AIRWAY RESPONSES TO AEROSOLIZED METHACHOLINE AND CITRIC ACID IN PONIES WITH RECURRENT AIRWAY OBSTRUCTION (HEAVES)

By

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ABSTRACT

AIRWAY RESPONSES TO AEROSOLIZED METHACHOLINE AND CITRIC ACID IN PONIES WITH RECURRENT AIRWAY OBSTRUCTION (HEAVES)

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We measured lung function and airway reactivity in response to aerosols of methacholine and citric acid in ponies with a history of heaves (principals) and in ponies with no history of respiratory disease (controls). Principals were paired with controls, and measurements were made when principals were in clinical remission (Period A), following barn exposure when principals had acute airway obstruction (Period B), and 1 and 2 weeks after they were returned to pasture (Periods C and D). Differences between groups were primarily found at Period B. Barn housing (Period B) decreased dynamic compliance, increased pulmonary resistance, and caused airway hyperreactivity to methacholine and citric acid aerosols in principals but not in controls. Inhalation of 10% citric acid aerosol by principals at Period B induced changes only in pulmonary resistance. We conclude that ponies in clinical remission from heaves do not exhibit nonspecific airway hyperreactivity. Hyperreactivity exists only during acute exacerbations of airway obstruction.

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I. INTRODUCTION

Asthma is a common respiratory disease that affects between three and seven percent of the American population,[1,2] and annually causes over four thousand deaths in this country.[3] It is a leading cause of morbidity among infants and children and is responsible for more lost school days in the United States than any other single chronic condition.[4] Despite the impressive degree of morbidity and mortality that exists with asthma, it is still true that "in almost no other field is the gap between diagnostic and therapeutic knowledge and its general application so great."[5]

Eberle, a Philadelphia physician, defined asthma in 1830 as a "paroxysmal affection of the respiratory organs, characterized by a great difficulty of breathing, tightness across the breast, and a sense of impending suffocation, without fever or local inflammation."[6] Despite tremendous strides forward in our understanding of the pathophysiology of the respiratory tract since that time, bronchial asthma continues to defy rigorous definition. In 1962, the American Thoracic Society attempted to define asthma in terms of the clinical and functional abnormalities that occur.[7] By this definition, asthma is "a disease characterized by increased responsiveness of the airways to various stimuli, manifested by prolongation of forced expiration, which changes in severity either spontaneously or as a result of therapy." This definition, by focusing attention on airway hyperreactivity as an

essential feature of the disease, has paved the way for two and one half decades of intensive research on the physiological, immunological, biochemical, and pharmacological basis for this response. Widespread application of pulmonary function testing and bronchoprovocation techniques has made it possible to identify asthmatic patients who do not exhibit the classical signs of episodic wheezing and dyspnea. Unfortunately, the definition is sufficiently nonspecific that it also allows the criteria for asthma to be met when other pathological processes are clearly present.[8] An additional problem with this definition arises from the observation that some asthmatic individuals, especially those who develop the condition late in life or suffer from it for many years, show little or no response to currently available treatments.[9] As a result of such limitations, there is still no agreement on a universal definition of asthma. Clearly, a more complete understanding of the physiological and pathological abnormalities that occur in bronchial asthma is needed to resolve this dilemma.

Physiological studies on healthy volunteers and on asthmatic patients have greatly expanded our knowledge about this complex disease. There are, however, limits to which this type of investigation can be carried. Studies on the structural derangements in asthmatic lungs are largely based on information from the relatively few patients who die in status asthmaticus.[10] It is unlikely, therefore, that we have an accurate picture of the changes that occur in the airways of patients who have mild to moderately severe asthma.[10]

As these limitations are reviewed, it is clear that there is a need for an animal model that parallels the clinical, physiological, and pathological abnormalities of human asthma. The dilemma posed by the

difficulty in defining asthma becomes apparent when one considers that "a disease can be modelled only when the disease itself is adequately defined."[11] Despite the problems involved in defining this particular disorder, it is widely accepted that the common denominator underlying the asthmatic diathesis is a nonspecific hyperirritability of the tracheobronchial tree.[1] Clinical and animal research has revealed important clues as to the mechanisms of altered airway responsiveness in asthma. Evaluation of the currently available animal models of bronchial hyperreactivity has lead to the conclusion that recurrent airway obstruction, or "heaves," in horses and ponies "may be the closest truly spontaneous disease yet described in animals that parallels the human disease of asthma."[8] The purpose of the study described in this thesis was to further investigate the phenomenon of airway hyperreactivity in ponies with heaves by characterizing their response to aerosol delivery of a cholinergic agonist (methacholine) and a nonspecific irritant receptor stimulant (citric acid).

II. GENERAL LITERATURE REVIEW

A. Bronchial Hyperreactivity in Asthma

From an etiological standpoint, asthma is a heterogeneous disease. It is useful for epidemiological and clinical purposes to classify the forms of this disorder by the principal stimuli that incite or are associated with acute episodes. The pathogenetic mechanisms responsible for producing the clinical signs of asthma appear to be multiple and complex. It is important to emphasize, however, that nonspecific airway hyperresponsiveness is the central common feature in all forms of asthma. Thus, the distinction between various types of asthma may be artificial, and the response of patients within a given subclassification may be initiated by more than one type of stimulus.[1] With this reservation in mind, asthma can be described in two broad groups: allergic and idiosyncratic.[1]

Allergic or extrinsic asthma usually affects children and young adults, and is frequently seasonal. This form of asthma is believed to occur as a result of inhalation, or less often ingestion, of an antigen that reacts with an immunoglobulin E (IgE) antibody.[6] Allergic asthma is often associated with a personal or family history of allergic diseases, a positive response to intradermal skin testing, increased serum concentrations of IgE, and/or a positive response to provocation tests involving the inhalation of specific antigen.[12] Based on these defined immunological criteria, however, a significant segment of the

asthmatic population cannot be readily classified. These patients are said to have idiosyncratic or intrinsic asthma. Bronchoconstriction in patients with idiosyncratic asthma can be triggered by diverse stimuli such as exercise, laughter, aspirin ingestion, and emotional stress.[1]

The observation that allergic patients exposed to allergens can develop both early and late reactions in the respiratory tract dates back more than a century.[13] The early or immediate asthmatic response (EAR) occurs 10-30 minutes after allergen exposure and represents an IgE-mediated reaction to allergen.[14] This aspect of the biphasic response has proven to be relatively easy to reproduce in experimental animals. Consequently a variety of in vivo model systems are available for study. Bronchospasm and edema characterize this phase of the asthmatic response.[15] A characteristic of the EAR is that beta-adrenergic agonists are effective in preventing or reversing bronchoconstriction.[16] The late asthmatic response (LAR) occurs 2-12 hours after allergen exposure and appears to be largely due to the inflammatory component of allergic asthma.[14,15] An important initial observation was made by Herxheimer[17] in 1952, when he pointed out that patients with LARs had more severe asthma than patients without these reactions. It has subsequently been found that allergen-induced LARs are associated with a persistent increase in bronchial responsiveness to histamine and methacholine, while subjects with only EARs after inhalation challenge do not have this heightened responsiveness.[18]

As the foregoing discussion would imply, inhalation challenge testing has provided both the physician and researcher with a valuable tool. It is equally as useful for the identification and monitoring of

asthmatic patients as it is for in vivo investigations into the pathogenesis of the disease. Bronchoprovocation may be defined as the administration of a stimulus to susceptible individuals, followed by measurements of the resulting bronchospasm.[19] Inhalation challenge is a method of testing for bronchial reactivity following inhalation of either a specific antigen to which the patient may be sensitive or a "nonspecific" pharmacological agent. Early reports indicated that bronchospasm could be precipitated in asthmatic patients by injections of pilocarpine[20] and histamine.[21] It has subsequently been shown that other drugs, including serotonin,[22-24], bradykinin,[25,26] prostaglandin $F_{2\alpha}$,[27,28] and various cholinergic agents such as acetylcholine, methacholine, and carbachol[29] have the same effect. Other stimuli such as exercise,[30] rapid respiratory maneuvers,[31,32] or inhalation of citric acid,[33] dust,[32,34] distilled water,[35] or cold air[36] can also provoke bronchoconstriction in susceptible individuals.

Standardization of procedures for performing inhalation challenge testing using histamine and methacholine has greatly improved our understanding of asthma, and of bronchial hyperreactivity in particular.[37-44] A variety of pulmonary function parameters can be serially evaluated in human subjects during inhalation challenge testing. A dose-response curve to the inhaled agonist is created by plotting the pulmonary function parameter of interest against the cumulative dose of agonist administered.[19] The parameter used most widely to indicate a positive test is FEV_1 (forced expiratory volume at 1 second). The provocative dose that causes a fall in FEV_1 of 20% from the lowest post-saline value is determined from the dose-response curve

by interpolation.[19,38,39] By definition, asthmatics have hyperreactive airways, that is, they characteristically develop bronchoconstriction to a greater extent in response to smaller concentrations of various stimuli than do healthy individuals.[45] Over 99% of patients with current symptomatic asthma have a bronchoconstrictor response to an inhaled stimulus that is outside the normal range. Their sensitivity to an agonist such as methacholine varies from one hundred to several thousand times that of normal subjects.[40] In establishing the diagnosis of asthma, bronchial provocation tests are more sensitive than standard pulmonary function tests when the latter are close to normal.[46] The mean sensitivity of former asthmatics to inhalation challenge is approximately one tenth that of current asthmatics.[41] In general, there is good agreement between bronchial hyperresponsiveness and the clinical severity of asthma.[47] Concordance between airway reactivity and asthmatic state is not total, however. For example, not all patients with occupational asthma are hypersensitive to methacholine.[48] Conversely, nonasthmatic patients with hay fever are nearly as sensitive to $PGF_{2\alpha}$ as asthmatic patients, although the two groups are clearly separable with histamine or methacholine challenge.[28]

It has been recognized for some time that bronchial hyperreactivity is not unique to asthma.[49,50] Nonspecific bronchial hyperreactivity is encountered in many patients with allergic rhinitis,[37,49,50] chronic obstructive bronchitis,[51] and in normal subjects following some viral upper respiratory tract infections.[33] A very small proportion of healthy individuals may also have hyperreactive airways.[37,41,52-54] Airway responsiveness can be temporarily heightened by events that cause

inflammation. These include vaccination,[55] natural exposure to allergens,[56] specific occupational chemical sensitizers,[48,57,58] and inhalation of ozone,[59,60] or noxious fumes such as chlorine,[38] nitrogen dioxide,[61] and sulphur dioxide.[62] The induction of increased bronchial reactivity by such means offers a way to reproduce this phenomenon, but these studies are probably only relevant to the study of asthma if the acquired bronchial hyperreactivity resembles the "endogenous" hyperreactivity associated with the disease.[29] Acquired and endogenous hyperreactivity differ in both degree and duration, the bronchial sensitivity in asthma being more severe and persistent.[29,40] In asymptomatic patients with a history of asthma, hyperreactivity is often present for over a year and may persist for up to 20 years.[40,63,64] It may be necessary for asthmatic subjects to be repeatedly exposed to antigen in order for bronchial hyperreactivity to persist.[29]

Cockcroft and co-workers[65] surveyed the bronchial responsiveness to inhaled histamine in a large, randomly selected population. They concluded that the distribution of histamine threshold values was unimodal, the asthmatic subjects representing a subgroup within the hyperresponsive distribution tail rather than a separate distribution peak. Such a unimodal distribution supports the concept of asthma as a heterogeneous polygenic and environmental disease. It also lends credence to a continued search for common pathophysiological mechanisms to account for hyperreactivity of the tracheobronchial tree under a diversity of normal and abnormal conditions, and to the use of animal models of bronchial hyperreactivity to investigate the mechanisms involved.

B. Animal Models of Airway Hyperreactivity

It has been said that bronchial asthma is a disease that can be characterized clinically by reversible airway obstruction, physiologically by increased bronchial reactivity to specific and/or nonspecific stimuli, and pathologically by airway inflammation.[66] The relationship of the clinical signs to the physiological changes has been partly addressed in the foregoing discussion. A number of animal models have been employed in an attempt to understand the link between the physiological and the pathological alterations seen in human asthma.

1. Specific antigen-induced bronchial hyperreactivity

Bronchospasm induced by specific antigen challenge has been the basis of most animal models of asthma. The traditional immunological model of antigen-induced airway constriction has been the guinea pig, which is easily sensitized to foreign proteins and in which the lung is the primary target organ in the anaphylactic reaction.[67-72] Most such studies have used antigens such as bovine or egg albumin, which bear little resemblance to those incriminated in human extrinsic asthma. In addition to the guinea pig, other species including the sheep,[73-75] the monkey,[76-79] and the dog[80-89] have been used to assess airway reactivity and pulmonary mechanics in antigen-induced bronchoconstriction. In all allergic bronchoconstriction models, sensitized animals respond to antigen aerosol with increases in respiratory frequency and pulmonary resistance and decreases in dynamic lung compliance.[90] These abnormalities are associated with decreases in arterial oxygen tension.[83] When functional parameters after antigen challenge have returned to normal, bronchial responses to stimuli such as histamine, acetylcholine, and propranolol may be exaggerated.[72]

Dogs are often natively allergic to Ascaris suum as a result of prior parasitic infestation with roundworms, which cross react with A. suum. If this antigen is administered to a sensitized dog as an aerosol, airway constriction can occur.[80-82] The systemic administration of histamine produces physiological changes similar to those induced by specific antigen challenge.[85] Histamine is released after systemic and local airway administration of specific antigen in allergic dogs.[91-93] Similar to the effects in human beings, canine plasma histamine levels correlate with circulatory and respiratory changes that occur during these reactions.[85] Pretreatment with a combination of H_1 and H_2 antihistamines is not effective in preventing or altering specific antigen-induced reactions.[94] Studies by Bleeker and co-workers[85] have shown that the products of arachidonic acid metabolism may be important in modulating immediate hypersensitivity, as the release of immunological mediators (PGD_2 and leukotriene C_4) into plasma and bronchoalveolar lavage fluids is temporally related to the development of hypotension and bronchospasm, as well as to an increase in the number of neutrophils in respiratory secretions.

As the A. suum sensitive canine model is only remotely related to clinical circumstances, considerable interest has been shown in the subset of the canine population that are ragweed-sensitive.[81,86-89] A well-defined atopic disease entity occurs spontaneously in dogs.[95,96] Ragweed-sensitized dogs and some spontaneously atopic dogs show IgE-mediated hypersensitivity reactions that bear many similarities to human allergic asthma.[80,86,87,97] Common features include immediate and late-phase cutaneous responses, an immediate airway response after inha-

lation of specific antigen aerosol, and increased airway responsiveness to inhaled histamine and methacholine.[87,95-98] Although the spontaneous occurrence of antigen-induced airway constriction mediated by an IgE-like antibody makes this an attractive model of asthma, it is necessary to screen large numbers of mongrel dogs to obtain a colony of such animals.[99] Even when dogs from an established colony are used, approximately 35% of individuals do not demonstrate any increase in airway reactivity after antigen exposure.[86] Attempts to induce reaginic hypersensitivity in adult mongrel dogs have met with varying degrees of success.[99] Success in inducing such hypersensitivity has been obtained using puppies from atopic parents and starting the immunization program at 4 weeks of age.[100,101] In this situation, some correlation can be seen between skin and airway responses to antigen.[101] Ragweed-sensitized dogs exhibit nonspecific airway hyperresponsiveness for up to 4 months following antigen challenge,[86] but a late-phase bronchoconstriction has not been observed in such dogs.[99]

While immediate IgE-mediated reactions to allergen challenge are common in atopic asthmatics, the late phase responses are less common and poorly understood. It has been speculated that these late phase changes, which are poorly responsive to bronchodilators and often require corticosteroids for resolution, are important in the subacute and chronic symptoms of the more severely affected asthmatics.[102] The late response can prevented by the administration of cromolyn sodium or corticosteroids prior to antigen challenge.[13,16,103] The finding that inflammatory mediators are released during late-phase reactions in asthmatics after specific antigen exposure[104] and after exercise[105] or inha-

lation of cold dry air,[106] suggests that the release of inflammatory substances may be central to the pathogenesis of disease in a much larger proportion of asthmatic patients than only those who experience symptoms immediately after exposure to a specific antigen.[107] Late skin reactions that occur after allergy testing in man may be different from the LAR as late skin reactions do not occur in the absence of an early reaction.[46] It is of interest nonetheless, to examine the results of studies that suggest late-phase cutaneous responses represent an IgE-dependent response.[108,109]

Late reactions would appear to be a more suitable model for the study of asthma than are acute reactions to antigen. To date, late-phase bronchoconstriction following specific antigen challenge has only been reproduced experimentally in conscious rabbits[110] and sheep,[111] although the response can also be simulated with nonantigenic agents such as toluene diisocyanate.[8,48] In the rabbit model described by Shampain and colleagues,[110] neonatal rabbits were immunized with Alternaria tenius extract and then boosted regularly. This immunization schedule takes advantage of the fact that neonatal rabbits exposed to antigen within 24 hours of birth will develop no detectable antibody response other than IgE.[112] Such "IgE" rabbits exhibit changes in pulmonary function when challenged with aerosolized Alternaria extract at 3 months of age. An increase in pulmonary resistance and decrease in dynamic compliance begins within 15 minutes of challenge. These parameters again approach baseline or reach a plateau after 30 minutes and a second phase of bronchoconstriction begins after 105 minutes and lasts through the sixth hour. Rabbits immunized first at 7 days of age make

multiple antibody isotypes. The presence of IgG blunts, rather than enhances, pulmonary responsiveness. Further evidence in support of IgE mediation of the biphasic response was provided by transfusion of plasma containing anti-Alternaria IgE into control rabbits, producing, upon challenge, both early and late responses in pulmonary mechanics. These results are consistent with the report that asthmatic patients have increased levels of neutrophil chemotactic factor (a macromolecule associated with mast cell degranulation and IgE-mediated bronchoconstriction) during the late response.[113]

Abraham and co-workers[111] documented that a dual response to inhalation challenge with specific antigen occurs in conscious sheep with naturally-occurring Ascaris suum hypersensitivity. All animals selected for use in this study exhibited both a cutaneous reaction to intradermal injection of A. suum extract and a bronchoconstrictor response to previous inhalation challenge with A. suum antigen. By 5 hours after challenge, mean specific pulmonary resistance and arterial oxygen tension have returned to baseline values, but specific resistance increases again from 6.5 until 8 hours post challenge. Inhalation of cromolyn sodium prior to antigen challenge blocks both early and late responses, and parenteral steroid therapy 3 hours post challenge blocks the late response. The time course of both the immediate and late phase responses approximates the time course observed in asthmatics, and pharmacological modification of both responses in allergic sheep is similar to that found in human patients.

An ingenious in vivo model of the allergic reaction in man has recently been developed.[114] This model involves nasal challenge of an

allergic subject with specific allergens, followed by repetitive washing of the nose with a saline solution. Both early and late reactions are associated with the release of histamine, kinins and TAME (tosyl arginine methyl ester)-esterase activity into nasal washes.[107] Prostaglandin D_2 is liberated after the acute, but not the late phase response.[115] Since PGD_2 is a mast cell but not a basophil product, it seems likely that the basophil may play an important role in the late reactions.[107] The observation that mast cells and basophils differ in their responsiveness to corticosteroids,[116,117] is consistent with the fact that the acute response to antigen is not blocked by steroid pretreatment, while late reactions are sensitive to such intervention.[103,107] This work indicates that IgE-mediated stimuli can initiate a series of events that can have a prolonged physiological response. Comparison of in vitro[118-120] and in vivo studies implicates the mast cell in acute reactions and the basophil in late reactions.[107]

2. Hyperreactivity associated with inflammation

Bronchospasm induced by specific antigen challenge in a variety of animal species has been the basis of the models of asthma discussed to this point. Other models rely on changes induced by acute lung injury to provoke bronchial hyperreactivity. Pulmonary injury can be created by methods such as infusion of endotoxin or inhalation of air pollutants. Various species, particularly the dog,[45,121] have been used in ozone-exposure studies. It has been found that airway responsiveness does not increase in all dogs after exposure to ozone, but only in those animals that have an increase in the number of neutrophils in the epithelium of the proximal[122] and distal[123] airways. As the development

of hyperresponsiveness and inflammation appear to be so closely related, this model continues to be used to further define this association. Unfortunately, when hyperresponsiveness develops after ozone exposure in this canine model, it is short-lived.[124] This is in contrast to results obtained in healthy volunteers, 25% of whom have persistence of hyperreactivity for more than one week post ozone exposure.[125]

The development of techniques for bronchoalveolar lavage has allowed for serial assessment of pulmonary inflammation in individual animals. Using this technique, Fabbri and co-workers[123] determined that the level of bronchial responsiveness induced in dogs by ozone exposure was correlated with the number of neutrophils in the lavage fluid. Nadel and colleagues[45] performed an elegant series of studies to investigate whether airway epithelial inflammation is the cause of ozone-induced hyperirritability. When circulating neutrophils were depleted in dogs prior to ozone exposure, the expected hyperreactivity was prevented.[126] To test the hypothesis that neutrophils release mediators that induce airway hyperresponsiveness, circulating neutrophils were harvested, activated in vitro and the supernatent removed.[45] When this supernatent was aerosolized into the airways of healthy dogs, hyperresponsiveness of the airway smooth muscle resulted.[121] After finding that leukotriene B_4 (LTB_4), a leukotriene with potent neutrophil chemotactic properties, is released in significant quantities from inflamed canine tracheal epithelial cells,[127] hyperirritability was reproduced in normal dogs by aerosol administration of LTB_4.[45] Reproduction of airway hyperreactivity by this mediator suggests an important role for mediators released by the activated neutrophil in inducing hyperresponsiveness.

The guinea pig has been used extensively in studies examining the relationship between mucosal permeability and airway reactivity. Cigarette smoke is known to injure the airway mucosa, resulting in migration of neutrophils into the airway epithelium within 4 to 6 hours.[128] Further investigations in the guinea pig resulted in the conclusion that the observed increase in airway reactivity occurs during the fluid exudative phase of the inflammatory response before the neutrophils migrate into the epithelium.[129] This conclusion is in agreement with data that showed that smokers have increased airway permeability[130,131] that is not associated with increased airway reactivity.[132] This complex relationship between permeability and reactivity has been further studied in guinea pigs using intravenous and aerosol administrations of leukotrienes C_4, D_4, and E_4, components of the slow-reacting substance of anaphylaxis.[133] There are significant similarities between guinea pig[134] and human[135] studies. The leukotrienes produce potent, prolonged bronchoconstriction in both species. The time course of onset and resolution of bronchoconstriction is comparable to that elicited by inhaled antigen in each species, and is compatible with the leukotrienes having a causative role in allergic asthma.[136]

3. Nonspecific airway hyperreactivity

It has been recognized for some time that the hyperreactivity that develops following specific antigen challenge, despite being characteristic and reproducible in individual animals, is variable from animal to animal.[72] This latter characteristic is of interest in comparing animal models to human asthma. Studies by Douglas and colleagues[70,137]

suggest that there is a log normal distribution of nonspecific responsiveness to inhaled chemical mediators in guinea pigs, a situation analagous to that found in a random human population[65] and in dogs.[138] Attempts have been made to reproduce the human disease more closely by selecting animals at the hyperresponsive end of the distribution tail.[139] Another interesting analogy with the situation in human asthma is the apparent genetic predisposition for guinea pigs to develop nonspecific bronchial hyperresponsiveness as suggested by the data of Takino and co-workers.[139] Although rats have not been used as extensively as guinea pigs in airway reactivity studies, an inbred line of hyperreactive rats has also been developed by selecting animals at the hyperresponsive end of the distribution curve.[140] Further support for the hypothesis that acquisition of hyperreactivity requires both a genetic predisposition to the condition and an inciting event is provided by the work of Wardlaw,[141] who reported that injection of pertussis vaccine induced hypersensitivity in some strains of mice and not in others.

Considerable interest has been centered on selecting and breeding a population of dogs that possess both nonspecific airway hyperreactivity and allergic inhalant dermatitis (atopy). A line of basenji-greyhound crosses has been shown to possess these characteristics.[99] Marked airway hyperreactivity to methacholine, histamine, leukotriene D_4, and citric acid has been demonstrated in these basenji-greyhound dogs compared to mongrel dogs of similar size.[142,143] Moreover, the offspring of these basenji-greyhound dogs are also hyperreactive to methacholine and citric acid, whereas the offspring of allergic dogs lacking airway hyperreactivity are similarly unreactive to these stimuli.[144] In the

basenji-greyhound dog, aerosol challenge with specific antigen results in changes in airflow resistance and dynamic compliance of far greater magnitude than has been reported in the mongrel dog.[99] Additionally, increases in residual lung volume associated with antigen-induced bronchoconstriction occur in some of these basenji-greyhound dogs. Although the basenji-greyhound dog model has many similarities to human asthma, late bronchoconstriction following antigen challenge does not occur, and the bronchial hyperreactivity that these dogs exhibit is not associated with any clinical disease.[99] In addition, inhalation challenge testing on these dogs requires general anesthesia, a factor that may depress airway smooth muscle responses.[145,146]

C. A Pony Model of Bronchial Hyperreactivity

Spontaneous attacks of asthma-like signs are exceedingly rare in any nonhuman species except equids. The clinical signs associated with "heaves" or "broken wind" have been recognized since at least 333 B.C., when Aristotle described the characteristic active expiratory "heave" in affected horses.[147] The comparative importance of heaves to human asthma was first alluded to in 1726.[148] Although Percivall warned in 1853 that many horses with broken wind did not have emphysema, Stömmer and others described the pathological changes in "heavey" horses in terms of their similarities to those in human patients with emphysema.[148] It was almost a century before several investigators more accurately characterized the major pathological change in the lungs of affected horses as being a bronchiolitis.[149,150] Over a combined observation period of 23 months, Lowell[151] recorded 16 attacks among 6 heavey horses while hay was being fed, whereas there were only 3 attacks in a

combined total period of 54 months when hay was not fed. Lowell also observed that severe manifestations of heaves could appear and disappear within a period of days upon manipulation of the diet. He concluded that the episodes of dyspnea were a result of transient bronchial obstruction caused by hypersensitivity to some component of hay, to which horses were exposed by inhalation. Evidence for an allergic etiology for the disease,[151-154] combined with the facts that equine lungs are structurally similar to human lungs,[155] and that airway inflammation is the characteristic pathological alteration in both asthma and heaves,[156] provided justification for evaluating horses or ponies with heaves as a model for studying the pathophysiology of human asthma.

The work of Alexander,[157] Spörri and Leeman,[158] Gillespie and colleagues,[159,160] McPherson and Lawson,[161] Muylle and Oyaert,[162] and Sasse[163] characterized the pathophysiological changes that occur during spontaneous exacerbations of heaves. Increased work of breathing, increased change in intrapleural pressure (ΔPpl) during tidal breathing, decreased dynamic compliance, increased pulmonary resistance, prolonged nitrogen washout, decreased maximal expiratory flows and hypoxemia have been consistently demonstrated. Such indicators of diffuse small and large airway obstruction lead Sasse to introduce the term "chronic obstructive pulmonary disease" (COPD) to the equine medical literature.[163] Measurement of maximum ΔPpl and arterial oxygen tension was suggested to establish diagnostic criteria for COPD.[161]

The observation that human patients with bronchial asthma are more sensitive to histamine than normal subjects was published by Weiss and

co-workers[21] in 1929. Andberg, Boyd and Code[164] demonstrated that the intravenous administration of histamine to normal horses would induce a complex of clinical signs closely resembling those seen in heaves. Obel and Schmitterlöw[165] compared the histamine sensitivity of horses suffering from heaves with normal horses. In a landmark paper, they reported that affected horses exhibit bronchial hyperreactivity in response to intravenous histamine administration. Following work to determine the pulmonary effects of intravenous histamine administration in the conscious normal pony,[166] Derksen and colleagues[167] more fully characterized the changes in pulmonary mechanics that occur following intravenous histamine administration in ponies with a history of heaves. Dose-response curves were generated for affected ponies during periods of clinical remission, during acute dyspnea induced by barn exposure, and during recovery. It was found that bronchial reactivity differed between affected and control ponies only when ponies with a history of heaves had acute airway obstruction.

Bronchoprovocation testing by specific inhalation challenge is known to produce bronchoconstriction in affected horses and ponies. As Lowell[151] and others have shown, a crude natural inhalation challenge can be carried out by exposing horses to a barn environment, particularly one containing moldy hay or straw.[147] The onset of clinical signs following exposure to allergen can be sudden or gradual and shows considerable variability between individuals. The earliest response of an asymptomatic but affected horse to challenge occurs in about 2 hours. The more obvious "heaving" occurs 4 to 10 hours after exposure,[147] a time course similar to the LAR observed in asthmatic patients. Affected

horses are also sensitive to nebulization of culture extracts of specific thermophilic actinomycetes (Micropolyspora faeni) and fungi (Aspergillus fumigatus).[147,154,168]

It is interesting to consider the results of surveys for the presence of serum precipitating antibodies and studies on intradermal testing for antigen sensitivity. Lawson et al.[168] found that precipitating antibodies to M. faeni and A. fumigatus are present in the sera of both normal and COPD-affected horses, and many heavey horses without detectable precipitins respond clinically to inhalation challenge with those antigens. A positive reaction to an intradermal injection of antigen is evidence that a horse has been previously exposed to that antigen. Significantly more heavey horses show skin hypersensitivity to M. faeni than do normal horses, and 90% of COPD cases with skin hypersensitivity to M. faeni also show bronchial hyperreactivity to this antigen.[154] Using other antigens, positive skin reactions are as likely to occur in normal horses as in those with COPD. Halliwell and co-workers[153] found a higher percentage of positive responses at 4 hours in affected horses compared to controls, but a positive response to specific bronchial challenge was far more readily induced in animals with strong 30 minute reactions than those with strong 4-hour ones. Apparently, intradermal skin testing for antigen sensitivity is unreliable as a means of identifying affected horses that are in clinical remission at the time of examination.[147]

Considering the many similarities between equine heaves and human asthma, it seemed logical to ask whether horses or ponies with a history of heaves exhibit that central common feature of asthma, nonspecific

airway hyperreactivity. To determine this, we measured lung function and airway reactivity to histamine administered by aerosol in two groups of ponies.[169] Affected and control ponies were studied at four time periods: when affected ponies were in clinical remission, following barn housing (when affected ponies had acute airway obstruction), and at one and two weeks after return to pasture. Ponies with a history of heaves were found to be hyperreactive to aerosolized histamine during acute exacerbations of their disease, but not during clinical remission. Histamine dose-response curves for dynamic compliance, pulmonary resistance and respiratory frequency were generated for each time period. The dose of histamine required to reduce dynamic compliance to 65% of the value obtained after saline challenge ($ED_{65}C_{dyn}$) was calculated by interpolation from these curves. During the period of acute airway obstruction, $ED_{65}C_{dyn}$ decreased by 2.5-log doses in affected ponies, but was unchanged in controls. In individual affected ponies, $ED_{65}C_{dyn}$ decreased by as much as 4.5 log-doses between studies performed during the period of clinical remission and following barn exposure. This study provided conclusive evidence that affected ponies have hyperreactive airways during periods of acute airway obstruction. The following study was undertaken to determine whether the airways of affected ponies would similarly hyperreact to other nonspecific stimuli, namely methacholine and citric acid.

III. AIRWAY RESPONSES TO AEROSOLIZED METHACHOLINE AND CITRIC ACID IN PONIES WITH RECURRENT AIRWAY OBSTRUCTION (HEAVES)

A. Methods

Five ponies (19.0 +/- 3.9 years of age and weighing 201.6 +/- 16.8 kg) with a history of heaves were designated as the principal group. They were matched for age, gender, and weight with 5 ponies (control group) (17.8 +/- 2.1 years of age and weighing 195.8 +/- 18.7 kg) historically free of respiratory disease. Chronic tracheostomies were created in the midcervical region, and a carotid artery was relocated to a subcutaneous site. All animals were kept at pasture and were not exposed to hay, straw, or a barn environment for at least 2 months prior to the first measurements of airway reactivity. All principal ponies were in clinical remission at the end of this period.

For investigations of airway reactivity, principal and control ponies were paired. To ensure that both ponies received the same environmental exposure, each pair was always transported together, housed together, and studied on the same day. Baseline pulmonary function data were collected, and airway reactivity to methacholine and citric acid was assessed in all ponies after 2 months at pasture (Period A). Each pair was then housed in a barn, bedded on straw, and fed hay. An attempt was made to increase exposure to hay dust by shaking the hay in the stall 3 times daily. When the principal pony developed clinical signs of airway obstruction, pulmonary function and

airway reactivity were measured in both ponies (Period B). Animals were then returned to pasture and studied 1 week (Period C) and 2 weeks (Period D) after barn exposure.

1. Pulmonary function measurements

Ponies were tranquilized with xylazine (Rompun; Haver Lockhart, Shawnee Mission, KS) (0.5 mg/kg of body weight) and restrained in stocks. Each pony was intubated via a tracheostoma with a cuffed endotracheal tube (20 mm internal diameter, 45 cm long). A pneumotachygraph (Fleisch no. 4; Dynasciences, Blue Bell, PA) and an associated pressure transducer (Model PM5; Statham Instruments, Hato Rey, PR) were attached to the endotracheal tube. This system produced a signal proportional to air flow ($\dot{V}$) that was electronically integrated to give tidal volume (V_T) (Figure 1).

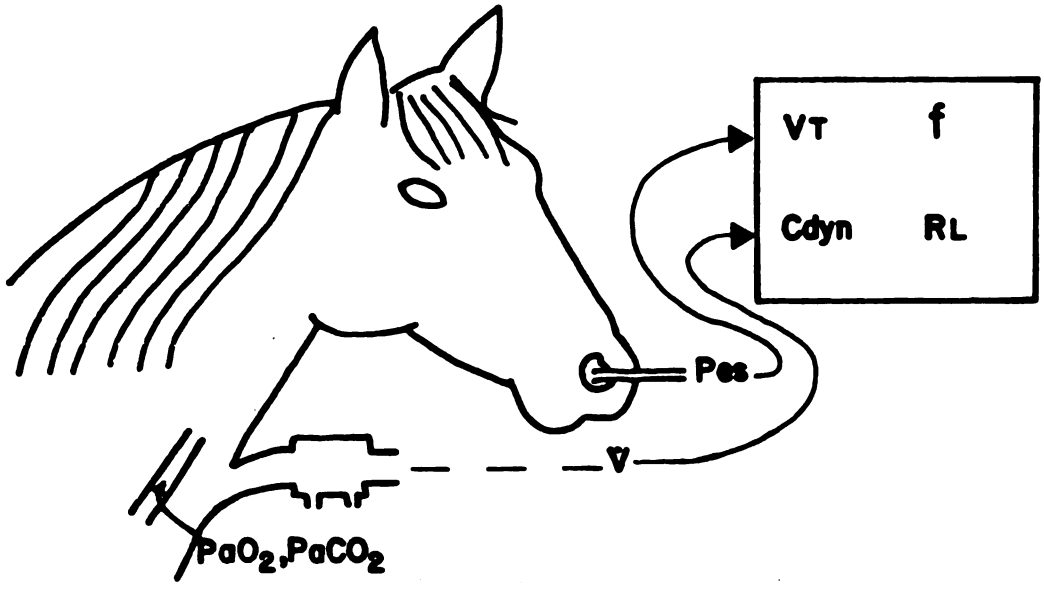

Figure 1. Schematic representation of instrumentation used for measurement of pulmonary function parameters.

After each experiment, the system was calibrated by forcing known volumes and flows of air through the pneumotachygraph, using a 3-L

calibrated syringe (Three L Super Syringe; Hamilton Syringe Co., Reno, NV) and a rotameter flow meter (Model 10A3500; Fischer and Porter Co., Warminster, PA).

An esophageal balloon (10 cm length, 3.5 cm perimeter, 0.06 cm wall thickness) was sealed over the distal end of a polypropylene catheter (3 mm internal diameter, 4.4 mm outside diameter, 140 cm length) that had several spirally arranged holes in the part covered by the balloon. The distance from the nares of the ponies to the midthoracic portion of the esophagus was visually approximated and marked on the esophageal balloon catheter with indelible ink. The esophageal balloon was passed via the nares into the midthoracic portion of the esophagus. Balloon volume was adjusted to 0.5 ml of air. The balloon was attached to a pressure transducer (Model PM 131; Statham) that was taped to the forelock. Transpulmonary pressure (P_L) was defined as the difference between atmospheric and esophageal pressure. Transpulmonary pressure, V_T, and flow signals were observed on an oscilloscope (Model VR12; Electronics for Medicine, White Plains, NY) and recorded on light-sensitive paper, and were entered into a pulmonary function computer (Model 6; Buxco Electronics, Sharon, CT) and data logger (Model DL12; Buxco) for the calculation of dynamic compliance (C_{dyn}), pulmonary resistance (R_L), respiratory frequency (f), and minute ventilation ($\dot{V}_E$).

The frequency responses of the pneumotachygraph transducer system and the esophageal pressure transducer system were matched to prevent phase differences between pressures and flows. This was accomplished by comparing pressure recorded with the esophageal catheter and transducer against pressure recorded with the pneumotachygraph transducer on the XY plotter while exposing all devices to the same oscillating pressure

source. Frequency responses were checked up to 5 Hz and were flat.[170]

In carotid artery samples, arterial oxygen tension (Pa_{O_2}), carbon dioxide tension (Pa_{CO_2}), and pH were measured immediately prior to airway reactivity measurements using a blood gas analyzer (Model ABL3; Radiometer, Copenhagen, Denmark).

2. Airway reactivity measurements

Methacholine and citric acid challenges were performed on adjacent days. Three pairs of ponies always received methacholine challenge on the day prior to citric acid challenge; the order was reversed for the other 2 pairs. The apparatus used for generation and delivery of methacholine aerosols to the pony lung is shown in Figure 2.

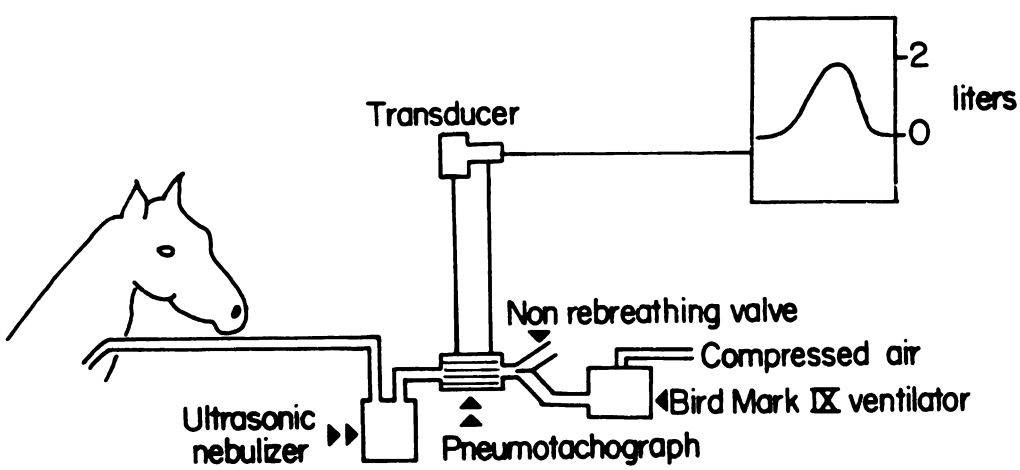

Figure 2. Diagrammatic representation of equipment used to generate and deliver aerosols of methacholine.

Aerosol methacholine delivery was standardized in the following protocol. Each pony was force-ventilated with air for 10 breaths (3 to 4.5 L, depending on body weight) to eliminate sighing and to provide a constant volume history. The ultrasonic nebulizer (Model 65; DeVilbiss,

Somerset, PA) was then turned on and the animal received 6 breaths (2 to 3.5 L) containing aerosol. The output of the nebulizer was 0.11 ml/2-L breath, with a particle size of 0.5 to 3.0 μm. The nebulizer was disconnected from the endotracheal tube and the pneumotachygraph was attached for 3 minutes to allow recording of P_L, V_T, and $\dot{V}$. Because forced ventilation perturbed breathing patterns immediately after aerosol delivery, we used only data obtained from recordings during the second and third minutes. Exactly 3 minutes after the end of the first challenge, we began the next aerosol challenge. The sequence of challenge was air, saline, and increasing concentrations of methacholine (0.0001, 0.001, 0.01, 0.1, 1, 3, and 10 mg/ml). Aerosol challenge was stopped when C_{dyn} decreased to approximately 50% of the value obtained after saline challenge, at which time considerable respiratory distress was evident. Although some ponies required a higher dose of methacholine to decrease C_{dyn}, 0.1 mg/ml was the maximal dose to which all ponies could be consistently and safely exposed. Methacholine dose-response curves were generated by plotting C_{dyn}, R_L, and f as a function of methacholine dose (Figure 3). By interpolation between points on the dose-response curves, we calculated the dose of methacholine required to decrease C_{dyn} to 65% of the value obtained after saline challenge ($ED_{65}C_{dyn}$). We also calculated R_L and f at the $ED_{65}C_{dyn}$ methacholine dose. The changes in C_{dyn} and R_L induced by administration of 0.1 mg/ml methacholine were calculated at each measurement period and were designated $\Delta\%C_{dyn}0.1$ and $\Delta R_L0.1$.

The delivery of citric acid is described in the following protocol. The ultrasonic nebulizer containing saline was attached to the endotracheal tube through a nonrebreathing valve, and the pony was

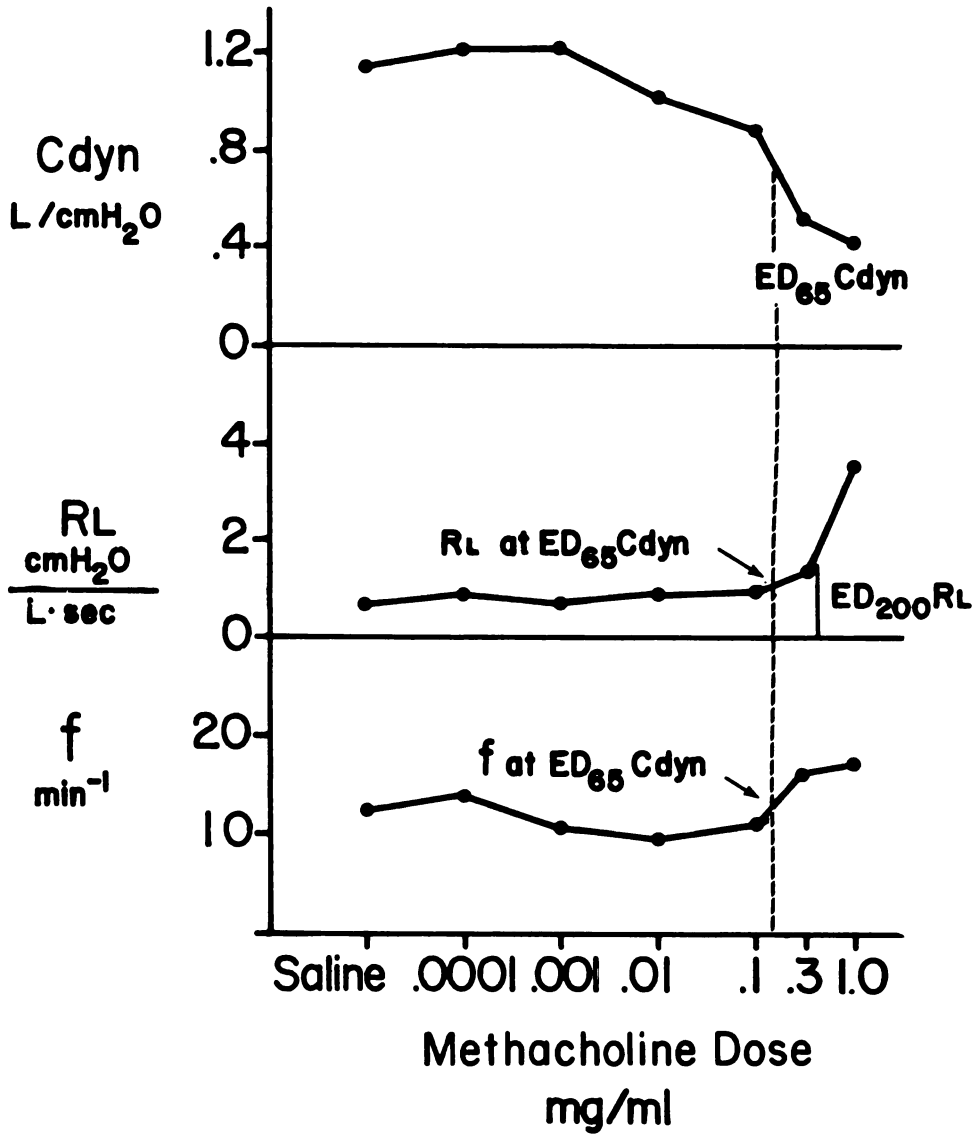

Figure 3. Relationship between methacholine dose and dynamic compliance (C_{dyn}), pulmonary resistance (R_L), and respiratory frequency (f). Doses of methacholine required to reduce dynamic compliance to 65% of the postsaline value ($ED_{65}C_{dyn}$) to double pulmonary resistance ($ED_{200}R_L$) are shown. Also shown are R_L and f at the $ED_{65}C_{dyn}$ methacholine dose.

allowed to breathe saline aerosol for 10 minutes. Subsequently, data were recorded every 2 minutes for 10 minutes, followed by the delivery of a 10% citric acid aerosol for 10 minutes. Data were recorded every 2 minutes for 10 minutes and at 5-minute intervals from 10 to 30 minutes after aerosol inhalation.

3. Statistical Analysis

To evaluate changes caused by barn exposure and return to pasture, data were analyzed using a two-way analysis of variance. When comparing differences between the principal and control groups of ponies, we used a split-plot factorial analysis of variance. When f values were significant at $p < 0.05$, means from each measurement period were compared using Tukey's ω procedure.[171]

B. Results

TABLE 1

LUNG FUNCTION OF PRINCIPAL AND CONTROL PONIES AT EACH MEASUREMENT PERIOD

	Measurement Period			
Parameter	A	B	C	D
Principal ponies				
Pa_{O_2}, torr	79 ± 3	65 ± 4	76 ± 4	85 ± 3
Pa_{CO_2}, torr	41 ± 1	42 ± 2	39 ± 4	39 ± 1
Cdyn, $L \cdot cmH_2O^{-1}$	0.57 ± 0.08	0.21 ± 0.03*†	0.48 ± 0.08	0.42 ± 0.07
R_L, $cmH_2O \cdot L^{-1} \cdot s$	0.95 ± 0.12†	2.37 ± 0.32*†	0.86 ± 0.17	1.07 ± 0.16
V_T, L	2.32 ± 0.36	1.70 ± 0.10	2.21 ± 0.31	2.08 ± 0.39
$\dot{V}_E$, $L \cdot min^{-1}$	29.5 ± 5.6	40.0 ± 10.9	30.5 ± 4.1	29.5 ± 3.1
f, min^{-1}	14.7 ± 4.9	22.9 ± 5.4	14.3 ± 2.1	16.2 ± 3.5
Control ponies				
Pa_{O_2}, torr	82 ± 3	76 ± 5	80 ± 5	86 ± 9
Pa_{CO_2}, torr	39 ± 1	43 ± 1	41 ± 1	38 ± 3
Cdyn, $L \cdot cmH_2O^{-1}$	0.72 ± 0.16	0.88 ± 0.18	0.82 ± 0.20	1.06 ± 0.22*
R_L, $cmH_2O \cdot L^{-1} \cdot s$	0.52 ± 0.08	0.44 ± 0.10	0.45 ± 0.08	0.73 ± 0.12
V_T, L	2.34 ± 0.23	2.41 ± 0.32	2.22 ± 0.32	2.11 ± 0.19
$\dot{V}_E$, $L \cdot min^{-1}$	28.0 ± 2.2	25.9 ± 2.1	25.0 ± 1.6	32.5 ± 4.8
f, min^{-1}	12.2 ± 0.9	11.1 ± 0.7	12.4 ± 2.4	15.9 ± 2.8

Definition of abbreviations: Pa_{O_2} = arterial oxygen tension; Pa_{CO_2} = arterial carbon dioxide tension; Cdyn = dynamic compliance, R_L = pulmonary resistance; V_T = tidal volume; $\dot{V}_E$ = minute ventilation; f = respiratory frequency.

* Significant difference from Period A

† Significant difference from control group

1. Lung function

Table 1 shows pulmonary function data of principal and control ponies at all measurement periods. At Period A, principal ponies had a slightly higher mean R_L than control ponies, despite the lack of clinical evidence of airway obstruction. After barn exposure (Period B), there was no change in any values in the control ponies, but a significant decrease in C_{dyn} and an increase in R_L occurred in ponies in the principal group. Both values were significantly different from those in the control group at Period B. Although Pa_{O_2} decreased in all principal ponies between Periods A and B, the magnitude of the decrease was variable. A trend towards decreased V_T was seen in the principal ponies

at Period B. After removal from the barn environment, there was improvement in pulmonary function in the principal ponies, with values for C_{dyn} and R_L at Periods C and D similar to those at Period A. Dynamic compliance in the control group increased at Period D, resulting in a difference from the principal group at this measurement period.

2. Responses to aerosolized methacholine

All ponies consistently responded to aerosolized methacholine by a decrease in C_{dyn} compared with the reference value obtained after aerosolized saline. In 38 of 40 observations (10 ponies at 4 time periods), an increase in R_L accompanied the decrease in C_{dyn}. Examples of dose-response curves of principal and control ponies at Periods A and B are provided in Figure 4. In Principal Pony 5 at Period A, the decrease in C_{dyn} was unaccompanied by an increase in R_L. At Period B, the decrease in C_{dyn} occurred at a lower dose of methacholine and was then accompanied by a large increase in R_L. In Control Pony 25, a decrease in C_{dyn} occurred at a similar dose of methacholine at Periods A and B, and in both cases was accompanied by modest increases in R_L.

The mean log $ED_{65}C_{dyn}$ of methacholine is shown in Figure 5. At Periods A, C, and D, log $ED_{65}C_{dyn}$ did not differ between principal and control groups. At Period B, log $ED_{65}C_{dyn}$ decreased significantly in principal ponies compared with that at Period A and was significantly lower than in control ponies at the same time period.

The mean change in R_L and the percent change in C_{dyn} induced by exposure to an aerosol of 0.1 mg/ml methacholine are shown in Figure 6. In control ponies, $\Delta R_L 0.1$ and $\Delta\%C_{dyn}0.1$ did not differ between measurement periods. In the principal group at Period B, $\Delta R_L 0.1$ and $\Delta\%C_{dyn}0.1$

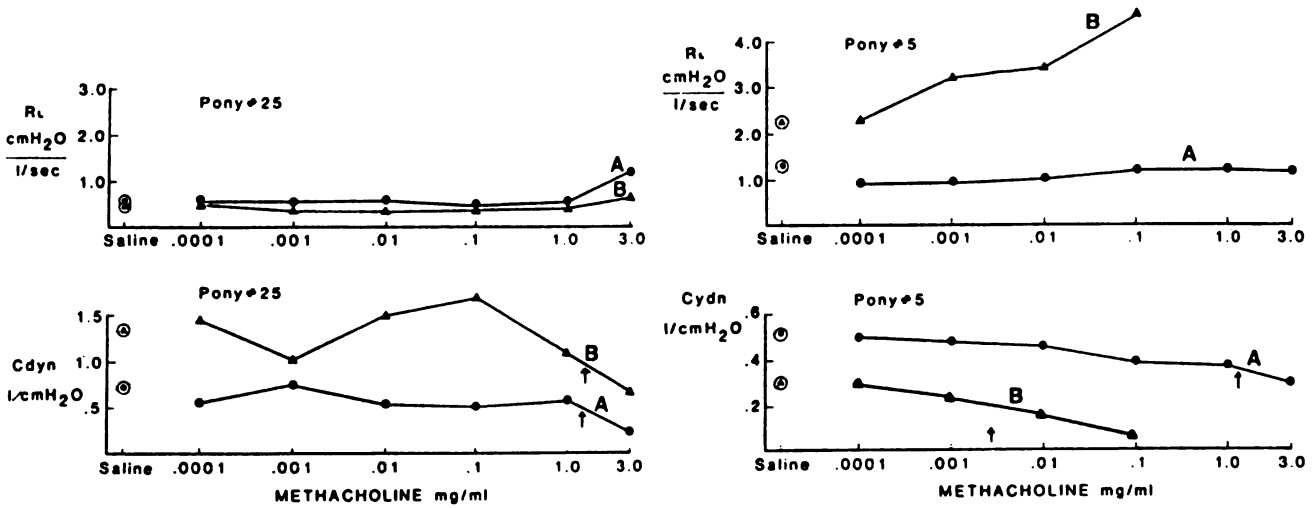

Figure 4. Dose-response curves of Control Pony 25 (left panel) and Principal Pony 5 (right panel). Pulmonary resistance (R_L) and dynamic compliance (C_{dyn}) are shown as a function of increasing methacholine dose at 2 time periods. At Period A (closed circles), Principal Pony 5 was in clinical remission. At Period B (triangles), Principal Pony 5 had acute airway obstruction. Arrows indicate $ED_{65}C_{dyn}$, the dose of methacholine required to reduce C_{dyn} to 65% of the value observed after aerosol saline (circled symbols). In Principal Pony 5, R_L did not increase in response to methacholine at Period A; $ED_{65}C_{dyn}$ decreased at Period B in the principal but not in the control pony.

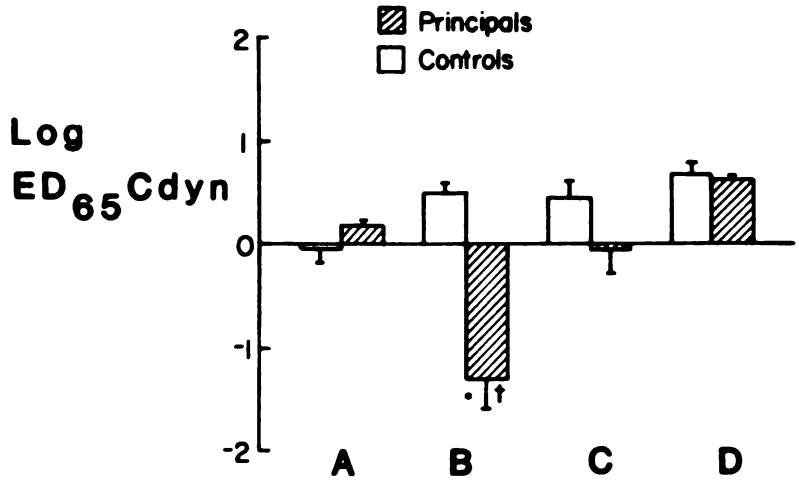

Figure 5. Logarithm of the concentration of methacholine required to reduce dynamic compliance to 65% of the value obtained after aerosol saline (log $ED_{65}C_{dyn}$) in principal and control ponies. Methacholine concentration was in mg/ml; A, B, C, and D indicate different time periods. At Period A, principal ponies were in clinical remission. At Period B, they had acute heaves. Periods C and D were 1 and 2 wk after Period B (asterick = significant difference of principal group from Period A value; dagger = significant difference between principal and control groups within a time period).

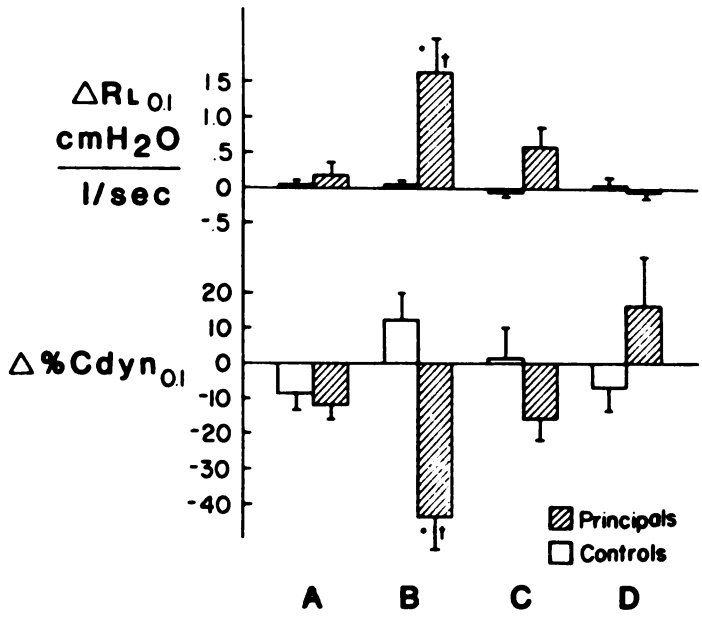

Figure 6. Change in pulmonary resistance and dynamic compliance induced by 0.1 mg/ml methacholine aerosol ($\Delta R_L 0.1$ and $\Delta\%C_{dyn}$ 0.1, respectively); A, B, C, and D indicate different time periods. At Period A, principal ponies were in clinical remission. At Period B, they had acute heaves. Periods C and D were 1 and 2 wk after Period B (asterick = significant difference of principal group from Period A value; dagger = significant difference between principal and control groups within a time period).

were significantly different from those in the control ponies. These parameters also differed within the principal group between Periods A and B.

Pulmonary resistance at the $ED_{65}C_{dyn}$ methacholine dose for both groups at all measurement periods is shown in Figure 7. Pulmonary resistance at $ED_{65}C_{dyn}$ followed the pattern of baseline R_L, always being higher in principal ponies than in control ponies and increasing significantly in the principal group in response to barn exposure. We calculated the change in R_L that occurred between saline exposure and R_L at $ED_{65}C_{dyn}$ ($\Delta R_L ED_{65}$). $\Delta R_L ED_{65}$ did not change significantly between time periods and did not differ in control and principal animals. Respiratory frequency at $ED_{65}C_{dyn}$ did not differ between groups or at any measurement period.

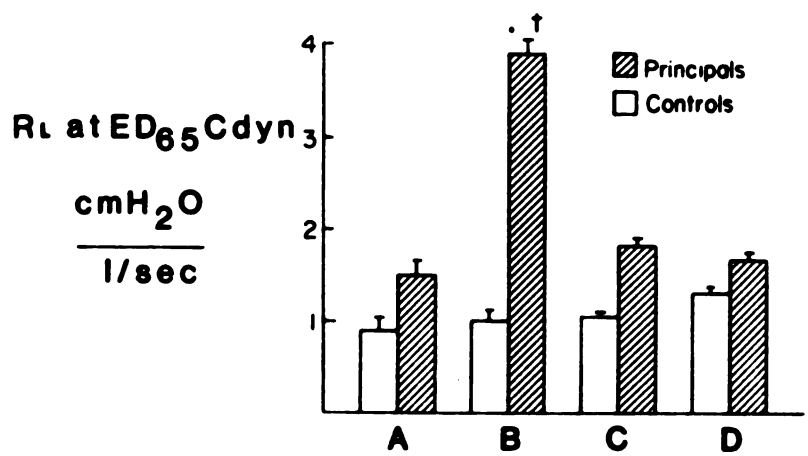

Figure 7. Pulmonary resistance at an $ED_{65}C_{dyn}$ dose of methacholine in principal and control ponies; A, B, C, and D indicate different time periods. At Period A, principal ponies were in clinical remission. At Period B, they had acute heaves. Periods C and D were 1 and 2 wk after Period B (asterick = significant difference of principal group from Period A value; dagger = significant difference between principal and control groups within a time period).

3. Responses to citric acid aerosol

Mean C_{dyn} for principal and control ponies after sequential saline and citric acid inhalation is shown in Figure 8. Inhalation of saline or citric acid had no effect on C_{dyn} in either group.

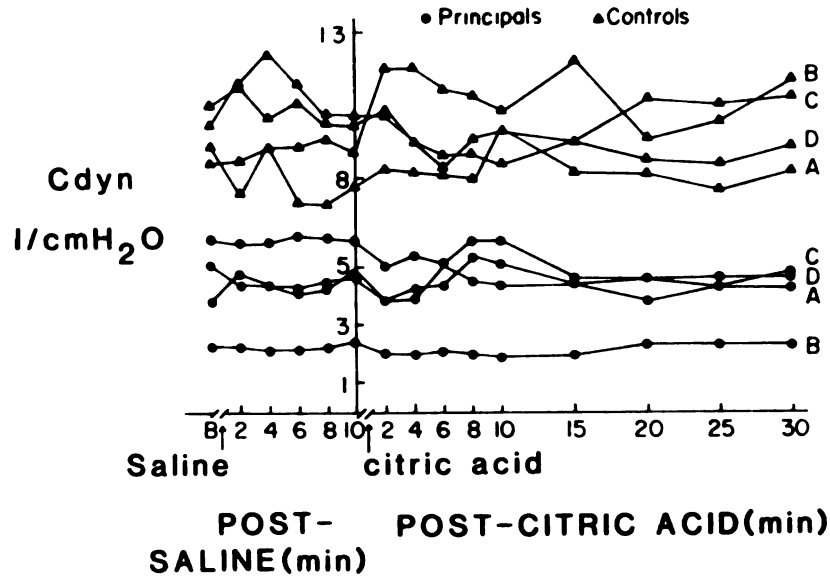

Figure 8. Dynamic compliance (C_{dyn}) of principal and control ponies after aerosolized saline and 10% citric acid; A, B, C, and D indicate different time periods. At Period A, principal ponies were in clinical remission. At Period B, they had acute heaves. Periods C and D were 1 and 2 wk after Period B. Saline and citric acid failed to alter C_{dyn} in either group of ponies.

Mean values for R_L are shown in Figure 9. Neither saline nor citric acid caused a change in R_L of control ponies at any measurement period. In the principal group, the response to citric acid was highly variable in both magnitude and time of occurrence. The biggest changes in R_L after citric acid inhalation were seen in principal ponies at Period B.

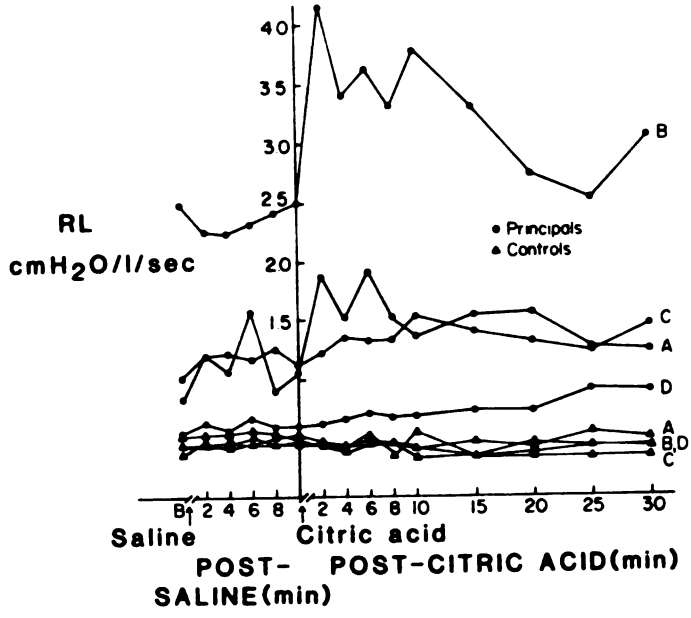

Figure 9. Pulmonary resistance (R_L) of principal and control ponies after aerosolized saline and 10% citric acid; A, B, C, and D indicate different time periods. At Period A, principal ponies were in clinical remission. At Period B, they had acute heaves. Periods C and D were 1 and 2 wk after Period B. Saline failed to alter R but citric acid increased R_L in principal ponies, particularly at Period B.

We calculated the maximal changes in R_L that occurred after saline inhalation (ΔR_L saline) and after citric acid (ΔR_L citric acid) (Figure 10). Maximal ΔR_L citric acid occurred 2 minutes after citric acid inhalation in 3 ponies, 15 minutes after citric acid in a fourth, and the fifth pony had a biphasic response with peaks at 2 and 30 minutes. Split-plot analysis of data showed a significant overall difference between principal and control groups in response to citric acid but not in response to saline.

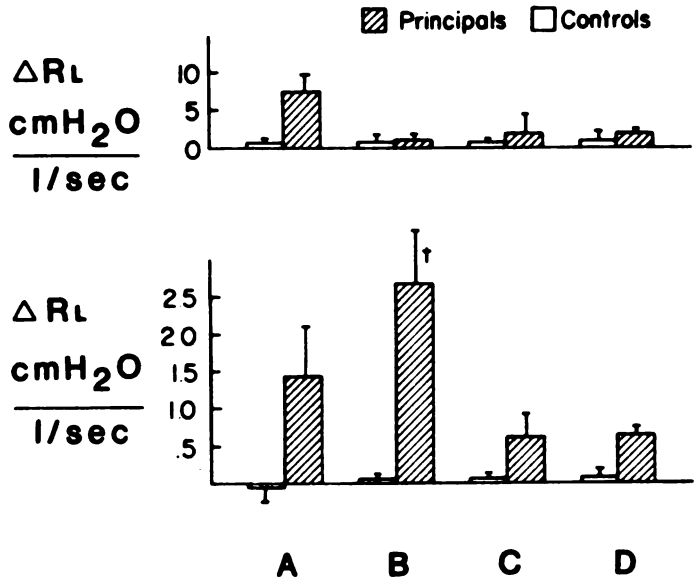

Figure 10. Change in resistance (ΔR_L) after aerosolized saline (upper panel) and 10% citric acid (lower panel) in principal and control ponies; A, B, C, and D indicate different time periods. At Period A, principal ponies were in clinical remission. At Period B, they had acute heaves. Periods C and D were 1 and 2 wk after Period B (dagger = significant difference between principal and control groups within a time period).

It can be seen in Figure 10 that ΔR_L saline did not differ between or within groups at any time period. At Period B, ΔR_L citric acid was significantly greater in principal than in control ponies. Within the principal group, ΔR_L citric acid was highest at Period B and declined significantly after removal from a barn environment (Periods C and D). Exposure to citric acid aerosol did not affect respiratory rate in either group of ponies at any measurement period.

C. Discussion

The exposure of principal ponies to a barn environment resulted in changes in lung function similar to those previously reported by our group.[167,169] A reduction in C_{dyn} and an increase in R_L reflect the airway obstruction that is characteristic of horses with heaves.[158-163,167,169] The higher airway resistance of principal ponies at Period A compared with that in control animals may have been due to residual airway obstruction despite 2 months at pasture. The small but significant increase in C_{dyn} of control ponies at Period D remains unexplained.

Both principal and control ponies responded to aerosols of methacholine by decreasing C_{dyn} and increasing R_L. In comparison to control animals, ponies with a history of heaves were hyperreactive to aerosolized methacholine at Period B, reacting at a lower dose of methacholine and undergoing a larger change in C_{dyn} and R_L in response to an 0.1 mg/ml dose of methacholine. We used $ED_{65}C_{dyn}$ as a measure of bronchial reactivity because a decrease in C_{dyn} of 35% exceeds the normal variability in C_{dyn} observed in ponies.[172] Furthermore, an $ED_{65}C_{dyn}$ dose of methacholine did not result in an increase in respiratory rate, which might have caused frequency-dependent decreases in C_{dyn}. We attempted to calculate the dose of methacholine needed to increase R_L by a factor of 2 ($ED_{200}R_L$). We were unable to do so 30% of the time, because as shown by the dose-response curve of Principal Pony 5 at Period A (Figure 4), some ponies did not double R_L even when C_{dyn} decreased to almost 50% of the value recorded after saline administration. For this reason, we used $\Delta R_L 0.1$ to compare the effects of treatment periods on resistance. In our previous study this with pony model,[169] we were unable to express

reactivity by calculating the change in R_L in response to a given aerosol dose of histamine because of the extreme reactivity of our principal ponies at Period B. In the present study, a methacholine aerosol of 0.1 mg/ml consistently induced a change in resistance of less than 0.05 cm $H_2O \cdot l^{-1} \cdot sec^{-1}$ in control animals. In principal ponies, the $\Delta R_L 0.1$ was similar to that in control ponies and quite repeatable at Periods A and D. In contrast, at Period B, there was more than an eightfold increase in resistance in response to the same dose of methacholine. This hyperreactivity waned over 2 weeks after barn exposure.

The hyperreactivity to aerosol methacholine only at time Period B in principal ponies is consistent with our previous observation of hyperreactivity to both intravenously administered and aerosolized histamine at this same time period.[167,169] At this time period, bronchoalveolar lavage fluid contains increased numbers of neutrophils, indicative of airway inflammation.[173] There was no consistent difference in the R_L response to aerosolized methacholine and histamine,[169] i.e., the R_L induced by an $ED_{6s}C_{dyn}$ dose of either agonist was similar in control and principal ponies at all time periods.

In heaves, the principal lesion is bronchiolitis with excess mucus production and leukocyte accumulation. While these lesions may be the cause of increased R_L and decreased C_{dyn} at Period B, constriction of central airways may also be occurring. Because hyperreactivity is present only in principal ponies at Period B, decreased baseline airway caliber must be considered as a possible cause of hyperreactivity.[174] This explanation appears unlikely, as principal ponies were not hyperreactive at Period A, despite their resistance being significantly higher than that of control animals at this time period. Furthermore,

when we examined the change in reactivity of principal ponies between Period A and subsequent measurement periods as a function of change in dynamic compliance, there was no correlation. Several control ponies also had changes in lung function over the 4 measurement periods that were not associated with changes in reactivity.

Even though statistical analysis demonstrated an overall difference between principal and control ponies in response to citric acid inhalation, the greatest change in R_L occurred in principal ponies at Period B. This is consistent with our previous observations of hyperreactivity to aerosolized methacholine and intravenously administered[167] and aerosolized[169] histamine occurring only during periods of acute airway obstruction. Curiously, unlike the response to methacholine, which involved an increase in R_L and a decrease in C_{dyn}, the response to citric acid was characterized by an increase in R_L unaccompanied by a decrease in C_{dyn}, suggesting the response was occurring primarily in the central airways.

The primarily central airway response can be explained in one of two ways. First, citric acid may have been deposited purely in the central airways as a result of the aerosol delivery system. Citric acid was inhaled because ponies would not tolerate ventilation for more than 2 minutes, whereas methacholine was delivered by positive pressure ventilation. In subjects with diffuse airway obstruction, as occurs in ponies with heaves, rapid inhalation results in central deposition of aerosol.[175] Slower inhalation[175] or forced ventilation[176] such as we used with methacholine favors deposition of aerosol in peripheral airways. Secondly, aerosol may have been deposited throughout the airways, but the mechanism of response to methacholine may have existed only in

central airways. There are two mechanisms that have been proposed to explain the response of airways to citric acid.[32,177-179] The response to short-term exposure (2 minutes or less) is transient and caused by a vagally-mediated reflex. This response is blocked by administration of anticholinergic drugs.[32,178] Longer exposure to citric acid aerosol causes a more delayed and protracted response. Calcium chelation and release of leukotrienes have been suggested as mechanisms of this response.[178,179] It is unlikely that the latter mechanism is restricted to central airways. In basenji-greyhound dogs in which these mechanisms have been investigated,[178] both a decrease in C_{dyn} and an increase in R_L occur in response to citric acid delivered by forced ventilation. If the response to citric acid is vagally mediated, vagal efferents would have to be predominantly distributed to the central airways to explain our data. The distribution of vagal efferents is unknown in the horse, but in the dog vagal efferents are extensively distributed throughout the tracheobronchial tree.[180] It is, therefore, most likely that the different responses to citric acid compared with those to methacholine are due to varying sites of aerosol deposition.

Small airways obstruction is the classic lesion of heaves.[156] Lesions in central airways have not been described. The decrease in C_{dyn} and increase in R_L in response to aerosolized methacholine could be explained either by diffuse peripheral airway obstruction or by generalized constriction of the tracheobronchial tree. Therefore, investigations using methacholine do not allow differentiation of the site of airway reactivity. With histamine aerosol, we observed primarily a decrease in C_{dyn} with a lesser increase in R_L, suggesting hyperreactivity of peripheral airways. In contrast, the selective response of R_L

seen with citric acid clearly demonstrates that the central airways are also hyperreactive, despite the lack of description of lesions at this level.

Heaves in horses has frequently been compared to human asthma,[149,151,165] because, like atopic asthma, recurrent airway obstruction is characteristic of the disease and an allergy is suspected. With regard to airway reactivity, heaves clearly differs from asthma. Whereas asthmatics have hyperreactive airways during periods of clinical remission, ponies do not. Airway hyperreactivity in heaves occurs only during acute exacerbations of the disease.

IV. SUMMARY AND CONCLUSIONS

An ideal animal model of asthma would be a naturally-occurring syndrome that would duplicate the clinical, biochemical, physiological, immunological, and pathological characteristics of the human disease. Fundamental studies using such a model would facilitate the emergence of clinical and diagnostic correlates to the benefit of those involved in clinical investigation, diagnostics, and patient care. Unfortunately, despite investigations into a plethora of in vivo asthma models, the ideal animal model does not appear to exist. Given this limitation, it becomes important to critically examine each available model system for its potential advantages and uses.

The pony model of asthma has several advantages over other model systems. Heaves is a spontaneous disease, and the clinical signs resolve when affected indivuals are removed from a barn environment. We have shown that nonspecific airway hyperreactivity to aerosolized histamine,[169] methacholine, and citric acid is present during acute exacerbations of airway obstruction. Thus, ponies with heaves fulfill the criteria of the 1962 American Thoracic Society definition of asthma.[7] Because of their large size, it is practical in ponies to make multiple physiological measurements and to collect multiple samples of tissue and body fluids. It has proven to be possible to serially study individual animals over an extended time period. The investigation reported here was conducted on conscious ponies under xylazine tranquilization. These

animals, however, are generally cooperative and trainable. Studies on unmedicated ponies should, therefore, be possible. From this perspective, ponies are more suitable than horses for such investigations. In addition, there is some epidemiological evidence that heaves may be more prevalent in ponies than in horses.[181]

A considerable body of clinical and experimental evidence supports the theory that inflammatory cells sequestered within the lungs mediate airway hyperreactivity. Available data suggest that the inflammatory reaction is the underlying process responsible for the histological appearance of the airways in asthma.[10] This reaction can account for mucus hypersecretion and epithelial cell loss, as well as airway edema.[10] Most of the pathological features of asthma can be accounted for by the release of mast cell and mast cell-associated inflammatory mediators.[66] In contrast, the mechanism(s) of airway hyperreactivity have not yet been well defined, although they may be linked to airway inflammation.[66] Identification of a broad spectrum of mediators derived from a variety of cell types has widened the scope of investigations into the role of mediators in airway hyperreactivity. Inhalation challenge and bronchoalveolar lavage procedures have evolved into convenient methodologies for these studies. The pony model of asthma demonstrates both nonspecific bronchial hyperreactivity and airway inflammation[173] during episodes of acute airway obstruction. Because of this, and its other similarities to human asthma, continued investigations using the pony model may help elucidate the complex interrelationship between cells, mediators, neural mechanisms, and airway responsiveness.

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