There is clinical evidence to suggest that the parasympathetic nervous system plays a role in the pathogenesis of chronic obstructive pulmonary disease in the horse, because many cases respond to atropine administration.[8] The parasympathetic nervous system also plays a role in the pathogenesis of experimentally induced airway diseases in dogs, guinea pigs, and rabbits and some forms of asthma in man.[9-13] I therefore wondered if vagal reflexes were also involved in the pathogenesis of 3MI induced pulmonary toxicosis. The purpose of this paper is to report changes in pulmonary function occurring in the early stages of 3MI induced pulmonary toxicosis, to correlate functional changes with pathologic lesions and to determine the role of vagal reflexes in the mechanism of disease.

Materials and Methods

Ten ponies between 2 years and 15 years of age ($\bar{x}$ = 8.5 years) weighing 167.9 $\pm$ 8.7 kg ($x \pm$ SEM) were used in the experiments. Animals had been on pasture for the previous 2 months and all were vaccinated for the common viral respiratory diseases. Animals were regularly observed during this period, to detect any signs of respiratory disease.

Surgical Preparation

Horses were prepared for experiments by surgically exposing the vagus nerves, cannulating a carotid artery, and performing a tracheostomy. Anesthesia was induced with intravenous sodium thiamylal (10 mg/kg BW) and maintained with inhalation anesthesia using halothane. With the pony in left lateral recumbency the right cervical region was prepared for aseptic surgery. A 10 cm linear skin incision was made just dorsal to the jugular vein in the mid cervical region. The vagus nerve was exposed and a 1 cm section dissected free. A silk suture was looped around the nerve and tied loosely. The wound was closed in a routine manner, allowing the ends of the silk suture to exit through the skin. The same procedure was repeated on the right side of the neck and in addition a 1.19 ID, 1.70 OD (PE190) polyethylene catheter was placed in the right carotid artery and allowed to exit through the skin at a site distant from the incision. Lastly a ventral midline tracheostomy was performed in the mid cervical region. Animals were allowed to recover for 24 hours. The carotid catheter was flushed every 4 hours with 2 ml of heparinized saline.

Methods of Pulmonary Function Testing

Twenty-four hours after surgical preparation, animals were tranquilized with intravenous xylazine[a] (0.5 mg/kg of body weight) and restrained in stocks. The methods of pulmonary function measurement have been previously described.[14] Briefly, air flow ($\dot{V}$) and tidal volume (V_T), measured using a pneumotachograph[b] transducer system[c] attached to a cuffed endotracheal tube and inserted into the trachea via a tracheostoma, were recorded on light sensitive paper.[d] Transpulmonary pressure (P_{tp}) was measured as the pressure difference between the mid portion of the thoracic esophagus and the airway opening, using identical catheter systems. From the recording of P_{tp}, $\dot{V}$ and V_T, dynamic compliance (C_{dyn}), respiratory rate (RR) and minute ventilation ($\dot{V}_{min}$) were calculated.

Quasistatic pressure-volume curves were generated on an x-y plotter,[e] using an air driver pressure cycled ventilator.[f] The deflation limb of the quasistatic pressure-volume curve was empirically described as a single rising exponential, using a digital computer.[g,15]

$$V = V_{max} (1-e^{-\alpha P_{tp}}) \quad (1)$$

Where V = lung volume at a given P_{tp}, V_{max} is the volume at which the slope of the curve is 0 (i.e., P_{tp} is infinite) and α describes the rate of rise of the curve from functional residual capacity (FRC) to V_{max}. Quasistatic compliance (C_{stat}) was calculated from the first derivative

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[a] Rompun, Haver Lockhart, Shawnee Mission, Kansas
[b] Dynasciences, Bluebell, Pennsylvania
[c] Model PM5, Statham Instruments, Hato Rey, Puerto Rico
[d] Model VR6, Electronics for Medicine, White Plains, New York
[e] Model XY575, Esterline Angus Co., Indianapolis, Indiana
[f] Mark 9, Bird Co., Palm Springs, California
[g] Model PDP11 Digital Equipment Co., Maynard, Massachusetts

of equation #1 at P_{tp} = 3 cm H_2O. Functional residual capacity was measured by helium equilibration and total lung capacity (TLC) was defined as the total lung volume at P_{tp} = 30 cm H_2O.

Total respiratory system resistance (R_{tot}) was measured using a forced oscillation technique. During hyperventilation induced apnea, the respiratory system was oscillated at its resonant frequency and airway opening pressure (P_{ao}) and flow were plotted on an x-y plotter.[d] Total respiratory system resistance was calculated as the slope of the resulting line. Specific conductance (SG_{tot}) was calculated as the ratio of conductance (R_{tot}^{-1}) and FRC.

Experimental Protocol

Ten ponies were randomly divided into two groups. Arterial blood gas tensions, pulmonary mechanics and lung volumes were determined in all ponies 24 hours post surgery to establish baseline values. Immediately after these measurements were taken, the four ponies in group 1 received 0.5 liters of corn oil while the six ponies in group 2 received 100 mg/kg of 3MI in 0.5 liters of corn oil, both via nasogastric tubes. Measurements were repeated 24 hours after treatment and if R_{tot} had not increased and C_{dyn} had not decreased from baseline values, again at 48 hours post treatment. Subsequently, the vagus nerves of ponies in group 2 were exposed through the surgical wounds using the silk sutures and were transected. Ten minutes after bilateral vagotomy, arterial blood gases, pulmonary mechanics and lung volumes were measured again.

Data Reduction and Statistical Treatment

Curve fitting routines were performed by a digital computer, using the nonlinear least squares method of Bevington.[16] Data were analyzed using the students t test for paired data.[17] Significance was determined at $P < 0.05$.

Postmortem Examination

After the last measurement was taken, the six animals in group 2 were euthanized with an overdose of pentobarbital and exsanguinated. After the gross appearance of the lung was noted, the lungs were removed and minimum lung volume determined in 5 ponies by water displacement. The minimum lung volume was compared to that of 5 ponies free of clinically apparent lung disease, euthanatized and exsanguinated in the same manner.

Random sections of tissue were taken from the lungs, fixed in buffered formalin, paraffin embedded, sectioned at 5 microns, and stained with H and E. Additional fixed tissues were post-fixed in osmium tetroxide, dehydrated in graded alcohols, critical point dried, coated with 20 nm of gold and viewed under a JEOL JSM-35C scanning electron microscope.

Results

Gas exchange, pulmonary mechanics and lung volumes remained unchanged in ponies in group one up to 48 hours after corn oil treatment. Twenty-four hours after 3MI treatment, C_{dyn} and R_{tot} had not changed from baseline values in two ponies in group 2. In these ponies, measurements were repeated 48 hours post treatment. Figures 4-1 and 4-2

Figure 4-1 Respiratory rate (RR) ($\bar{x}$ + SEM), tidal volume (V_T) and minute ventilation ($\dot{V}_{min}$), measured during a prechallenge period (PC), after 3-methylindole (3MI) treatment and after vagotomy (VC). Stars indicate significant difference from preceding value.

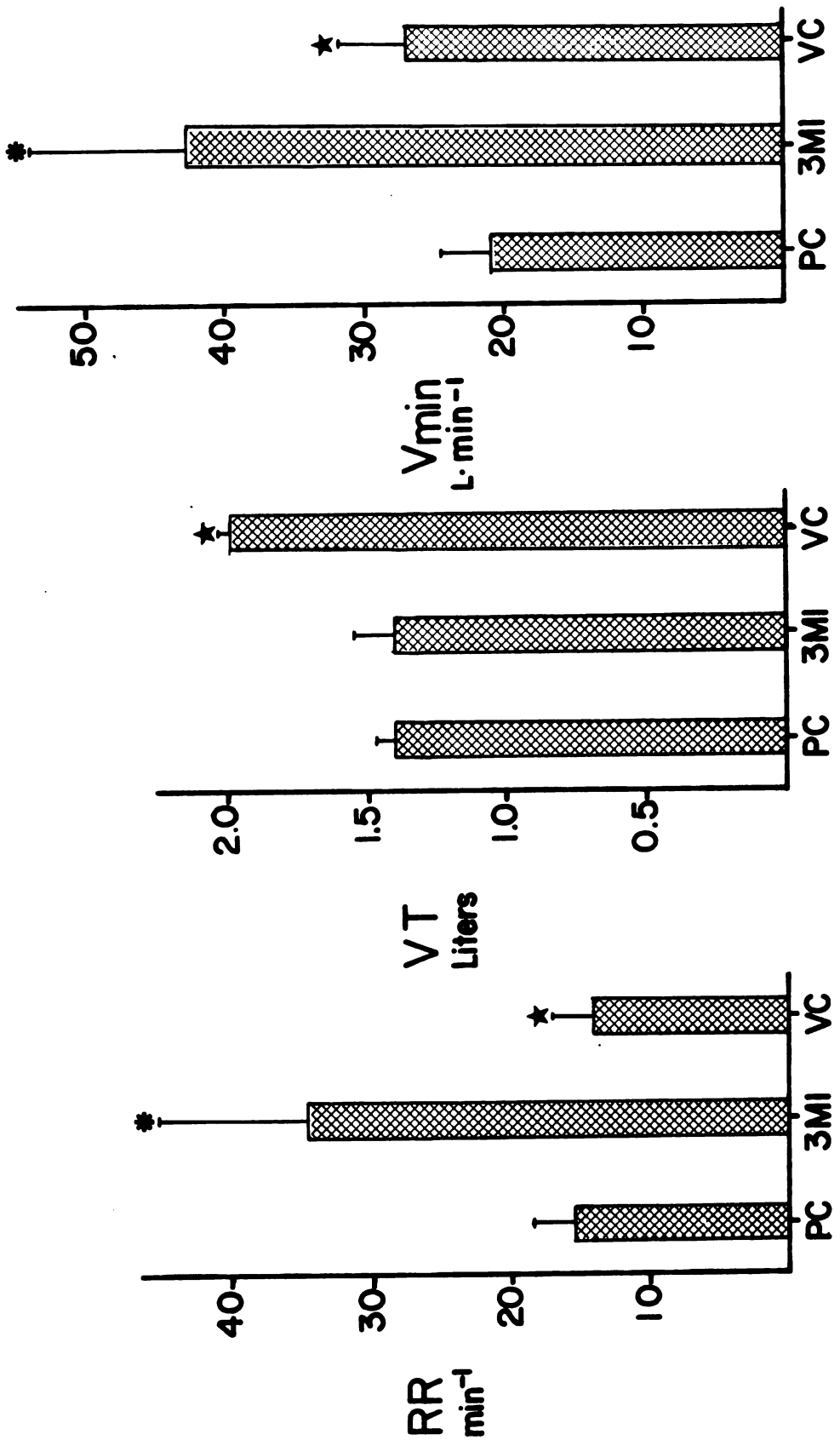
RR
min⁻¹
40
30
20
10
PC
3MI
VC
VT
Liters
2.0
1.5
1.0
0.5
PC
3MI
VC
Vmin
L·min⁻¹
50
40
30
20
10
PC
3MI
VC

Figure 4-1

Figure 4-2 Arterial O_2 tension (PaO_2) ($\bar{x}$ + SEM), arterial CO_2 tension ($PaCO_2$), and pH, measured during a prechallenge period ($\overline{PC}$), after 3-methylindole (3MI) treatment and after vagotomy (VC). Stars indicate significant difference from preceding value.

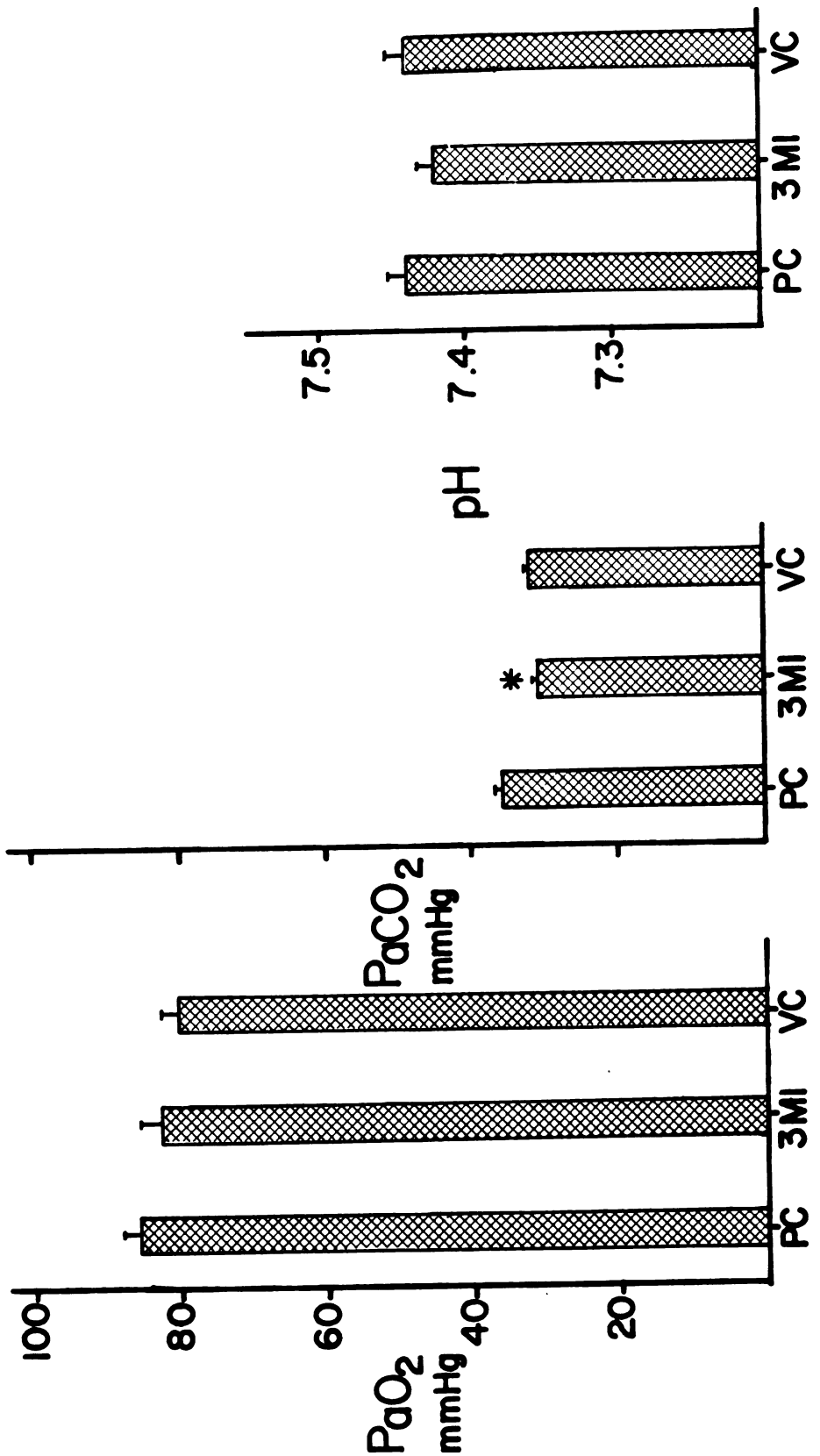

Figure 4-2

Figure 4-3 Functional residual capacity (FRC) ($\bar{x} \pm$ SEM) and total lung capacity (TLC) measured during a prechallenge period (PC), after 3-methylindole (3MI) treatment and after vagotomy (VC). Stars indicate significant difference from preceding value.

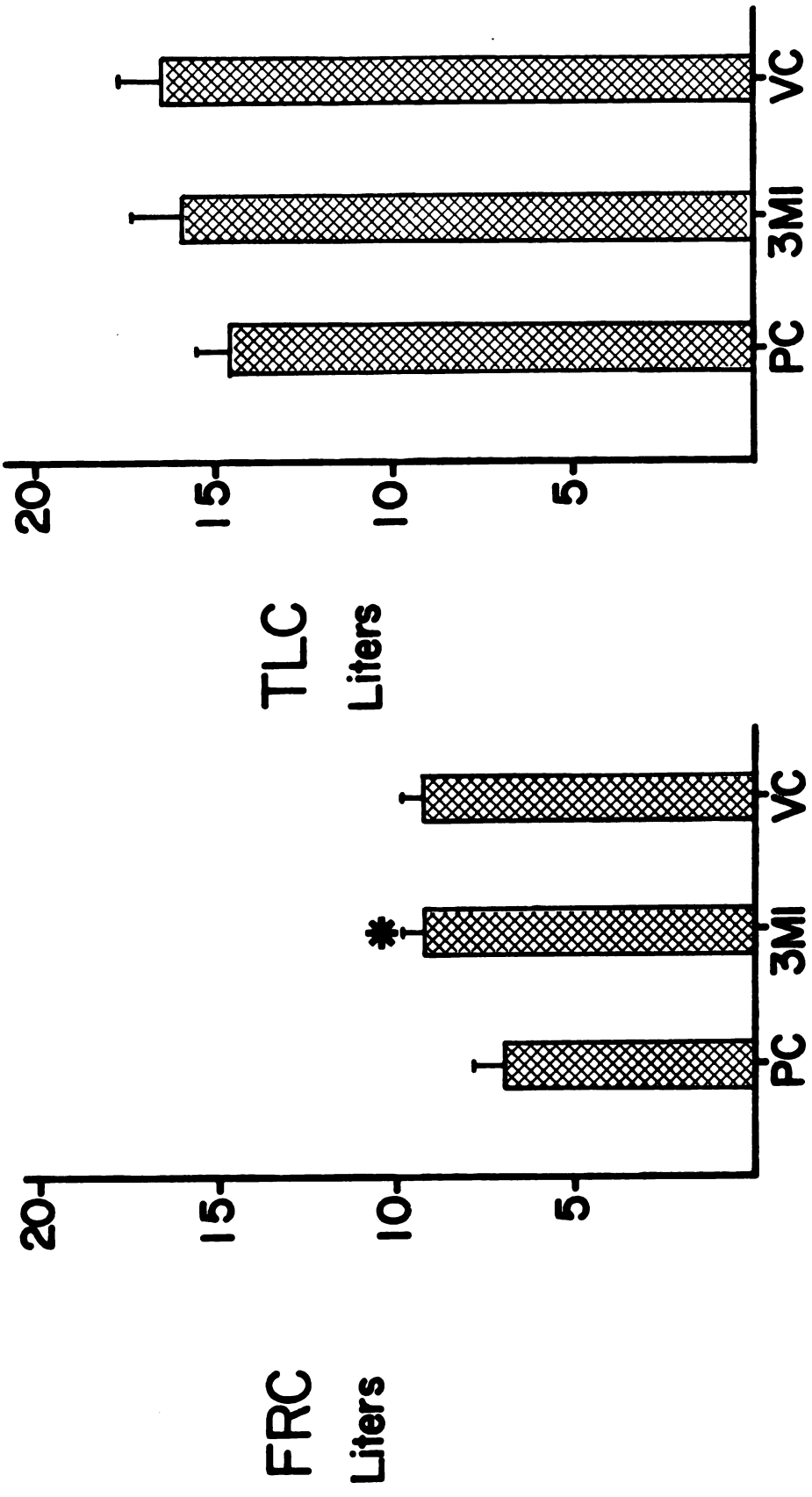

Figure 4-3

Figure 4-4 Total respiratory system resistance (R_{tot}) and specific conductance (SG_{tot}) measured during a prechallenge period, (PC), after 3-methylindole (3MI) treatment and after vagotomy (VC). Stars indicate significant difference from preceding value.

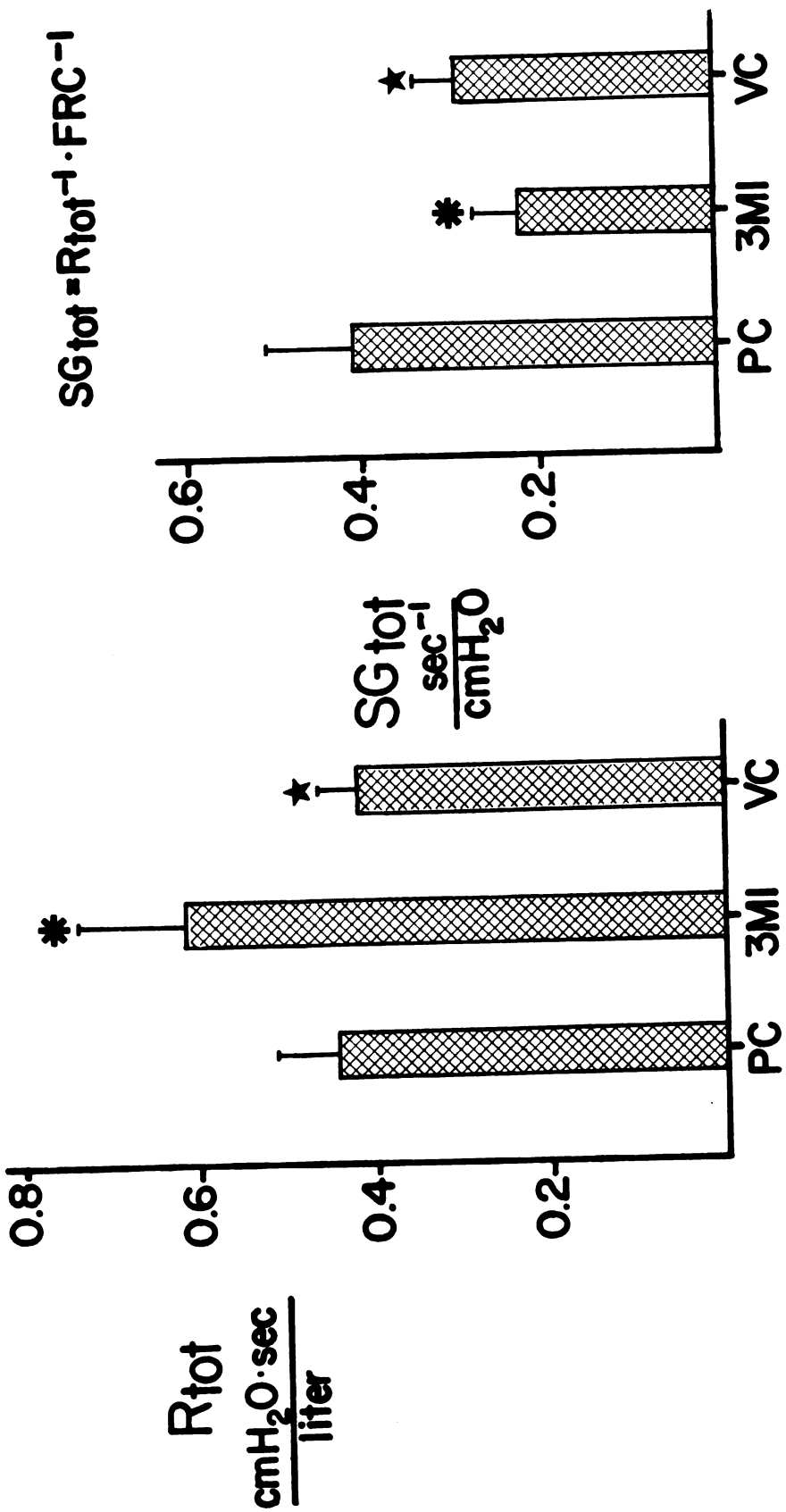
R_{tot}
$\frac{cmH_2O \cdot sec}{liter}$
0.8
0.6
0.4
0.2
PC
3MI
VC
SG_{tot}
$\frac{sec^{-1}}{cmH_2O}$
0.6
0.4
0.2
PC
3MI
VC
$SG_{tot} = R_{tot}^{-1} \cdot FRC^{-1}$

Figure 4-4

Figure 4-5 Dynamic compliance (C_{dyn}) measured during a prechallenge period (PC) after 3-methylindole (3MI) treatment and after vagotomy (VC). Stars indicate significant difference from preceding value.

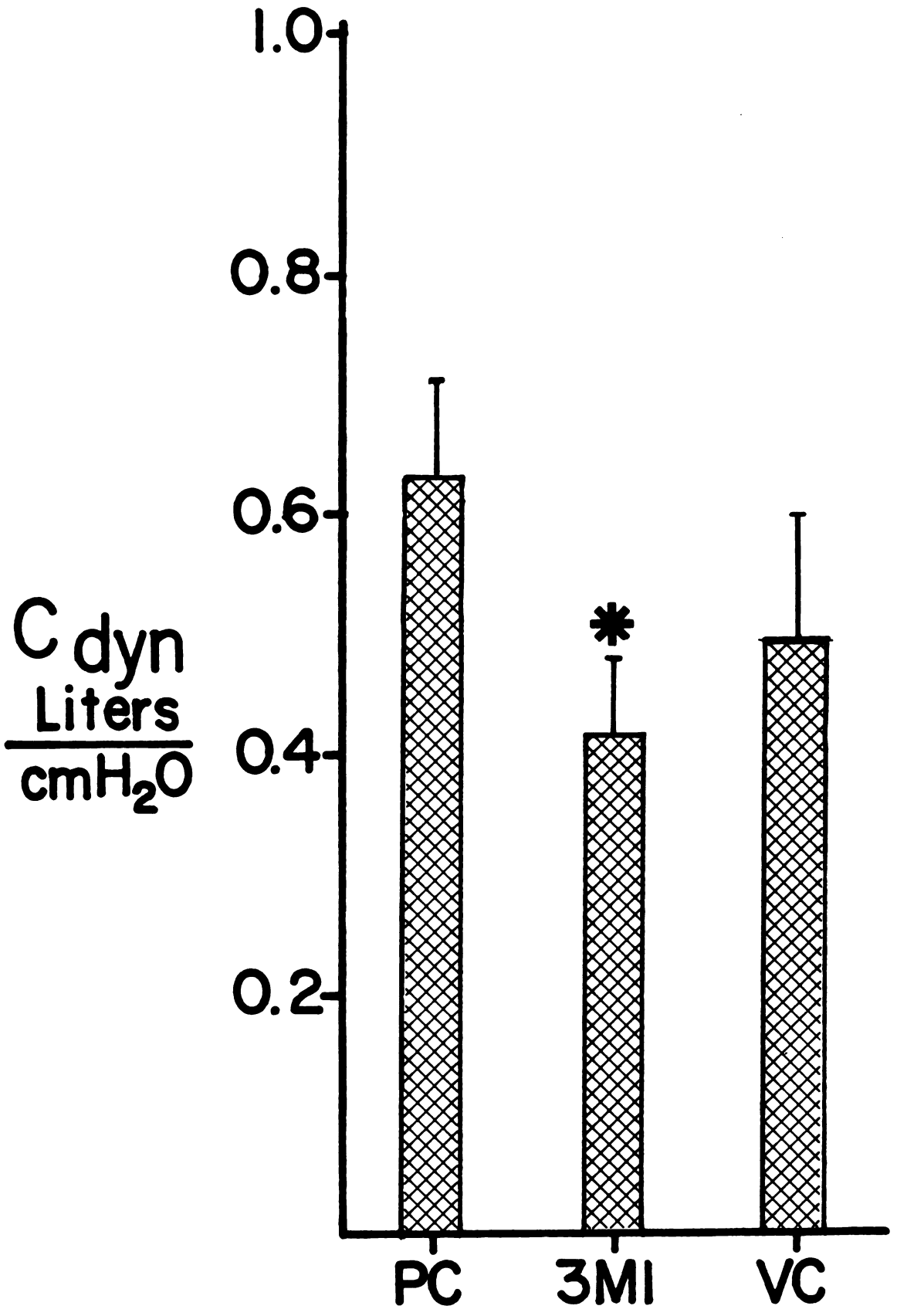

Figure 4-5

Figure 4-6 Photomicrograph, showing bronchioles with epithelial degeneration and cellular debris in their lumen.

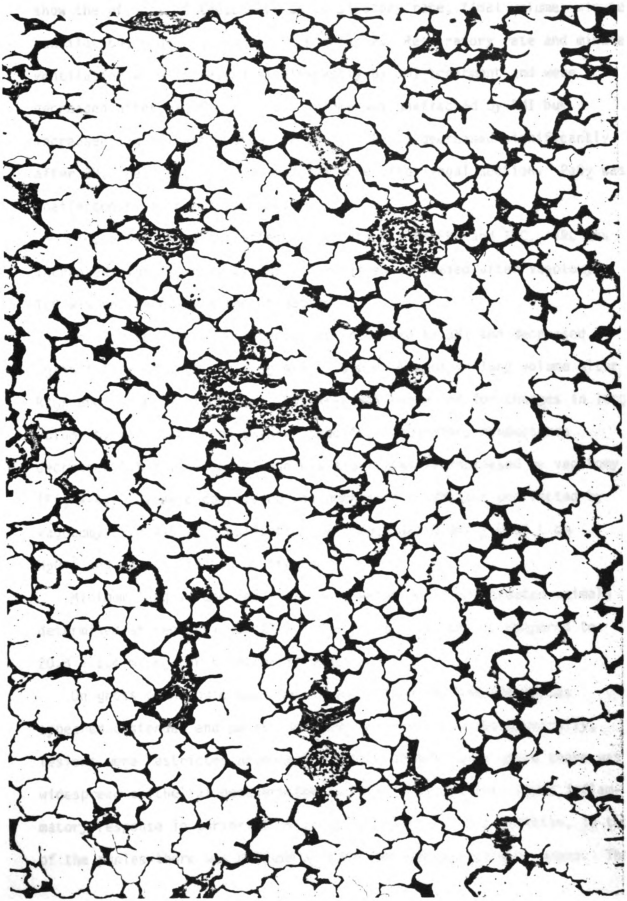

Figure 4-6

show the effects of treatments on respiratory rate, tidal volume, minute ventilation, PaO_2, $PaCO_2$ and pH in group 2. Respiratory rate and minute ventilation were increased significantly by 3MI treatment and were decreased after vagotomy. Tidal volume was unaffected by 3MI but increased significantly after vagotomy. $PaCO_2$ decreased significantly after 3MI treatment and remained the same after vagal section. PaO_2 was unaffected by either treatment.

Figure 4-3 shows the effect of treatments on FRC and TLC. FRC was increased significantly by 3MI and remained increased after vagotomy. TLC was unaltered by either treatment.

Total respiratory resistance was increased by 3MI and decreased again following vagotomy (Fig 4-4). Since changes in lung volume alter R_{tot} and since 3MI increased FRC, R_{tot} was corrected for changes in lung volume by calculation of SG_{tot}. Specific respiratory conductance decreased 46% following 3MI and was significantly increased by vagotomy (Fig 4-4). Dynamic compliance was decreased by 3MI but unaffected by vagotomy (Fig 4-5). Quasistatic compliance was 0.887 ± 0.04 L cm H_2O^{-1} and was unaffected by treatments.

Minimum lung volume per kg of body weight of 5 3MI treated animals determined at necropsy was 43.3 ± 2.4 ml/kg ($\bar{x} \pm$ SEM) as compared to 20.8 ± 1.4 ml/kg for 5 untreated ponies.

On gross pathologic examination lungs from 3MI treated horses appeared distended and palpation revealed crepitus. Histologically, lesions were restricted principally to the bronchioles, where there was widespread epithelial degeneration and a mild to moderate mixed inflammatory response in peribronchiolar areas (Fig 4-6). In addition, in two of the ponies there was diffuse alveolar and peribronchiolar edema. The

edema fluid was cell free but contained fibrillar material suggestive of fibrin. Scanning electron micrographs indicated that bronchiolar epithelial surfaces were damaged and were covered by scattered accumulations of cellular debris. Surfaces of the larger airways were normal.

Discussion

Derangement of pulmonary function after oral administration of 3MI was characterized by decreased C_{dyn} and SG_{tot} and an increased FRC and MV. A decrease in C_{dyn} may be produced by decreased static compliance (C_{stat}) or by the production of time constant inequalities between parallel lung units.[18] In addition, if significant time constant inequalities pre-exist, an increase in RR will decrease C_{dyn}.[19] In normal ponies, C_{dyn} is not frequency dependent over a range of 15 to 60 breaths per min.[14] Therefore it is unlikely that the decreased C_{dyn} after 3MI was due to increased RR acting on preexisting time constant inequalities. Since C_{stat} was unchanged by 3MI, the observed decrease in C_{dyn} was most probably due to the production of time constant inequalities, characteristic of small airway obstruction.

In dogs, resistance to flow resides mainly in large airways with peripheral airways contributing approximately 20% of pulmonary resistance of FRC.[20] If the distribution of resistance is similar in ponies, the decreased conductance caused by 3MI may be attributed to a decrease in caliber of central airways, a massive small airway obstruction or a combination of large and small airway obstruction.[21]

Factors that may increase FRC include small airway closure, increased expiratory time constants of peripheral lung units and tonic activation of inspiratory muscle groups. Muller et al reported

recently that the increase in FRC seen in persons after histamine administration may be due in part to persistence of inspiratory muscle activity during exhalation, while Slocombe et al report that in calves the increased FRC caused by histamine is abolished by vagotomy, suggesting stimulation of pulmonary receptors with afferents in the vagus is the cause of the increased FRC.[h,21] In the present study, vagotomy did not reverse the increase in FRC. It is therefore unlikely that pulmonary vagal reflexes were involved and the increase in FRC was probably caused by airway obstruction and prolongation of expiratory time constants.

Increased minimal volume results from premature small airway closure which results from decreased elastic recoil of the lung, increased smooth muscle tone in airways or plugging of airways by secretions or debris. We did not evaluate the elastic properties of the lung at MV but the pressure volume behavior of the lung above FRC was not changed by 3MI. Thus the increase in minimum volume observed may be attributed to small airway obstruction resulting from either increased airway smooth muscle tone or accumulation of secretions or debris.

The decrease in C_{dyn}, increase in R_{tot}, FRC and MV after 3MI treatment suggest small airway obstruction. It is surprising that in the face of small airway disease horses were able to maintain normal arterial oxygen tensions. Data suggest however that despite normal PaO_2, gas exchange was impaired. After 3MI treatment, tidal volume remained at baseline values but RR increased significantly. If dead space volume did not change, alveolar ventilation must have increased, resulting in the

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[h] Slocombe RF, Robinson NE: Vagotomy abolishes the histamine induced increase in functional residual capacity in neonatal calves. Fed Proc 40:387, 1981.

significant decrease in $PaCO_2$. Since the alveolar gas equation states that

$$PAO_2 = K_1 - K_2\ PaCO_2 \qquad (2)$$

where PAO_2 = alveolar oxygen tension and K_1 and K_2 are constants, a decrease in $PaCO_2$ must have resulted in an increased PAO_2.[22] Thus, if PAO_2 increased and PaO_2 was unchanged by 3MI, the alveolar arterial oxygen tension difference increased indicating impaired gas exchange.

Considering that minute ventilation more than doubled after 3MI, the decrease in $PaCO_2$ is small. This suggests either that CO_2 production or the dead space tidal volume ratio increased. While our data do not differentiate these possibilities, the tachypnea induced by 3MI may have increased both parameters. Following vagotomy, $PaCO_2$ remained the same as following 3MI despite a decrease in minute ventilation. The increased tidal volume resulting in a smaller deadspace tidal volume ratio would adequately explain these latter observations.

The increase in V_T and decrease in RR and R_{tot} after vagotomy are similar to changes reported in normal conscious ponies.[23] The marked decrease in RR after vagotomy suggests that the tachypnea induced by 3MI was a vagally mediated response. Vagal afferents known to affect RR include aortic chemoreceptors, pulmonary J receptors located in the interstitium, and irritant receptors located in the submucosa of conducting airways.[24] Since PaO_2 and arterial PH were unchanged by treatments and $PaCO_2$ decreased after 3MI treatment, stimulation of aortic body chemoreceptors did not occur. Therefore the tachypnea was due to stimulation of pulmonary afferent receptor systems.

The decrease in R_{tot} and increased SG_{tot} after vagotomy suggests dilation of large or small airways. Since C_{dyn} remained unaltered after

vagotomy, it appears that dilation of small airways did not occur. Interruption of normal parasympathetic bronchomotor tone to large airways would adequately explain the observed decrease in R_{tot} and increase in SG_{tot} after vagotomy.[14]

Histopathologically, 3MI pneumotoxicosis was characterized by necrotizing bronchiolitis and alveolar emphysema without involvement of bronchi. In addition to bronchiolitis, alveolar edema was also present in two of six horses. Alveolar edema has not been previously reported in horses after 3MI treatment. Bronchiolitis with acinar over-inflation is also characteristic of naturally occurring chronic obstructive lung disease in the horse,[1-3] but the role of 3MI pneumotoxicosis in the disease is presently unknown.

Histologic findings support the physiologic data and both suggest that 3MI toxicosis is characterized by small airway obstruction and acinar over-inflation without involvement of bronchi. Tachypnea produced by 3MI appears to be due to stimulation of pulmonary receptors with afferents in the vagus.

3-methylindole induced pulmonary toxicosis may have broad biologic implications because the fecal flora of man, horses and other domestic species is capable of producing 3MI from tryptophan and some of its metabolites.[25] In addition, potentially significant amounts of 3MI are found in tobacco smoke.[7]

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CHAPTER 5

Pulmonary Function in Ovalbumin Induced Allergic Lung Disease in the Awake Pony: Role of Vagal Mechanisms

Introduction

In some experimental and spontaneous lung diseases, vagal mechanisms are believed to be involved in the pathogenesis of airway obstruction[1,2,3] while in others the vagus nerve plays no significant role.[4,5,6] Alleviation of bronchoconstriction by vagal blockade may be attributed to several different mechanisms. Bronchoconstriction may be induced by a vagal reflex originating in pulmonary receptors, by a central nervous system induced increase in efferent vagal activity, or by an increase in sensitivity of airway smooth muscle to vagal influences.[7] In only one disease model has a true vagal reflex bronchoconstriction been described. Gold et al[1] showed that in the sensitized mongrel dog, unilateral Ascaris suum aerosol challenge induces bilateral bronchoconstriction reversible by unilateral vagal blockade. In order to study vagal mechanisms in another immunologically mediated lung disease, I assessed the derangement in pulmonary function and the effect of vagal blockade during ovalbumin induced allergic lung disease in the sensitized conscious pony. The equid was chosen for study because it is the only domestic animal that commonly suffers from chronic recurrent airway disease[8] and because of its size, intubation of mainstem bronchi could be relatively easily accomplished in the awake animal allowing investigation of vagally mediated reflexes following antigen challenge of only one lung.

Materials and Methods

Eight grade ponies between 2 and 10 years of age ($\bar{x}$ = 3.9 years) weighing 149.5 $\pm$ 17.8 kg were used in the experiments. Four animals had bilateral cervical vagal loops and exteriorized carotid arteries. Prior

to use, animals had been on pasture for at least two months and all were vaccinated against the common viral respiratory diseases. Animals were regularly examined to detect any signs of respiratory disease.

Sensitization of Ponies

Animals were sensitized with 10 mg ovalbumin dissolved in 2 ml of phosphate buffered saline solution and emulsified in 2 ml of complete Freund's adjuvant. The emulsified ovalbumin was divided and injected deep into the right and left triceps and semimembranosus muscles. This protocol was repeated two months later. Aerosol challenges were conducted at least 2 weeks after the last sensitization.

Aerosol Challenge

In bilateral challenge experiments an endotracheal tube was inserted into a tracheostoma. An ultrasonic nebulizer (Devilbis model 65) was attached to the endotracheal tube via a one-way valve assembly so that animals inhaled through the nebulizer. Forty ml of solution were aerosolized in 20 minutes. In unilateral challenge experiments the right and left mainstem bronchi were intubated with specially prepared cuffed tubes via a tracheostoma created in the lower 1/3 of the cervical trachea. The distal ends of the endobronchial tubes were coated with a thin layer of anesthetic cream to prevent excessive coughing during intubation. A side hole catheter, incorporated into the distal end of the tubes, was used to measure bronchial pressure. Isolation of the lungs was verified by 1) visual inspection of the cuffs of the tubes using a fiberoptic bronchoscope, and 2) ventilation of the left lung with 80% He, 20% O_2 mixture for 10 minutes and failure to measure

helium in the gas expired from the right lung. In unilateral challenge experiments, the ultrasonic nebulizer and one-way respiratory valve assembly were used to deliver 20 ml of solution in 20 minutes.

Pulmonary Function Measurements

Experiments were performed with animals restrained in stocks and tranquilized with intravenous xylazine (0.5 mg/kg of body weight). The methods and reproducibility of pulmonary function measurements in ponies have been previously described.[9,10] Briefly, air flow ($\dot{V}$) and tidal volume (V_T), measured using a pneumotachograph (Fleisch #4, Dynasciences, Blue Bell, PA) and transducer (Model PM5, Statham Inst., Hato Rey, PR) attached to a cuffed endotracheal tube and inserted into the trachea via a tracheostoma, were recorded on light sensitive paper (Model VR6, Electronics for Medicine, White Plains, New York). Transpulmonary pressure (P_{tp}) was measured as the pressure difference between the mid portion of the thoracic esophagus and the airway opening, using identical balloon catheter systems attached to a differential pressure transducer (Model P131, Statham Inst., Hato Rey, PR). From the recording of P_{tp}, $\dot{V}$ and V_T, dynamic compliance (C_{dyn}), respiratory rate (RR) and minute ventilation ($\dot{V}_{min}$) were calculated.

Quasistatic pressure-volume curves between functional residual capacity (FRC) and total lung capacity (TLC) were generated on an x-y plotter (Model XY575, Esterline Angus, Indianapolis, IN) using an air driven pressure cycled ventilator (Mark 9, Bird Corp., Palm Springs, CA). The deflation limb of the quasistatic pressure-volume curve was empirically described as a single rising exponential. The curve was fit by computer to the equation

$$V = V_{max} (1-e^{-\alpha P_{tp}}) \quad (1)$$

Where V = lung volume at a given P_{tp}, V_{max} is the volume at which the slope of the curve is 0 (i.e., P_{tp} is infinite) and describes the rate of rise of the curve from FRC to V_{max}.[11] Quasistatic compliance (C_{stat}) was calculated from the first derivative of equation #1 at P_{tp} = 3 cm H_2O. Functional residual capacity was measured by helium equilibration and TLC was defined as the total lung volume at P_{tp} = 30 cm H_2O.

Prior to measurement of total respiratory system resistance (R_{tot}), animals were force ventilated 4 times up to an airway opening pressure of 30 cm H_2O to ensure a constant volume history and to create a period of apnea lasting between 2 and 30 sec. During this period of apnea, the respiratory system was oscillated at its resonant frequency and airway opening pressure (P_{ao}) and flow were plotted on photorecording x-y plotter (Model VR6, Electronics for Medicine, White Plains, NY). Total respiratory resistance was calculated as the slope of the resulting line. In the experiments in which left and right lungs were intubated separately, the two endobronchial tubes were connected with a y-tube so that a volume history was provided simultaneously to both lungs. The right and left lungs were then oscillated separately at their resonant frequencies and bronchial opening pressure and flow were plotted on the x-y plotter. Left and right lung resistances (R_{totL} and R_{totR}) were calculated as the slope of the resulting lines.

Vagal Blockade

In 4 ponies with bilateral cervical vagal loops, the vagus was reversibly blocked by circulating coolant at a temperature of -2°C through copper coils, wrapped around both loops. In an earlier study on

the same ponies,[12] we established criteria of bilateral cervical vagal blockade: tachycardia, slow deep breathing and paresis of the cricoarytenoideus dorsalis muscle. The latter was determined by lack of movement of the arytenoid cartilages during tidal breathing, as observed through an endoscope (Model BF type B2, Olympus Co., New Hyde Park, NY).

In the remaining ponies, vagal blockade was achieved by vagal sectioning, performed under local anesthesia.

Experimental Protocol

Ponies were divided in two groups of 4 animals each. Group 1 ponies had bilateral vagal loops and both lungs were challenged with aerosol antigen via the endotracheal tube, while in group 2 ponies only the left lung was challenged through the left endobronchial tube.

Group 1 ponies: Bilateral aerosol antigen challenge. The four ponies with bilateral cervical vagal loops and exteriorized carotid arteries were challenged with 40 ml of saline, 2 g of bovine γ globulin in 40 ml saline and 2 g of ovalbumin in 40 ml of saline on separate days. Pulmonary function measurements were made during a baseline period, and hourly after the beginning of challenge for 5 hours. In the ovalbumin group, measurements were also made during two periods of vagal blockade, at 1½ and 4½ hours after the beginning of challenge.

Group 2 ponies: Unilateral aerosol antigen challenge. The left lungs of group 2 animals were challenged with 1 g of ovalbumin in 20 ml of saline. Respiratory rate, R_{totL} and R_{totR} were measured during a baseline period, hourly after the beginning of challenge for 4 hours, and after both ipsilateral and bilateral vagal sectioning which was

performed after the 4 hour measurement. Subsequently, animals were euthanized and subjected to postmortem examination.

Postmortem Examination

Animals were euthanized with an overdose of pentobarbital and exsanguinated. After the gross appearance of the lung was noted, minimal volume (MV) of both challenged and unchallenged lungs was determined by water displacement.

Random sections of tissue were taken from the dorsal, middle and ventral regions of the lung and from a main stem bronchus. Sections were fixed in phosphate buffered formalin, sectioned at 5 microns and stained with H & E. Qualitative comparisons were made between challenged and unchallenged lungs and between regions of lungs.

Statistical Treatment

The effect of aerosol challenge and vagal blockade on pulmonary function variables was analyzed using two-way analysis of variance in a randomized complete block design.[13] Differences between means were determined using the Student-Newman Keul's test. The effects of aerosol challenge were assessed by comparing the 1, 2, 3, 4 and 5 hour measurement periods with baseline values while the effects of vagal blockade were assessed by comparison of the first period of vagal blockade with the 1 and 2 hour measurement periods and the second period of vagal blockade with the 4 and 5 hour measurement periods. In the unilateral challenge experiments, the effect of ipsilateral and bilateral vagal section were compared with the 4 hour measurement period. At necropsy minimum volume data were analyzed using the students' t test for paired data. Significant was set at $\alpha < 0.05$.

Figure 5-1 Respiratory rate (RR).($\bar{x}$ + SEM), tidal volume (VT) and minute ventilation ($\dot{V}$ min) measured during a prechallenge period (PC), hourly after challenge for 5 hours and during two periods of vagal blockade (VB). Round stars indicate significant differences from prechallenge value, while pointed stars indicate significant effect of VB, compared to adjacent measurement periods.

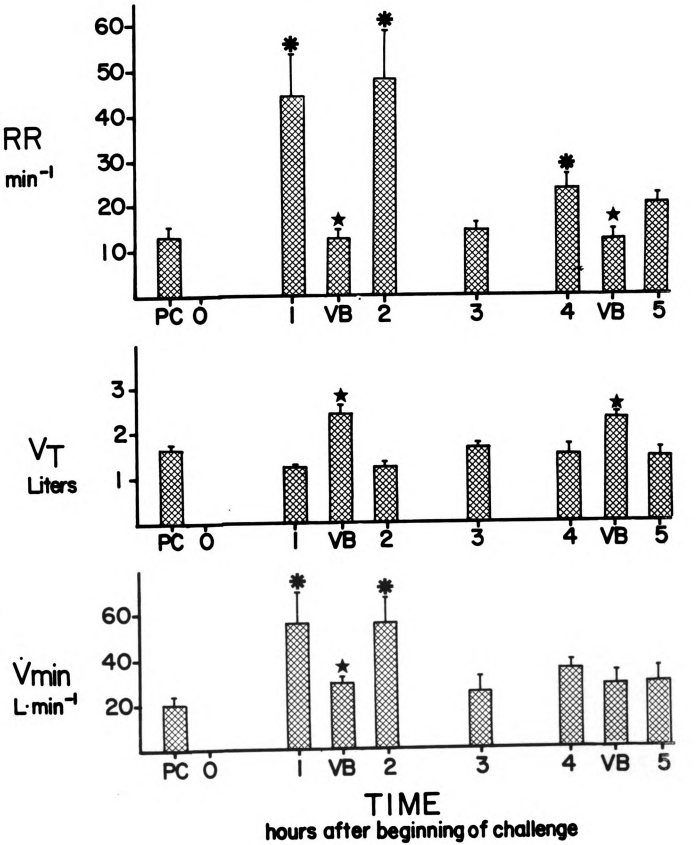
RR
min⁻¹
60
50
40
30
20
10
PC 0 1 VB 2 3 4 VB 5
V_T
Liters
3
2
1
PC 0 1 VB 2 3 4 VB 5
Vmin
L·min⁻¹
60
40
20
PC 0 1 VB 2 3 4 VB 5
TIME
hours after beginning of challenge

Figure 5-1

Figure 5-2 Total respiratory system resistance (R_{tot}) ($\bar{x} \pm SEM$) and dynamic compliance (C_{dyn}) measured during a prechallenge period (PC) hourly after challenge for 5 hours and during 2 periods of vagal blockade (VB). Round stars indicate significant differences from prechallenge value while pointed stars indicate significant effect of VB, compared to adjacent measurement periods.

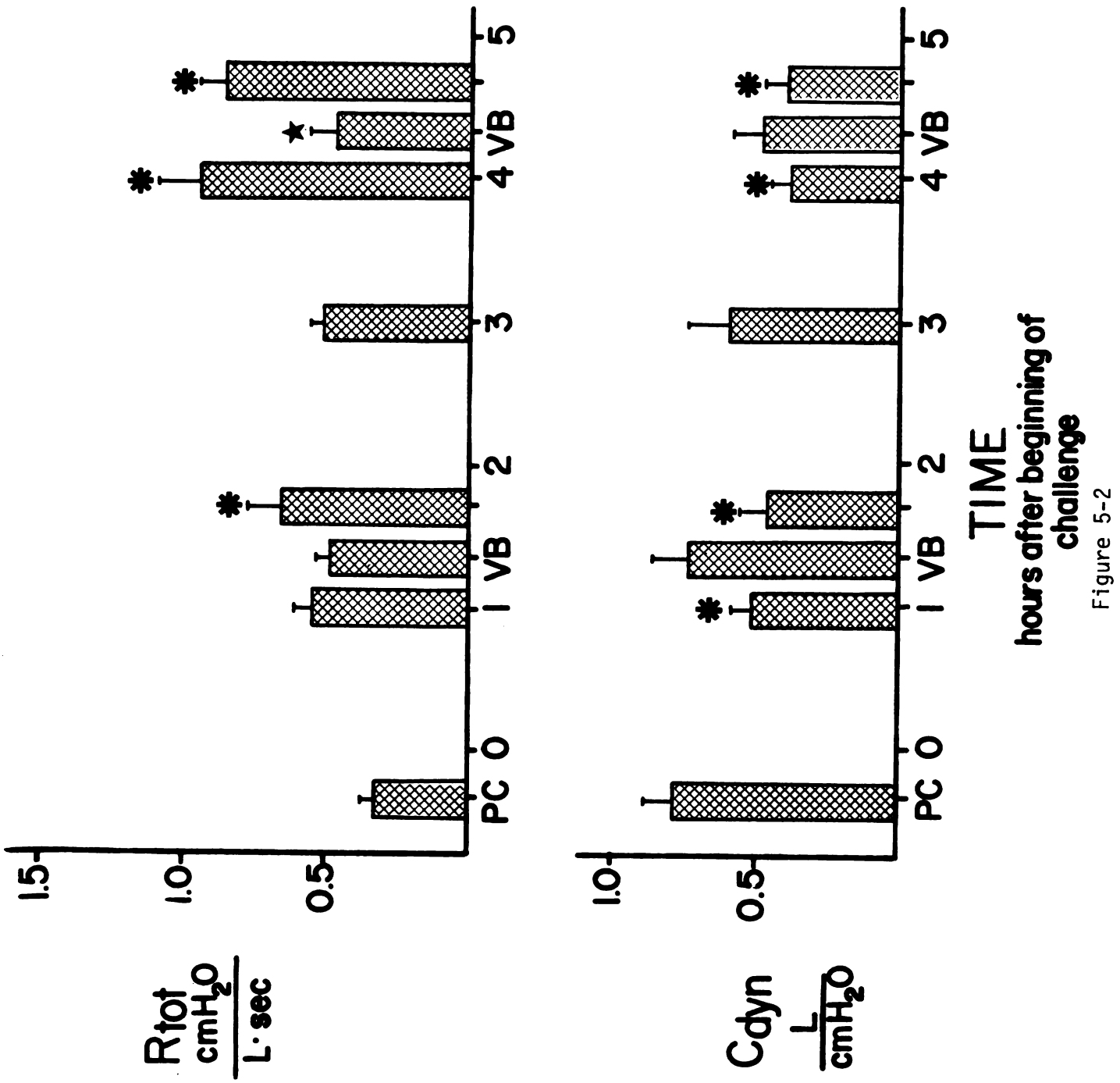
1.5
1.0
0.5
R_{tot} $\frac{cmH_2O}{L \cdot sec}$
PC 0
1 VB
2
3
4 VB
5
1.0
0.5
C_{dyn} $\frac{L}{cmH_2O}$
TIME
hours after beginning of challenge

Figure 5-2

Figure 5-3 Respiratory rate (RR) ($\bar{x}$ + SEM), and left and right lung resistance (R_{totL} and R_{totR}) measured during a prechallenge period (PC) hourly after challenge for 4 hours and during ipsilateral and bilateral vagal blockade (IVB and VB). Single star indicates significant difference from prechallenge value. Asterisk indicates significant effect of VM, compared to the 4 hour measurement period. Double stars indicate significant difference from all other measurement periods.

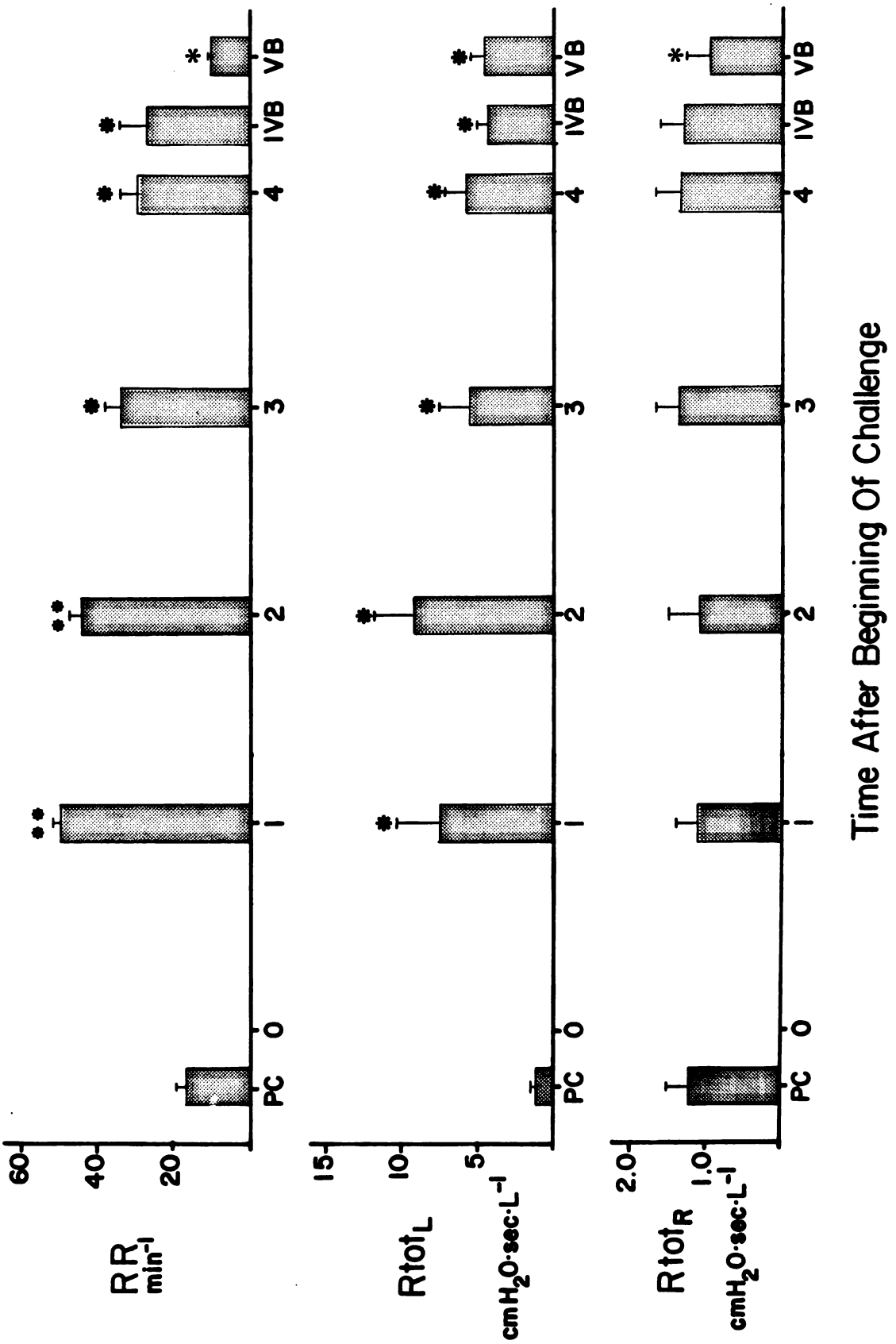
RR min⁻¹
60
40
20
Rtot L
cmH2O·sec·L⁻¹
15
10
5
Rtot R
cmH2O·sec·L⁻¹
2.0
1.0
PC
0
1
2
3
4
IVB
VB
Time After Beginning Of Challenge
hours

Figure 5-3

Table 5-1 Group 1 ponies: Bilateral aersol antigen challenge. Arterial blood gas tensions and lung volumes ($\bar{x}$ + SEM) during a prechallenge period, hourly after challenge for 5 hours and during two periods of vagal blockade (VB).

	Baseline	1 Hour	VB I	2 Hour	3 Hour	4 Hour	VB II	5 Hour
pH	7.42±0.01	7.40±0.02	7.40±0.02	7.42±0.02	7.42±0.02	7.41±0.02	7.40±0.02	7.41±0.02
PaO_2 torr	85.2±2.7	75.3±4.3*	74.3±3.3.6*	72.9±4.4*	72.4±4.9*	72.7±3.3*	74.7±4.5*	75.0±3.4*
$PaCO_2$ torr	41.4±3.2	42.7±2.3	41.0±3.5	39.6±3.3	39.0±2.4	38.9±2.1	38.7±2.5	36.9±2.6
FRC liters	12.5±0.71	11.8±0.9	10.9±0.8	11.8±0.7	11.1±1.2	11.0±0.4	11.4±0.6	11.8±0.7
TLC liters	20.9±2.1	18.1±1.2	18.9±1.3	19.1±1.3	19.1±2.1	18.4±1.1	18.9±0.6	18.6±0.6
C_{stat} $L.cmH_2O^{-1}$	1.26±0.216	0.919±0.10	1.12±0.15	1.00±0.11	1.12±0.10	1.03±0.12	0.961±0.07	0.924±0.15

FRC = Functional residual capacity
TLC = Total lung capacity
C_{stat} = Quasistatic compliance
* indicates significant decrease from baseline value

Results

Group 1 ponies: Bilateral aerosol antigen challenge. Aerosol challenge using saline or bovine γ globulin did not alter arterial blood gas tensions, pulmonary mechanics, lung volumes or rectal temperature. Fig 5-1 shows the effect of ovalbumin aerosol challenge on RR, V_T and $\dot{V}_{min}$. Respiratory rate and $\dot{V}_{min}$ were increased significantly at 1, 2 and 4 hours post challenge and at 1 and 2 hours post challenge respectively, but tidal volume did not change with time. During both periods of vagal blockade, RR and $\dot{V}_{min}$ decreased while V_T increased.

The effect of ovalbumin aerosol challenge on R_{tot} and C_{dyn} is shown in Fig 5-2. Dynamic compliance decreased significantly after challenge at all measurement periods and was not significantly altered by vagal blockade. Total respiratory resistance increased significantly at 2, 4 and 5 hours post challenge. Vagal blockade decreased R_{tot} during the second period of vagal blockade.

PaO_2 decreased significantly 1 hour after ovalbumin aerosol challenge and remained depressed at all subsequent measurement periods. $PaCO_2$, arterial blood pH, lung volumes, pressure-volume characteristics of the lungs and rectal temperature were not changed by ovalbumin challenge or vagal blockade (Table 5-1).

Group 2 ponies: Unilateral aerosol antigen challenge. Challenge of the left lung increased RR significantly at 1 and 2 hours after challenge. At 3 and 4 hours, RR decreased but was still significantly higher than the prechallenge value (Fig 5-3). Ipsilateral vagal sectioning did not affect RR but bilateral section reduced RR below prechallenge levels. Left lung resistance was increased after challenge and remained elevated at all measurement periods, while R_{totL} was not

Figure 5-4 Photomicrograph of a brochiole 5 hours after ovalbumin challenge, showing acute obstructive bronchiolitis.

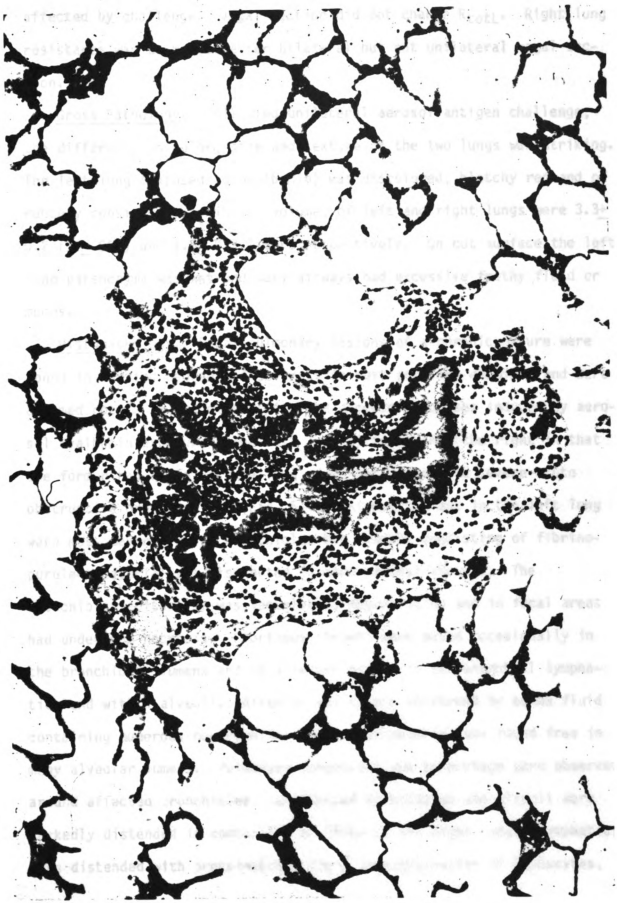

Figure 5-4

affected by challenge. Vagal section did not change R_{totL}. Right lung resistance was decreased after bilateral but not unilateral vagal section.

Gross Pathology. Following unilateral aerosol antigen challenge, the difference in color, size and texture of the two lungs was striking. The left lung (exposed to ovalbumin) was discolored, blotchy red and of rubbery consistency. Minimal volume, of left and right lungs were 3.3± 0.2 ($\bar{x}$ ± SEM) and 1.5± 0.3 liters respectively. On cut surface the left lung parenchyma was wet and many airways had excessive frothy fluid or mucus.

Histopathology. Focal pulmonary lesions of a chronic nature were found in varying degrees of severity in most sections examined and were assumed not to be associated with the disease processes induced by aerosol ovalbumin exposure. The left lung differed from the right in that the former had acute diffuse lung edema and widespread severe acute obstructive bronchiolitis (Fig. 5-4). Airway lesions in the left lung were most severe in the bronchioles but exudate consisting of fibrinopurulent material was also noted in more central airways. The bronchiolar epithelium was vacuolated, degenerating and in focal areas had undergone necrosis. Fibrinous thrombi were noted occasionally in the bronchiolar lumens and to a lesser extent in peribronchial lymphatics and within alveoli. Alveolar walls were thickened by edema fluid containing numerous neutrophils. A similar material was found free in many alveolar lumens. Pulmonary congestion and hemorrhage were observed around affected bronchioles. Unaffected bronchioles and alveoli were markedly distended in comparison to those of the right lung. Lymphatics were distended with proteinaceous fluid and margination of leucocytes,

predominantly neutrophils, was noted occasionally in peribronchiolar vessels. Mild to moderate numbers of eosinophils were found associated with the airways and the lymphatics but were not observed in excessive numbers in the alveolar edema fluid. The lesions appeared most severe in the ventral lung.

Discussion

Bilateral aerosol challenge with saline or bovine γ globulin did not significantly alter pulmonary function. Therefore the response to inhaled ovalbumin antigen was immunologic in nature and not due to nonspecific airway irritation.

The pulmonary response to bilateral aerosol antigen challenge of sensitized ponies with ovalbumin was biphasic. The early response, evident at 1 and 2 hours after the onset of challenge was characterized primarily by tachypnea, while the late response was characterized primarily by an increased R_{tot}. Response to antigen challenge depends upon the manner of sensitization. Large quantities of antigen in complete Freund's adjuvant encourages delayed responses while sensitization with antigen saline solutions or antigen in incomplete Freund's adjuvant encourages an immediate response.[14] Since we sensitized with antigen in complete Freund's adjuvant, the early tachypneic response was unexpected.

Tachypnea is observed in experimentally induced allergic lung disease in animals and acute attacks of asthma in man.[15,16] Mechanisms proposed for the increased RR include an increase in $PaCO_2$ and pH or decrease in PaO_2, a rise of core temperature, anxiety, antigen induced changes in mechanical properties the lungs, and stimulation of pulmonary receptors with vagal afferents.[15] In these experiments, rectal

temperature, $PaCO_2$ and pH did not change and anxiety was not likely to be involved because challenge with saline and bovine γ globulin did not alter RR. Although the PaO_2 decreased, from 85.2 torr to 75.3 torr, the tachypneic response was too severe to be explained solely on this basis.[17] In addition the tachypneic response followed a clearly different time course than both the PaO_2 and the pulmonary mechanics changes. Therefore it is most likely that in the pony, as in the dog[15] afferent vagal pathways mediate the ventilatory response to inhaled antigen. The time course of tachypnea in unilateral and bilateral challenge experiments was similar. In addition, in unilateral challenge experiments, tachypnea was only abolished after bilateral vagal blockade, suggesting that in the horse pulmonary afferent vagal fibers cross over to the contralateral vagus nerve within the thorax. Pulmonary receptors which may have been involved in the stimulation of respiration include irritant receptors present in the submucosa of conducting airways and J receptors, located in the pulmonary interstitium.[18] Our data do not allow identification of responsible receptor systems.

After bilateral aerosol antigen challenge, R_{tot} increased gradually and was greatest 4 hours after challenge. If the central airways account for the majority of resistance to airflow in horses as they do in dogs[19] the 3-fold increase in R_{tot} at 4 hours after challenge combined with a small decrease in C_{dyn} suggests that large airways were involved in the antigen induced airway narrowing.[20]

Vagal blockade decreased R_{tot} at 4½ hours after challenge. Although in normal ponies vagal blockade also decreases R_{tot}[9] the decrease in R_{tot} in this experiment was too large to be explained by interruption of normal bronchomotor tone alone. This suggests the involvement of a

vagal mechanism such as a vagal reflex originating in pulmonary receptors, a central increase in efferent vagal activity or an increase in sensitivity of airway smooth muscle to normal vagal tone. Vagal blockade did not reduce R_{tot} below baseline value as it should have done if vagal mechanisms alone were responsible for the increased R_{tot}.[9] Therefore, local mechanisms also play a role in aerosol antigen induced airway caliber changes. This latter conclusion is supported by the failure of vagal blockade to reduce R_{tot} below baseline value at 1½ hours after challenge.

In order to determine the relative importance of vagal and local mechanisms in aerosol antigen induced airway narrowing in the horse, we challenged the left lungs of ponies through an endobronchial tube and determined R_{totL} and R_{totR} hourly for 4 hours after the beginning of challenge and following unilateral and bilateral vagal sectioning. We reasoned that if vagal reflexes, originating in the challenged lungs or a challenge induced increase in efferent parasympathetic bronchomotor activity were responsible for airway narrowing in this disease model, unilateral aerosol antigen challenge would result in airway narrowing in both lungs, abolished by either unilateral or bilateral vagal blockade. If aerosol antigen challenge increased the sensitivity of airway smooth muscle to normal vagal tone or if the baseline airway caliber was important, left unilateral challenge would result in increase in R_{totL} only, abolished by either unilateral or bilateral vagal blockade, while if local mechanisms were important in airway caliber changes, unilateral challenge would only cause an increase in R_{totL}, unaffected by vagotomy.

Since unilateral aerosol antigen challenge of the left lung resulted in a marked increase in R_{totL}, without altering R_{totR}, a vagal reflex

bronchoconstriction originating in the left lung or a central increase in parasympathetic bronchomotor tone were not responsible for the airway narrowing. The increase in R_{totL} was of greater magnitude than the increase in R_{tot} following bilateral challenge. Because the endobronchial tubes were more peripherally located and had less deadspace, differences in amount and location of aerosol deposition may have been responsible for this discrepancy.

Although a trend was apparent, unilateral and bilateral vagal blockade did not significantly decrease R_{totL}, suggesting that increased responsiveness of airway smooth muscle to normal vagal tone or decreased baseline airway caliber was not the most important mechanism in the pathogenesis of antigen induced airway narrowing. Thus these data clearly indicate that local mechanisms such as direct effects of mediators of inflammation on airway smooth muscle or mechanical obstruction of airway lumens with debris or edema fluid are of critical importance in the antigen aerosol induced airway obstruction in ponies.

Although local mechanisms appear to be the most important in the increase in R_{totL} following unilateral challenge, data suggest a minor role for increased responsiveness of airway smooth muscle to normal vagal tone. Following vagal blockade there was a trend towards a decrease in R_{totL}. Although this decrease was not statistically significant because of variability in response to both challenge and vagal blockade, the magnitude of decrease in R_{totL} was larger than the decrease in R_{totR} and similar to the magnitude of decrease in R_{tot} during the second period of vagal blockade in the bilateral challenge experiment. If a decrease in baseline airway caliber was important, the decrease in R_{totL} following vagal blockade would have

been much larger than the decrease in R_{totR}. Therefore these data suggest that in challenged lungs, the effect of vagal blockade on airway smooth muscle is enhanced, i.e., that following antigen aerosol challenge airway smooth muscle responds more vigorously to normal vagal tone. This mechanism may have been responsible for the decrease in R_{tot} during the second period of vagal blockade in the bilateral challenge experiment, but because of an enhanced local effect was of minor importance in the response to antigen aerosol in the unilateral challenge experiment.

A change in C_{dyn} can be produced by changes in FRC, by an alteration in the elastic properties of the lung, or by the production of time constant inequalities between parallel lung units.[20] In addition, if significant time constant inequalities preexist, an increase in RR will cause a fall in C_{dyn}.[21] Since neither the quasistatic pressure-volume curve nor FRC changed with bilateral aerosol antigen challenge, the fall in C_{dyn} observed must have been caused by the production of time constant inequalities. Significant time constant inequalities did not preexist in these ponies, as in a previous study using the same animals we demonstrated that within a range of frequencies between 15 and 60 breaths per minute, C_{dyn} did not change.[9] The increase in R_{tot} and decrease in C_{dyn} following challenge suggests that both central and peripheral airways narrowed in response to challenge. Similar findings were reported by Drazen et al and Mills et al[22,3] in guinea pigs and Karczewski[2] in rabbits who concluded that antigen challenge in these species results in generalized bronchoconstriction.

Minimal volume increased but FRC was not changed following ovalbumin challenge, suggesting that ovalbumin challenge resulted in airway closure at lung volumes greater than MV, but not at FRC. Since the helium

equilibration method for FRC measurement only detects gas volumes in communication with the airways, gas trapping may have gone undetected. However in 3-methylindole (3MI) induced diffuse small airway disease in ponies, we measured a similar increase in MV, and a significant increase in FRC suggesting gas trapping at both these lung volumes in the 3MI disease model[23] and showing that helium equilibration could measure an increase in FRC. Our data therefore suggest that FRC did not increase following ovalbumin aerosol challenge. In persons and sheep, allergic lung disease increases FRC,[24,25] while in dogs and monkeys, no increase in FRC has been observed.[26,27] The reasons for these species differences are not clear, as the severity of the airway response to challenge does not correlate well with increases in FRC.

The results of this study show that ovalbumin aerosol challenge of sensitized ponies causes both large and small airway obstruction, characterized physiologically by an increase in R_{tot} and MV, and decrease in C_{dyn} and PaO_2 and pathologically by acute fibrinopurulent obstructive bronchiolitis, bronchitis, pulmonary edema and alveolar distension. Results further show that local mechanisms such as direct effects of mediators of inflammation on airway smooth muscle or mechanical obstruction of airway lumens with debris or edema fluid are of critical importance in the pathogenesis of airway obstruction in this disease model. In addition, increased sensitivity of airway smooth muscle to normal vagal tone may also play a role in the pathogenesis of ovalbumin challenge induced airway obstruction. Tachypnea following ovalbumin challenge is caused by increased activity of pulmonary receptors.

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CHAPTER 6

Response of the Locally Sensitized Equine Lung to Aerosol Ovalbumin Challenge: Role of Vagal Mechanisms

Introduction

In the previous chapter I investigated the role of vagal mechanisms in the response of the equine lung to aerosol antigen challenge.[1] Ponies were systemically sensitized by intramuscular injection of ovalbumin in complete Freund's adjuvant. During an initial aerosol challenge, resistance increased gradually and was 300% above baseline at 4 hours after challenge. Vagal mechanisms were involved in the genesis of tachypnea and both local and vagal mechanisms were responsible for the increase in total respiratory system resistance.

Since pilot studies suggested that aerosol challenge following both systemic and local sensitization of the lung results in more severe dyspnea of rapid onset, in the present study I investigated the pulmonary response to aerosol challenge in ponies sensitized both systemically and locally. In addition, I studied the role of local and vagal mechanisms in the response to challenge and correlated the physiologic findings with histologic lesions in the lung.

Materials and Methods

Four grade ponies between 2 and 10 years of age ($\bar{x}$ = 5.7 years) weighing 199.4 $\pm$ 28.3 kg were used in the experiments. The animals had bilateral cervical vagal loops and exteriorized carotid arteries.[2] Prior to use, ponies had been on pasture for at least two months and all were vaccinated against the common viral respiratory diseases. Animals were regularly examined to detect any signs of respiratory disease.

Sensitization of Ponies

Ponies were sensitized systemically by intramuscular injection and locally via aerosol. Ten mg ovalbumin dissolved in 2 ml of phosphate buffered saline solution and emulsified in 2 ml of complete Freund's adjuvant was divided and injected deep into the right and left triceps and semimembranosus muscles. This protocol was repeated two months later. Three weeks following the last intramuscular injection, an endotracheal tube was inserted into a tracheostoma. An ultrasonic nebulizer Devilbis model 65 was attached to the endotracheal tube via a one-way respiratory valve assembly. Two grams of ovalbumin in 40 ml of saline were aerosolized in 20 minutes.

Aerosol Challenge

In bilateral challenge experiments aerosol challenge was accomplished by delivery of 2 g of ovalbumin in 40 ml saline via a tracheostomy tube and one-way valve assembly using a Devilbis model 65 ultrasonic nebulizer. In unilateral challenge experiments the right and left mainstem bronchi were intubated with specially prepared cuffed tubes via a tracheostoma created in the lower 1/3 of the cervical trachea. The distal ends of the endobronchial tubes were coated with a thin layer of anesthetic cream to prevent excessive coughing during intubation. A side hole catheter, incorporated into the distal end of the tubes, was used to measure bronchial airway opening pressures. Seal of the cuffs was ascertained by 1) visual inspection using a fiberoptic bronchoscope, and 2) ventilation of the left lung with 80% He, 20% O_2 mixture for 10 minutes and failure to measure helium in the gas expired from the right lung.

In unilateral challenge experiments, the ultrasonic nebulizer and one-way respiratory valve assembly were used to deliver 1 gm ovalbumin in 20 ml of saline in 20 minutes.

Pulmonary Function Measurements

Experiments were performed with animals restrained in stocks and tranquilized with intravenous xylazine (0.5 mg/kg of body weight). The methods and reproducibility of pulmonary function measurements have been previously described.[3,4] Briefly, air flow ($\dot{V}$) and tidal volume (V_T), measured using a pneumotachograph[b] (Fleisch #4, Dynasciences, Blue Bell, PA) transducer (Model PM5, Statham Inst., Hato Rey, PR), attached to a cuffed endotracheal tube and inserted into the trachea via a tracheostoma, were recorded on light sensitive paper (Model VR6, Electronics for Medicine, White Plains, NY). Transpulmonary pressure (P_{tp}) was measured as the pressure difference between the mid portion of the thoracic esophagus and the airway opening, using identical catheter systems attached to a differential pressure transducer (Model P131, Statham Inst., Hato Rey, PR). From the recording of P_{tp}, $\dot{V}$ and V_T, dynamic compliance (C_{dyn}), respiratory rate (RR) and minute ventilation ($\dot{V}_{min}$) were calculated.

Quasistatic pressure-volume curves between functional residual capacity (FRC) and total lung capacity (TLC) were generated on an x-y plotter (Model XY 575, Esterline Angus, Indianapolis IN), using an air driver pressure cycled ventilator (Mark 9, Bird Corp., Palm Springs, CA). The deflation limb of the quasistatic pressure-volume curve was empirically described as a single rising exponential. The curve was fit by computer to the equation

$$V = V_{max} (1-e^{-\alpha P_{tp}}) \quad (1)$$

where V = lung volume at a given P_{tp}, V_{max} is the volume at which the slope of the curve is 0 (i.e., P_{tp} is infinite) and α describes the rate of rise of the curve from functional residual capacity (FRC) to V_{max}.[5] Quasistatic compliance (C_{stat}) was calculated from the first derivative of equation #1 at P_{tp} = 3 cm H_2O. Functional residual capacity was measured by helium equilibration and total lung capacity (TLC) was defined as the lung volume at P_{tp} = 30 cm H_2O.

Prior to measurement of total respiratory system resistance (R_{tot}) animals were force ventilated 4 times up to an airway opening pressure of 30 cm H_2O to ensure a constant volume history and to create a period of apnea lasting between 2 and 30 sec. During this period of apnea, the respiratory system was oscillated at its resonant frequency (3-5 Hz) and airway opening pressure (P_{ao}) and flow were plotted on a photorecording x-y plotter (Model VR6, Electronics for Medicine, White Plains, NY). Total respiratory resistance was calculated as the slope of the resulting line. In the experiments in which left and right lungs were intubated separately, the two endobronchial tubes were connected with a y tube so that a volume history was provided simultaneously to both lungs. The right and left lungs were then oscillated separately at their resonant frequencies and bronchial opening pressure and flow were plotted on an x-y plotter. Left and right lung resistances (R_{totL} and R_{totR}) were calculated as the slope of the resulting lines.

Vagal Blockade

The vagus was reversibly blocked by circulating coolant at a temperature of -2°C through copper coils, wrapped around both loops. In an

earlier study on the same ponies,[2] we established criteria of bilateral cervical vagal blockade: tachycardia, slow deep breathing, and paresis of the crycoarytenoides dorsalis muscle, determined by failure of the arytenoid cartilages to abduct during inhalation as observed through an endoscope (Model BF type B2, Olympus Co., New Hyde Park, NY).

Experimental Protocol

The left lungs of ponies were challenged with 1 g ovalbumin in 20 ml of saline. Arterial blood gas tensions, RR, R_{totL} and R_{totR} were measured during a baseline period, one hour after challenge and during both ipsilateral and bilateral vagal blockade. At least 3 months later, ponies were challenged through the endotracheal tube with 2 g ovalbumin in 40 ml saline. Pulmonary function measurements were made during a baseline period, one hour after challenge and following bilateral vagal blockade. Following this protocol animals were euthanized and subjected to postmortem examination.

Postmortem Examination

Animals were euthanized with an overdose of pentobarbital and exsanguinated. After the gross appearance of the lung was noted, minimum volume of the lungs was determined by water displacement. The minimum volume was compared to that of 5 ponies, free of clinical apparent lung disease, euthanized and exsanguinated in the same manner. Tissue sections were fixed in phosphate buffered formalin, sectioned at 5 microns, and stained with H & E.

Figure 6-1 Respiratory rate (RR) ($\bar{x} \pm$ SEM), left and right lung resistance (R_{totL} and R_{totR}) measured during a prechallenge period, one hour after unilateral challenge and following unilateral and bilateral vagal blockade. (IVB and VB). Stars indicate significant differences from prechallenge value. Asterisk indicates significant effect of VB compared to the challenge measurement period.

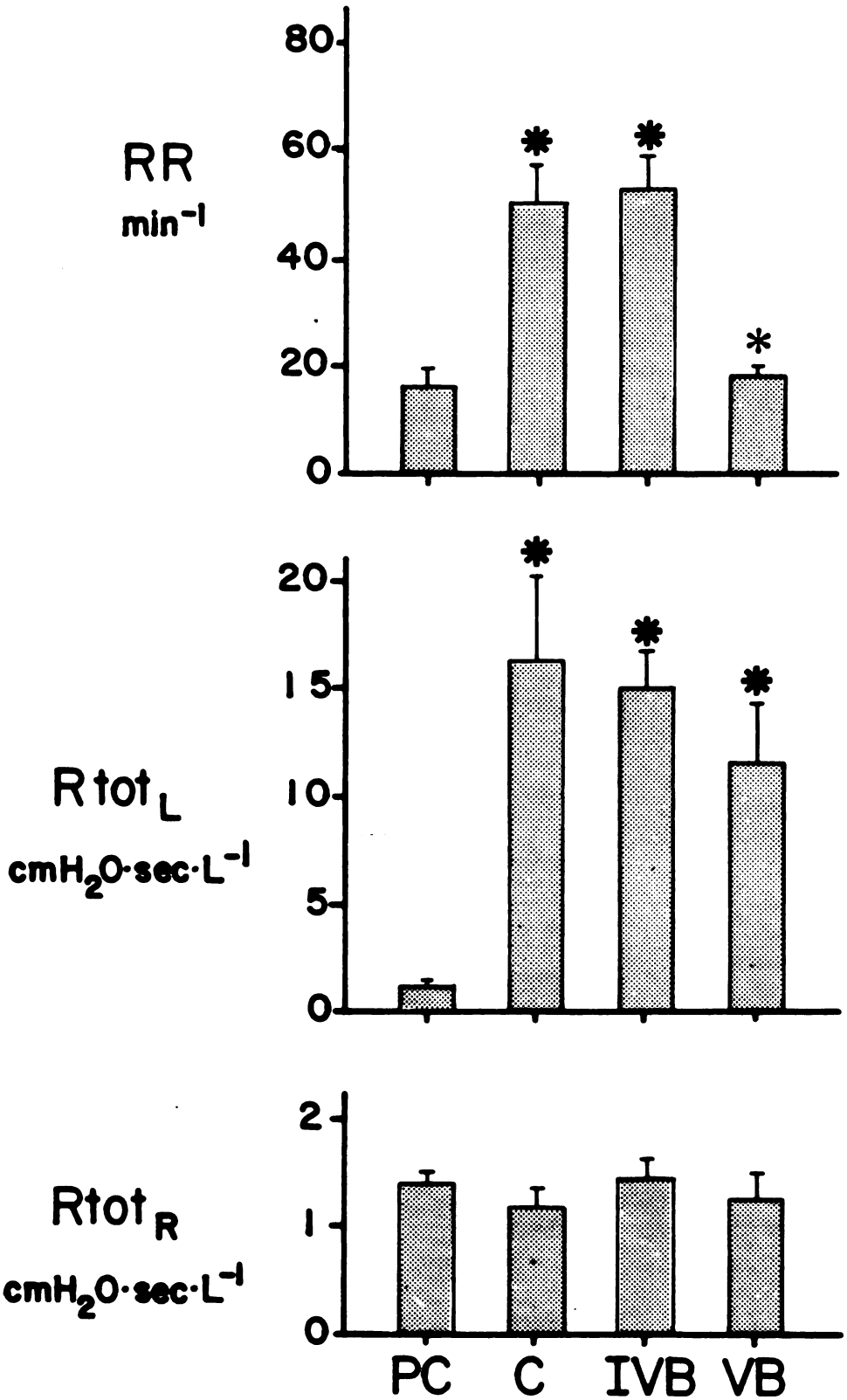
RR
min⁻¹
80
60
40
20
0
Rtot L
cmH2O·sec·L⁻¹
20
15
10
5
0
Rtot R
cmH2O·sec·L⁻¹
2
1
0
PC
C
IVB
VB

Figure 6-1

Figure 6-2 Respiratory rate (RR) ($\bar{x} \pm$ SEM), tidal volume (V_T), minute ventilation ($\dot{V}_{min}$), total respiratory system resistance (R_{tot}), dynamic and quasistatic compliance (C_{dyn} and C_{stat}), total lung capacity (TLC), functional residual capacity (FRC) and PaO_2, measured during a baseline period, one hour after bilateral challenge and following unilateral and bilateral vagal blockade (IVB and VB). Stars indicate significant differences from preceding value.

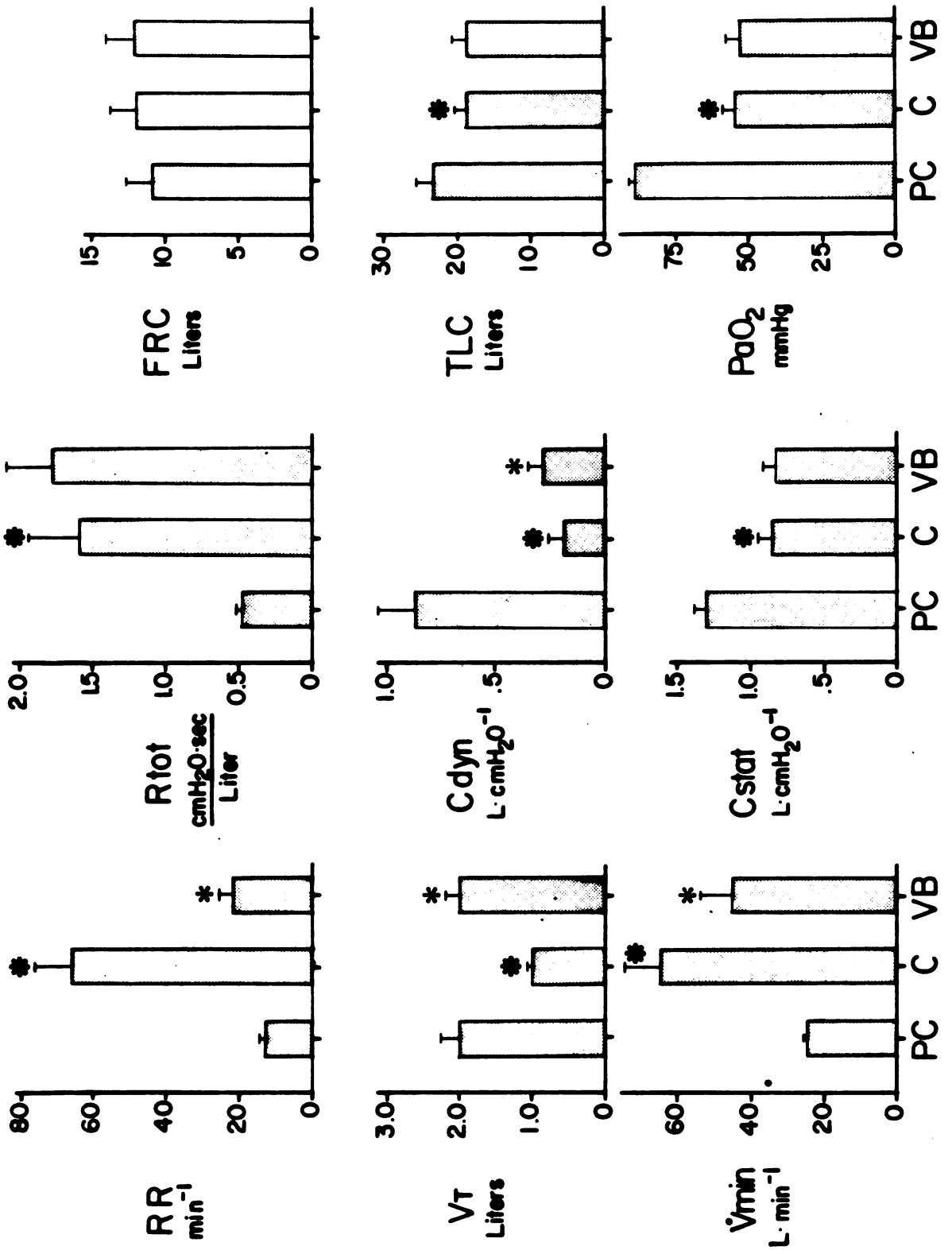
RR
min⁻¹
80
60
40
20
0
Rtot
cmH2O·sec
Liter
2.0
1.5
1.0
0.5
0
FRC
Liters
15
10
5
0
Vt
Liters
3.0
2.0
1.0
0
Cdyn
L·cmH2O⁻¹
1.0
.5
0
TLC
Liters
30
20
10
0
Cstat
L·cmH2O⁻¹
1.5
1.0
.5
0
PaO2
mmHg
75
50
25
0
Vmin
L·min⁻¹
60
40
20
0
PC
C
VB
*

Figure 6-2

Figure 6-3 Photomicrograph of a bronchiole in the challenged lung, 5 hours after unilateral ovalbumin challenge.

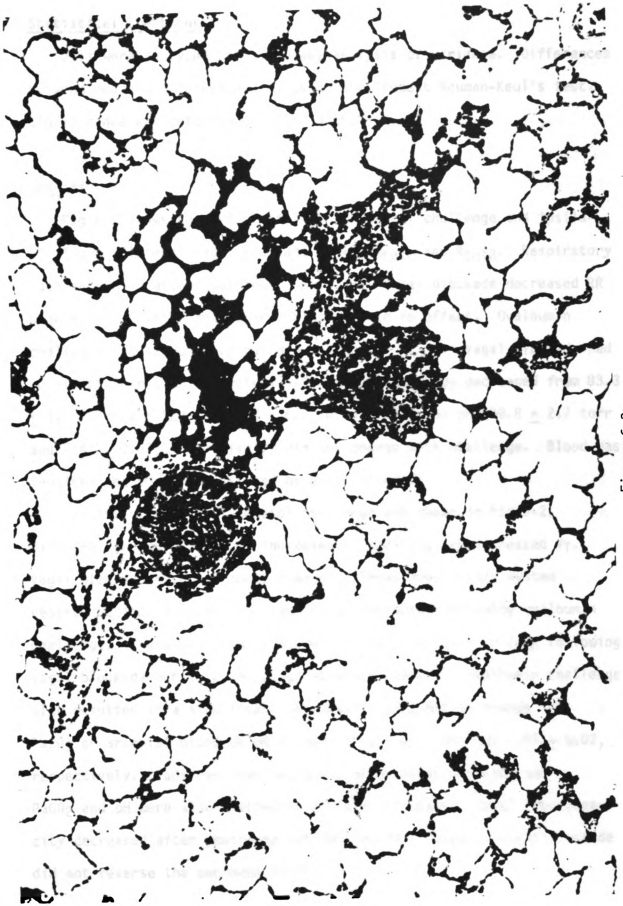

Figure 6-3

Statistical Treatment

Data were analyzed using two-way analysis of variance. Differences between means was determined by using the Student Neuman-Keul's test. Significance was determined at $P < 0.05$.[6]

Results

Fig 6-1 shows the effect of left unilateral challenge and ipsilateral and bilateral vagal blockade on RR, R_{totL} and R_{totR}. Respiratory rate increased after challenge. Bilateral vagal blockade decreased RR significantly while ipsilateral blockade had no effect. Ovalbumin challenge increased R_{totL} but did not change R_{totR}. Vagal blockade had no effect on either parameter. After challenge PaO_2 decreased from 83.3 $\pm$ 1.5 torr ($\bar{x} \pm$ SEM) to 66.7 $\pm$ 6.7 torr. $PaCO_2$ and pH (39.8 $\pm$ 2.7 torr and 7.41 $\pm$ 0.02, respectively) did not change with challenge. Blood gas tensions and pH were unaltered by vagal blockade.

Results of bilateral aerosol challenge are shown in Fig 6-2. Bilateral aerosol challenge increased RR and $\dot{V}_{min}$ and decreased V_T. Vagal blockade reversed these changes. Total respiratory system resistance increased and C_{dyn} and C_{stat} decreased following ovalbumin challenge. There was a small but significant increase in C_{dyn} following vagal blockade but R_{tot} and C_{stat} were not changed. Ovalbumin challenge also resulted in a significant decrease in PaO_2 but no change in $PaCO_2$ or arterial blood pH which were 40.0 $\pm$ 2.5 torr and 7.41 $\pm$ 0.02, respectively. PaO_2 remained depressed after vagal blockade and $PaCO_2$ and pH were also unaffected by this treatment. Total lung capacity decreased after challenge but FRC was not changed. Vagal blockade did not reverse the decrease in TLC.

Gross Pathology

Lungs failed to collapse after removal from the thorax, with clearly delineated rib impressions as a result of lung hyperinflation. The lungs were blotchy dark red and firm. Numerous petechia were present. Minimum volume per kg of body weight of the challenged lungs was 35.7 $\pm$ 1.3 ml/kg ($\bar{x} \pm$ SEM) as compared to 20.8 $\pm$ 1.4 ml/kg for the lungs of 5 control ponies.

Histopathology

The most striking lesions were present in the smaller airways but pathologic changes were not restricted to these areas. Peribronchiolar areas and bronchiolar lumens had large accumulations of a cellular exudate consisting principally of neutrophils, but with lesser numbers of eosinophils. Neutrophils were frequently present within the bronchiolar wall (Fig 6-3). The bronchiolar mucosa was extensively folded and the smooth muscle in the wall was especially prominent, suggesting the presence of considerable airway constriction.

The gas exchange areas of the lung were not uniformly affected. Multifocal areas of alveolar edema and hemorrhage were scattered throughout the lung. There were mild focal aggregates of neutrophils in alveolar walls and some of these areas also had accompanying congestion, hemorrhage and edema. Except for the foci of hemorrhage and edema the lung parenchyma was well inflated. Patchy hyperinflation was observed, particularly in subpleural areas.

The lumens of the bronchi and trachea had small accumulations of proteinateous fluid containing variable numbers of neutrophils. The mucosa was also infiltrated with neutrophils and the submucosa was

congested and had prominent focal aggregates of neutrophils. In contrast to the smaller airways, where the lumens were plugged with cellular exudate, the exudate in these larger airways was less cellular and did not obstruct the lumens. In addition to these acute pathologic changes, focal lesions of a more chronic nature were occasionally observed, and were assumed to be the result of pre-existing lung disease.

Discussion

The results of aerosol antigen challenge in this group of ponies which were sensitized by intramuscular and aerosol exposure are clearly different from the results of a previous study where ponies were sensitized only by the intramuscular route.[1] In the present study, following bilateral challenge, R_{tot} increased by 300% and C_{dyn} decreased to 18% of control within one hour. In the previous study, there was no significant increase in R_{tot} at 1 hour but 4 hours after challenge resistance had increased 300%, while C_{dyn} decreased to 75% of control. In addition, aerosol challenge following intramuscular sensitization alone produced a smaller decrease in PaO_2 and no change in C_{stat} and TLC. Therefore it appears that aerosol challenge following both systemic and local sensitization of the lung results in a more severe and more rapid response than occurs following challenge of ponies, sensitized by the systemic route alone. Differences in response to antigen challenge, dependent upon route and method of sensitization, have been previously reported in other species.[7]

In contrast to the differences in the mechanical response of the lung to challenge in the two studies, changes in RR were independent of

the route of sensitization. Both systemic and systemic and local sensitization resulted in tachypnea within 1 hour after challenge. The tachypnea may have been caused by increase in core temperature, anxiety, pulmonary mechanical changes, changes in arterial blood gas tensions and pH, or increased activity of pulmonary receptors, with their afferents in the vagus nerve.[8] Rectal temperature did not change during the experiments and anxiety is not likely to have played a role in the tachypnea, as challenge with saline or bovine γ globulin does not change RR in ponies.[1] Respiratory rate changes were independent of pulmonary mechanics changes, because pulmonary mechanics were unaltered by vagal blockade, while RR decreased. Although PaO_2 decreased following challenge, the magnitude of decrease is insufficient to solely account for the tachypnea.[9] Since vagal blockade reversed the increase in RR, these data suggest that in ponies as in dogs tachypnea following aerosol antigen challenge is mainly caused by increased activity of pulmonary receptors with their afferents in the vagus nerve. The conclusion is supported by results from the unilateral challenge experiment in which bilateral vagal blockade eliminated tachypnea. Since RR did not decrease following left unilateral vagal blockade alone, these results further suggest that vagal afferent fibers crossover to the contralateral vagus nerve in the thorax. In both the unilateral and bilateral challenge experiments, following vagal blockade RR did not decrease below baseline value as it should have done if vagal mechanisms alone were responsible for the tachypnea observed. Muir et al[9] reported in horses that a decrease in PaO_2 from 89.2 to 55.9 mmHg as occurred following bilateral ovalbumin challenge results in an increase in RR of approximately 12 breaths per minute. This increase is similar to the

difference between observed and expected RR following vagal blockade, suggesting that the decreased PaO_2 was responsible for the failure of RR to decrease below baseline value.

Following bilateral ovalbumin challenge, R_{tot} increased 300%. In the dog, the majority of resistance to flow resides in the central airways, while the peripheral airways contribute little to R_{tot}.[10] If this is also true in the horse, the increase in R_{tot}, combined with a large decrease in C_{dyn} in the bilateral challenge experiments, may be attibuted either to a modest large airway narrowing or to the massive small airway obstruction which was observed histologically.[11] Failure of vagal blockade to alter R_{tot} suggests that vagal mechanisms were not involved in the increased R_{tot} following challenge. This conclusion is supported by data from the unilateral challenge experiment because challenge of the left lung alone increased R_{totL} but did not change R_{totR} and because vagal blockade had no effect on R_{totL}. Since vagal mechanisms were not involved, the increase in R_{tot} following challenge must be due to local mechanisms, such as direct effect of mediators of inflammation on airway smooth muscle or mechanical obstruction of airways by debris or edema.

This conclusion is slightly different from that reached in systemically sensitized ponies.[1] In this latter group of ponies, vagal blockade partially reversed the increase in R_{tot} following bilateral challenge. We concluded that in addition to local mechanisms increased sensitivity of airway smooth muscle to normal vagal tone also played a role in the response of the lung to antigen challenge.[1]

The increase in R_{totL} following left unilateral ovalbumin challenge was nearly an order of magnitude greater than the increase in R_{tot} following bilateral challenge. Because the endobronchial tubes

were shorter and narrower than the endotracheal tube and located in a mainstem bronchus rather than the trachea, differences in amount and deposition of aerosol may account for this discrepancy. Using the same challenge technique we previously reported a similar difference in response to unilateral and bilateral antigen challenges in systemically sensitized ponies.[1]

Dynamic compliance can be decreased by a change in lung volume at which tidal breathing is accomplished by an alteration of the elastic properties of the lung or by a prolongation of peripheral time constants. The decrease in C_{dyn} following challenge was not caused by an increase in lung volumes as FRC did not change. Although C_{stat} decreased, the magnitude of this change was too small to solely account for the decrease in C_{dyn}. Therefore the marked decrease in C_{dyn} suggests that ovalbumin aerosol challenge resulted in prolongation of peripheral time constants, probably caused by the small airway obstruction which was observed histologically. Small airway obstruction probably also resulted in the increase in MV and decreased PaO_2. Since following small airway obstruction C_{dyn} may become frequency dependent,[12] the small but significant rise in C_{dyn} following vagal blockade may have been due to the concurrent decrease in RR.

Functional residual capacity was unaltered by challenge or vagal blockade. Following antigen challenge in some species FRC increases,[13] while in others FRC does not change.[14,15] The reason for this discrepancy is not clear as the change in FRC does not correlate with the severity of airway response as judged by changes in resistance and C_{dyn}.

The decrease in both TLC and C_{stat} following challenge may also have

resulted from diffuse peripheral airway obstruction and failure to recruit obstructed air spaces during inflation of the lung to 30 cm H_2O P_{tp}. In addition, because C_{stat} decreased and FRC was unchanged, specific compliance of the lung also decreased. A decrease in TLC following antigen challenge has also been reported in the guinea pig[16] but not in the monkey or dog.[14,15] In the guinea pig, this decrease in TLC was reversible by vagal blockade, suggesting that alveolar duct constriction was an important factor. This does not appear to be the case in the pony.

Distribution and severity of histopathologic lesions correlated with pulmonary function data. The most dramatic changes in pulmonary function were the large decrease in C_{dyn} and increase in MV. These changes were probably associated with the principal histologic lesions of severe necrotizing bronchiolitis and bronchiolar obstruction. The multifocal alveolitis and edema probably resulted in the change in the elastic properties of the lung and the decrease in TLC. Histologically, large airways were less affected by ovalbumin challenge than small airways. However, bronchoconstriction may have occurred in response to challenge and therefore we cannot determine whether the 3-fold increase in R_{tot} following challenge was due to massive small airway obstruction or large airway narrowing.

In this study we have shown that the tachypnea following ovalbumin aerosol challenge of conscious ponies is mediated via vagal afferents but that airway obstruction following challenge is caused by local mechanisms such as the obstruction of airway with exudate, debris, mucus and edema fluid. We also conclude that in this model of lung disease, changes in pulmonary function following challenge are predictive of the major histologic lesions.

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CONCLUDING DISCUSSION

When comparing the derangement in pulmonary function induced by the oral administration of 3MI and ovalbumin challenge of locally and systemically sensitized ponies, certain similarities are striking. Tachypnea characterized both the 3MI induced pulmonary toxiosis and the 2 allergic disease models and was mediated by pulmonary receptors with their afferent neurons in the vagus nerve. Tachypnea is characteristic of a variety of lung diseases in mammals and this study suggests that activation of pulmonary receptors with afferent neurons in the vagus nerve may be a stereo typical response to lung injury in the equid.

A decrease in Cdyn, an increase in MV and impairment of gas exchange occurred in both the 3MI model as well as the ovalbumin induced allergic disease models. These changes in pulmonary function are suggestive of small airway obstruction, which was confirmed at necropsy, as the major histopathologic lesion in all 3 pulmonary disease models was an obstructive bronchiolitis. Vagal blockade did not change Cdyn or gas exchange, suggesting that vagal mechanisms were not involved in the small airway obstruction in these pulmonary disease models. Interestingly, naturally occurring obstructive pulmonary disease in the horse and in persons is also characterized by obstructive bronchiolitis and, therefore, it appears that the small airways are a weak link in the mammalian pulmonary defence system. Following 3MI administration the increase in Rtot was 30% while following ovalbumin challenge Rtot increased 300%. This

suggests that the pulmonary lesions induced by 3MI were primarily in the small airways, while ovalbumin challenge also resulted in large airway obstruction. This was supported by histopathologic findings as no large airway lesions were present in 3MI treated horses while bronchitis was described following ovalbumin challenge. The studies presented in this dissertation suggest that the role of vagal mechanisms in airway obstruction in the 3 pulmonary disease models is minor in importance. Although R_{tot} decreased following vagal blockade in 3MI treated ponies, this change in R_{tot} could be attributed to the interruption of normal parasympathetic bronchomotor tone. In the allergic pulmonary disease models it was clearly demonstrated that local mechanisms were of major significance in the pathogenesis of airway obstruction. However, an increased sensitivity to normal vagal tone may have contributed to the airway obstruction in systemically sensitized ponies following ovalbumin challenge.

In some species, small airway obstruction is accompanied by an increase in FRC, while in others it is not. The reason for this discrepancy is not clear as the severity of the obstruction does not correlate with changes in FRC. In this study following 3MI treatment FRC increased while following ovalbumin challenge FRC did not change. The reason for this discrepancy remains unexplained.

Total lung capacity and quasistatic compliance decreased only following ovalbumin challenge in ponies sensitized both via the intramuscular and aerosol routes. Since the decrease in C_{dyn} in this model of lung disease was an order of magnitude larger than the decrease in C_{dyn} following 3MI treatment of ovalbumin challenge of systemically sensitized ponies, a more severe small airway obstruction may have resulted

in failure to recrute obstructed lung units at a P_{tp} of 30 mm H_2O. Morphometric studies were not performed on the histopathologic specimens and, therefore, this hypothesis could not be tested.

SUMMARY AND CONCLUSION

In the study reported in Chapter 1, pleural and esophageal pressures were compared in 6 standing sedated ponies. Pleural pressure was measured with blunt needles attached to transducers and inserted in the 10th intercostal space level with and 10 and 20 cm above the point of the shoulder. Two balloons (a condom and an esophageal balloon) attached to transducers measured esophageal pressure in the cranial, middle, and caudal portions of the thoracic part of the esophagus. Tidal volume was measured by integrating a flow signal derived from a pneumotachograph attached to an endotracheal tube inserted through a tracheostomy. Frequency responses of all measuring systems were matched. The change in pleural pressure during respiration was greatest in the middle and ventral portions of the thorax, less in the dorsal portion of the thorax and in the middle and caudal portions of the thoracic part of the esophagus, and least in the cranial portion of the thoracic part of the esophagus. The type of esophageal balloon had no effect on the measured pressure change and using either balloon, changes in esophageal pressure reflected local changes in pleural pressure. Regional variations in esophageal or pleural pressure during breathing caused variations in the calculated dynamic compliance. Pleural pressure gradients of 0.33 cm of water/cm of descent and 0.484 cm of water/cm of descent were recorded in the dorsal and ventral halves of the thorax, respectively, and may result in regional variations in lung

inflation similar to those observed in persons.

In Chapter 2, a surgical technique is described for preparation of chronic cervical vagal loops in ponies. Vagal blockade was induced by circulating methanol (-2°C) through coils which enclosed the loops. Vagal blockade increased tidal volume, heart rate, and systemic blood pressure and decreased respiratory rate. Atropine 0.04 mg/kg intravenously increased heart rate and systemic pressure but did not alter respiratory parameters indicating vagal cooling caused both afferent and efferent blockade. The effects of vagal blockade were rapidly reversed when refrigerated coils were removed.

In order to determine the short and long-term reproducibility of pulmonary function tests in ponies. Arterial blood gas tensions, pulmonary mechanics and lung volumes were measured in 4 sedated animals every hour for 6 hours and in 5 animals 4 times at 2 monthly intervals. (Chapter 3) Variability in blood gas tensions was small over both the short and long-term measurement periods, while the variability in total respiratory resistance (R_{tot}) and functional residual capacity (FRC) was small over the short term but larger over the long term. The variability in tidal volume (V_T), minute ventilation ($\dot{V}_{min}$), respiratory rate (RR) and dynamic and quasistatic compliance (C_{dyn} and C_{stat}) was relatively large over both the short and long term. When data from five ponies was pooled no significant change occurred in any of the variables over a period of six months.

Vagal blockade increased V_T and decreased RR and R_{tot}, but arterial blood gas tensions, $\dot{V}_{min}$, C_{dyn}, C_{stat}, FRC and lung and thoracic cage pressure-volume curves were unaffected.

Total respiratory resistance decreased with increasing lung volume with the vagus intact. Following vagal blockade the decrease in R_{tot} with lung volume was minimal.

Dynamic compliance was frequency independent over a range of 15-60 breaths min^{-1}, suggesting that significant inhomogeneity of peripheral time constants did not exist in our normal ponies.

Chapter 4 reports changes in arterial blood gas tensions, pulmonary mechanics and lung volumes, 24 to 48 hours after oral administration of either 500 ml of corn oil or 100 mg/kg body weight of 3-methylindole (3MI) in 500 ml of corn oil. In the latter group, variables were also measured after bilateral cervical vagotomy. Respiratory rate (RR) and minute ventilation ($\dot{V}_{min}$) were increased by 3MI treatment and decreased after vagotomy, suggesting that the tachypnea induced by 3MI was vagally mediated. Pa_{O_2} was unaffected but $PaCO_2$ decreased below baseline following 3MI and vagotomy. Both specific respiratory conductance (SG_{tot}) and dynamic compliance were decreased by 3MI. Following vagotomy SG_{tot} was increased but remained below baseline level, suggesting that local mechanisms were involved in the pathogenesis of airway narrowing. The increase in SG_{tot} following vagotomy may have been due to interruption of normal parasympathetic bronchomotor tone. Functional residual capacity, which increased following 3MI, was unaffected by vagotomy. Total lung capacity and quasistatic compliance were unaffected by either treatment. Minimal volume was larger in 3MI treated ponies than in a group of untreated ponies. Decreased dynamic compliance and specific respiratory conductance and increased functional residual capacity and minimal volume are all compatible with small

airway obstruction produced by the necrotizing bronchiolitis and bronchiolar obstruction observed histologically in 3MI treated ponies.

In Chapter 5, in awake sensitized ponies, we studied the effect of aerosol ovalbumin challenge on ventilation, pulmonary mechanics, lung volumes and gas exhange before and after vagal blockade. We also challenged the left lung and measured respiratory rate (RR), and right and left lung resistance (R_{totR}, R_{totL}) before and after both left and bilateral vagal section. Bilateral ovalbumin aerosol challenge increased RR, minute ventilation ($\dot{V}_{min}$), respiratory resistance (R_{tot}) and minimal volume, decreased dynamic compliance and arterial oxygen tension, and was without effect on functional residual capacity, total lung capacity, quasistatic lung compliance, and arterial carbon dioxide tension. Vagal blockade reversed the increase in RR, $\dot{V}_{min}$ and R_{tot} and increased V_T. Challenge of the left lung increased RR and R_{totL} but did not alter R_{totR}. Bilateral vagal section reversed the tachypnea but unilateral section did not. Histopathologic lesions included acute fibrino-purulent obstructive bronchiolitis, bronchitis, edema and alveolar distension. We conclude that local mechanisms are of critical importance in the pathogenesis of ovalbumin induced airway obstruction in ponies, that increased sensitivity of airway smooth muscle to normal vagal tone may also play a role and that tachypnea following challenge is caused by activity of pulmonary receptors with vagal afferent fibers.

Since pilot studies suggested that aerosol challenge following both systemic and local sensitization of the lung results in more severe dyspnea of rapid onset, in Chapter 6, in awake ponies, sensitized systemically by intramuscular injection and locally via aerosol, we studied ventilation, pulmonary mechanics, lung volumes and gas exchange before

and one hour after bilateral aerosol ovalbumin challenge and after left unilateral and bilateral vagal blockade. We also challenged the left lung and measured respiratory rate (RR) and right and left lung resistance (R_{totR} and R_{totL}) during the same measurement periods. Bilateral ovalbumin aerosol challenge increased RR, minute ventilation ($\dot{V}_{min}$), respiratory system resistance (R_{tot}) and minimum volume, decreased dynamic compliance, quasistatic compliance, PaO_2, tidal volume, total lung capacity and was without effect on functional residual capacity and $PaCO_2$. Bilateral and not unilateral vagal blockade decreased RR, $\dot{V}_{min}$, and increased V_T and C_{dyn}. Challenge of the left lung increased RR and R_{totL} but did not alter R_{totR}. Bilateral vagal blockade reversed the tachypnea but unilateral blockade did not. Pulmonary function changes following challenge in this group of ponies was more severe than in ponies sensitized only by intramuscular injection. Histopathologic lesions included acute fibrinopurulent obstructive bronchiolitis, bronchitis, and alveolar distension. We conclude that in this disease model local mechanisms are of critical importance in the pathogenesis of airway obstruction, that tachypnea following challenge is caused by increased activity of pulmonary receptors with vagal afferent fibers and that changes in pulmonary function following challenge are predictive of the major histologic lesions.

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