Criteria for Development of Animal Models of Diseases of the Respiratory System

The Comparative Approach in Respiratory Disease Model Development

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Advances in the understanding of human respiratory disease can come from careful clinical studies of the diseases as they occur in man, but such studies are naturally limited in terms of experimental manipulation. In the last 2 decades, an increasingly complex plethora of experimental respiratory disease models has been developed and utilized by investigators, but relatively less attention has been paid to the naturally occurring pulmonary diseases of animals as potential models. This paper is aimed at presenting selected examples of spontaneous pulmonary disease in animals that may serve as exploitable models for human chronic bronchitis, bronchiectasis, emphysema, interstitial lung disease, hypersensitivity pneumonitis, hyaline membrane disease, and bronchial asthma. Chronic bronchitis in dogs is characterized by chronic cough, excessive mucus production, and chronic inflammatory changes in bronchial walls. The disease affects mainly smaller-breed dogs of middle age or older. Equine chronic bronchitis tends to be a small airway disease with marked goblet cell proliferation and excessive mucus production, which may be accompanied by alveolar emphysema. Many animals develop bronchiectasis or bronchiolitis obliterans secondary to chronic suppurative bronchopneumonia, but chronic respiratory disease (CRD) of rats may be the most useful model of bronchiectasis. Models for emphysema must include actual alveolar destruction and ideally should be accompanied by appropriate pathophysiologic decrements. Many animals occasionally develop emphysema, but the disease has not been well documented, except possibly in horses. The interstitial lung diseases of man represent a complicated and poorly understood group of entities and near-entities. The same is true for animals, although interstitial lung disease in animals is much less common than bronchopneumonia. Cattle seem prone to develop interstitial lesions. Proliferative interstitial pneumonia of cattle includes many morphologic similarities to the spectrum of human interstitial pneumonitides. Fibrosing alveolitis of cattle is a morphologic end point that may have its origins in different forms of insterstitial injury. Hypersensitivity pneumonitis has been best detailed in cattle and in horses and is clinically, etiologically, immunologically, and morphologically similar to the disease in man. Hyaline membrane disease has been poorly documented in animals, with the possible exception of the neonatal respiratory distress syndromes of foals and piglets. Bronchial asthma is similarly not well established as a spontaneous disease in animals, although experimental models exist. Eosinophilic bronchiolitis of cattle may represent a useful asthma model but has been poorly detailed. In order to make them useful as models, more attention should be paid to detailing the clinical, morphologic, and etiologic aspects of these naturally occurring animal pulmonary diseases.

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THE VALUE and need for comparative medical studies in unraveling the complexity of disease need hardly be emphasized again. Their contributions have been voluminously documented in a seemingly endless number of symposia, workshops, seminars, monographs, reviews, lists, and books. Indeed, virtually any malady of any animal seems to have achieved "modeldom" in the hands of some skillful investigator. There is scarcely a single human disease that has not been touched by the application of scientific information derived directly from animal models. It seems reasonable, therefore, to wonder why an additional paper on models is necessary. What possibly can be added to the extremely long list of models already available?

The answer is not a simple one, but neither are the diseases in which the need for models is rooted. If one examines carefully the state of the art with respect to the contribution of models to our understanding of any single human disease, it quickly becomes apparent that the sum of that knowledge is a collection of small parts derived from several different sources. That is, no one model provides all of the clues, and no one clue constitutes a useful sum. It is from these bits and pieces of information from widely divergent backgrounds that a useful compendium can ultimately be achieved. Models come and models go, each offering its own personal clue and then moving on into a more senescent state of declining value. Keeping this evolutionary process viable requires that new models be continually added to replace those that are born, are studied, provide their clue, and pass away. Thus the continuing search for models is not only justified, it is necessary.

The respiratory tract has been a fertile ground for investigative studies. Poised as it is at the interface between animals and their environment, it is an ideal terrain for comparative research. In the last two decades, an increasingly complex plethora of experimental respiratory disease models has been developed and utilized by investigators, but relatively less attention has been paid to the naturally occurring pulmonary diseases of animals as potential models. We have chosen here to briefly highlight certain naturally occurring diseases of animals in the hope that more investigators will begin to attack and dissect them, forcing them to yield their clues to the understanding of parallel human conditions.

Canine Chronic Bronchitis

Recent work has revealed that a chronic bronchitis occurring in the dog closely resembles, both clinically and pathologically, the disease of chronic bronchitis in man.[1] The canine condition has been defined in clinical descriptive terms similar to the definition used in humans. Chronic bronchitis in the dog refers to the condition of subjects with chronic or re-

current excessive mucus secretion in the bronchial tree. This is a disease of adult dogs and is found from middle age onward. Although larger breeds, such as German sheperds, can be affected, chronic bronchitis typically occurs in smaller breeds, such as poodles and terriers, and is commonly seen in obese animals. The disease has an insidious onset and a progressive course: usually by the time the dog is presented it has been coughing for several months. Coughing is often precipitated by exercise or excitement, and in a number of cases it is productive, with gagging and expectoration. Radiographic examination discloses characteristic changes in approximately half the cases of chronic bronchitis; these changes take the form of increased bronchovascular markings extending to the periphery of the lung field and resulting in parallel and annular linear opacities.

The dominant feature at postmortem examination is the excess amount of mucus present in the airways; the mucus extends throughout the tracheobronchial tree, and there is often pooling at the tracheal bifurcation. Beneath the mucus, the mucosa appears roughened and opaque; microscopically, it is thickened by irregular and diffuse fibrosis, edema, and mononuclear cell infiltration. Most importantly, the mucus-secreting apparatus is increased in size: tracheobronchial mucous glands proliferate and enlarge so that they completely surround the airway lumina, and there is marked increase in the number of epithelial goblet cells. In addition, there is qualitative change in the mucus, with a shift in the production of the different types of mucosubstances.

Using the modified point count technique on samples collected at specified levels of the bronchial system, the bronchial wall components in young dogs, normal adult dogs, and dogs with chronic bronchitis have been quantitated.[2] The percentage of points falling on mucous glands was referred to as mucous gland percent (MGP), and the percentage falling on smooth muscle was designated smooth muscle percent (SMP). In a group of normal adults dogs, 10–14 years of age, and a group of normal young dogs, 3–5 months of age, the mean MGP was 1.3. This is much lower than the usual figure for normal adult human beings, in whom the MGP has been found to range from 8.8 to 11.03. Because the amount of bronchial mucous gland is much less in the dog than in man the Reid Index is particularly unsuitable for quantitation in the dog, owing to the lack of adequate numbers of representative sample sites. Mucous gland hypertrophy was confirmed in dogs with chronic bronchitis using the point counting technique. The MGP value for dogs with chronic bronchitis had a wide range, but they were significantly raised above those for normal adult and young dogs, the mean MGP for the group being 6.0 compared with 1.3 for normal animals.

The epithelial mucosubstances of the healthy canine bronchial system

have been studied by Spicer et al [3] and by Wheeldon et al.[2] The goblet cells of the canine bronchial epithelium were found to contain largely sulfomucins, but the mucous glands produced both sulfomucin and sialomucin as well as neutral mucins. In man, nearly all the goblet cells contain some acid mucosubstances that consist of periodate-reactive sulfomucins and sialomucins. The mucous glands of the human bronchial system contain mostly acid mucosubstances, the majority of which are sialomucin. The sialomucins in human respiratory mucus are of two types, one susceptible to neuraminidase and the other resistant to it. Both types of sialomucin have been identified in the canine respiratory tract. Generally speaking, the mucosubstances of the canine and human respiratory tracts are similar; however, there are differences in distribution, notably the predominance of sulfomucin in the goblet cells of the epithelium of the canine tracheobronchial tree. Dogs with naturally occurring chronic bronchitis have decreased amounts of epithelial sulfomucins together with a corresponding increase in epithelial sialomucins, as judged by the combined high iron diamine alcian blue technique.

Equine Chronic Bronchitis and Emphysema

The situation with respect to chronic obstructive pulmonary disease (COPD) in horses is not altogether clear. This broad group of debilitating respiratory conditions is often referred to among horsemen as "heaves" or "broken wind," in recognition of the typical double expiratory effort seen clinically. Unfortunately, as eloquently summarized by Breeze,[4] we have little detailed knowledge of the clinical and functional abnormalities, epidemiology, and pathology of equine COPD as it presently occurs in the United States, principally because all these aspects have never been adequately described in the same series of horses. The drawback in investigating only one facet of the problem, no matter how thoroughly this is undertaken, lies in satisfactorily defining the population under study so that it may be clearly identified by others. This has been responsible for many unwarranted generalizations and misunderstandings. In attempts to be more specific, a number of new terms have been introduced including chronic alveolar emphysema, emphysema, chronic bronchitis, chronic obstructive pulmonary disease (COPD), chronic asthmoid bronchitis, mucoid bronchiolitis, and bronchiolitis. Unfortunately, these terms have made the situation more confusing, because such diagnoses have frequently been applied in a manner that defies accepted methods of disease definition and differentiation. The rather confused state of the art is summarized in Table 1. The clinical signs characteristic of "heaves" can be produced by a wide variety of pulmonary lesions.

Table 1—Varieties of Equine Bronchopulmonary Disease Associated with the Clinical Syndrome of Chronic Respiratory Failure

A. Forms of chronic obstructive disease
1. Chronic bronchiolitis with acinar overinflation
2. Chronic bronchiolitis, acinar overinflation, and pulmonary eosinophilia
3. Chronic bronchiolitis, acinar overinflation, and destructive emphysema
4. Chronic bronchiolitis, acinar overinflation, pulmonary eosinophilia, and destructive emphysema
5. Destructive emphysema without chronic bronchiolitis
6. Chronic bronchiolitis without acinar overinflation or destructive emphysema
B. Other lesions associated with chronic respiratory failure
1. Hypersensitivity pneumonitis (extrinsic allergic alveolitis)
2. Chronic bronchopneumonia
3. Chronic interstitial pneumonia
4. Pneumocystosis
5. Various pulmonary granulomata
6. Pulmonary neoplasia

* Modified from Breeze.[4]

If the overall classification of this group of diseases is confusing, so are their pathologic changes. The pathology of equine chronic respiratory disease is known in general terms, but detailed investigations have been few. Chronic bronchiolitis is far more common than is destructive emphysema and may be characterized by diffuse epithelial hyperplasia, goblet cell hyperplasia, mucous plugging, and occasional bronchiolitis fibrosa obliterans. Mucosal and peribronchial infiltrates are common, and peribronchial fibrosis may be seen. Pulmonary eosinophil infiltrates are sometimes encountered. Acinar overdistention, or "air trapping," is seen far more often than is emphysema of the destructive type. Not all lesions are found in any single case, and there is a considerable variation in the degree or severity of the lesions.

It is clear that considerable work remains with respect to adequately defining the true nature of this group of conditions in horses. Future work must be aimed at careful definitional studies, for there is little chance of discovering etiologic or pathogenetic mechanisms, or of assessing different forms of treatment if a disease problem cannot be defined. It is fortunate that in human medicine many of the terms and diseases involved in chronic obstructive pulmonary disease are defined. At the same time, a note of caution should be sounded in adopting existing medical terminology too readily. Terms such as asthma and chronic bronchitis have already been applied to some of these equine conditions without any evidence that diseases similar to those defined in man exist in the horse. This approach can only lead to confusion in comparative medicine. There seems little doubt, on the other hand, that within the present confusion there lies

an extremely interesting group of equine lung diseases awaiting investigational exploitation to assess their considerable potential comparative importance as models of human respiratory disease.

Chronic Bronchitis and Bronchiectasis in Rats

Chronic bronchitis in man is defined as a clinical disorder characterized by excessive mucus secretion in the bronchial tree manifested by chronic productive cough not due to other known specific causes. Enlargement of the tracheobronchial mucous glands is the characteristic morphologic finding, together with an increase in the number of mucus-secreting cells in the surface epithelium at all levels of the airways. The clinical signs are difficult to detect and evaluate in most animal species; therefore the morphologic changes of chronic mucus hypersecretion are relied upon heavily in defining an animal model of chronic bronchitis.

When studying chronic bronchitis in rats, one important factor must always be kept in mind. Rats are very susceptible to a serious contagious syndrome referred to as chronic respiratory disease (CRD). Many agents have been suggested as causing chronic respiratory disease, with *Mycoplasma pulmonis* infection being frequently mentioned. Many terms have been used to describe the lesions. However, a fairly well-defined clinicopathologic entity emerges from all the reports. Young rats appear perfectly well, but by early adulthood (1 or 2 months), clinical signs (such as abnormal breathing sounds) may be apparent. Many rats develop catarrhal rhinitis and suppurative otitis media. Varying numbers develop pneumonia characterized by peribronchial infiltration of lymphoid cells, sometimes with distention of bronchi and bronchioles by mucus and polymorphonuclear leukocytes. Severe bronchiectasis with squamous metaplasia of bronchial epithelium may develop, with one or more lobes of the lung being converted to a mass of multiple large abscesses. In spite of the severe pulmonary lesions, mortality usually remains low. A number of procedures have been used to eliminate chronic respiratory disease from commercial breeding stocks, giving use to such terms as specific pathogen-free (SPF) rats. However, because of the contagiousness of the disease, even SPF rats frequently develop chronic respiratory disease unless strict surveillance procedures are maintained on the animals.

Chronic bronchitis, characterized primarily by chronic mucus hypersecretion, as in man, does not occur spontaneously in rats. As discussed above, chronic respiratory disease characterized by bronchitis and bronchiectasis does occur in many rat colonies. In general, this disease, because of the more inflammation and involvement of the pulmonary parenchyma, does not qualify as a good animal model for chronic bronchitis,

but it may be a useful model for describing the destructive airway changes leading to bronchiectasis. "Bronchitis" rats, however, have been used in a number of studies such as mucus transport in the tracheobronchial tree and the effect of exposure to irritant gases. In these situations, one should recall that the bronchitic rats have been naturally infected and the stage and severity of the disease may differ greatly among rats.

Lindsey et al [5] have provided the most definitive study of chronic respiratory disease in rats and have firmly implicated *Mycoplasma pulmonis* as the primary microbial cause. In their study, dramatic hyperplasia of bronchial epithelium accompanied lesions in peribronchial lymphoid tissue. This resulted in marked increase in height and piling up of epithelium along bronchial walls. Mucus production increased markedly. As the process continued, epithelial cells along the luminal surface underwent squamous metaplasia with loss of cilia. Simultaneously, neutrophils continued to accumulate in the bronchial lumens. Peribronchial lymphoid cuffing, hyperplasia of bronchial epithelium, and continued influx of neutrophils into the bronchi seemed to provide a vicious cycle for development of advancing lesions along the bronchial arborizations and in the surrounding parenchyma. The accumulation of lymphoid cells in bronchial walls and marked hyperplasia of bronchial epithelium often appeared directly related to stagnation and progressive accumulation of purulent exudate in more distal bronchi and bronchioles. As these changes increased in severity, the more distal parenchyma became atelectatic, and often the purulent exudate extended into terminal bronchioles and alveolar spaces. When neutrophils were present in alveolar spaces they were always found to be mixed with macrophages. Both of these cellular elements were sometimes so densely packed into the collapsed alveoli as to give the appearance of a solid tissue. Accumulation of peribronchial lymphoid cells often was accompanied by lesions in adjacent alveoli. These alveoli were lined by cuboidal epithelium and filled with neutrophils and macrophages.

The occurrence of bronchiectasis seemed clearly associated with extreme accumulation and subsequent impoundment of purulent exudate anywhere along the bronchial tree. As the exudate continued to accumulate distally, the hyperplastic bronchiolar epithelium appeared to invade alveolar spaces, thus forming bronchiectatic airways in close association with the pleural surface. In a few instances, discrete abscesses were found in lung parenchyma. Some of these were incompletely surrounded by bronchiolar epithelium, giving the impression that true abscesses were formed in such areas as a result of the destruction of bronchiolar epithelium in association with massive accumulation of purulent exudate.

It has always seemed interesting to us that CRD has been largely recognized by the research community in a negative sense. That is, it is something people do *not* want in their laboratory rats because it represents a serious underlying disease complication that has for years and years plagued investigators using rats as research animals. Without trying to detract from the importance of CRD as a disease that can restrict the usefulness of the rat for certain research purposes, we would like to suggest that it is high time that pulmonary disease researchers began to recognize the value of CRD in a positive sense as a frequent and naturally occurring model for destructive airway disease. It is entirely possible that the contributions that properly studied rats with CRD might make to our understanding of the pathogenesis of chronic airway damage and bronchiectasis could, in time, outweigh the years of historical damnation that the rat has suffered because of this fascinating affliction.

Emphysema

Pulmonary emphysema is defined as an anatomic alteration of the lung characterized by abnormal enlargement of air spaces distal to terminal, nonrespiratory bronchioles and accompanied by destructive changes of alveolar walls. The term has also been applied to abnormal increases in distal airspace size due to hyperinflation atrophy, or hypoplasia, but the currently accepted definition requires evidence of alveolar wall destruction. The definition does not require noticeable loss of function and does not address coexisting lung disorders; however, emphysema is often accompanied by functional impairment and the changes of a chronic obstructive lung disease complex. There are four major types of emphysema, based on the primary anatomic location of lesions. The centrilobular (centriacinar) type primarily involves the respiratory bronchioles. Panlobular (panacinar, diffuse or diffuse generalized) emphysema is a general involvement of respiratory bronchioles, alveolar ducts, and alveoli. Paraseptal emphysema is the destruction of respiratory epithelium along lobular septae. A fourth type is one in which the lobule is irregularly involved, an example being paracicatricial emphysema, in which destruction is found adjacent to scars in the lung.

The literature relative to the forms of emphysema occurring in animals as well as methods for inducing experimental emphysema has been reviewed by Karlinsky and Snider.[6] Emphysema is occasionally seen in any species of animals, but the details are often lacking. A reasonable example of the kind of confusion that can result is illustrated by the situation in horses. Clearly, some horses develop destructive emphysema, and elegantly detailed pathophysiologic studies are available.[7] Unfortunately, as

highlighted by Breeze,[8] there is a tendency for a variety of different morphologic forms of equine COPD to be grouped together, whether or not emphysema is present.

It is not certain whether uncomplicated emphysema as a single age-related disease entity exists in animals. Boatman and Martin [9] described emphysematous lesions in lungs of rabbits, which they believed to be secondary to ischemia resulting from pulmonary capillary lesions. Of the rabbits observed, emphysema was seen in 52% of those over 2.5 years of age; however, associated inflammatory changes were present in the lungs, and the degree of lung expansion was not controlled throughout the fixation period. Emphysematous changes have been observed in lungs of most species in association with other disease processes. Inflammatory changes in bronchioles of dogs may extend into alveolar ducts, leading to alveolar wall destruction and formation of large bullae at edges of lobes.[10] "Spontaneous" emphysema has been observed in rats in the presence of inflammation of airways and parenchyma.[11,12] A larger terminal airspace size was found in 38-week-old rats than in 10-week-old rats, but alveolar wall disruption was not documented.[13] McNulty [14] reported 12 cases of naturally occurring emphysema among 776 nonhuman primates necropsied at the Oregon Primate Center, but the lungs were also infested with the lung mite, *P simicola*. Boatman et al [15] found emphysema in two of three *Macaca nemestrina* females over 18 years of age and no emphysma in the lungs of subjects under 10 years of age, but the emphysematous lungs were also infested with lung mites (*P simicola*). It is possible that emphysema does occur as an uncomplicated, age-related spontaneous disease in monkeys, but the small number of observations in aged subjects does not permit that conclusion at this time.

The blotchy mouse presents a spontaneous model that may be useful for certain studies of emphysema, although the condition is not age-related and occurs in immature subjects. These mice have a genetically determined effect that prevents the generation of the lysine-derived aldehyde necessary for cross-linking of collagen and elastin.[16–18] Their lungs have enlarged terminal airspaces, attenuated alveolar walls, and functional characteristics typical of emphysema.[19]

For investigators utilizing animals models for emphysema, a constant awareness must be made of the interspecies differences in subgross anatomy of mammalian lungs.[20] In human lungs, the divisions distal to the terminal bronchiole are the gas-exchanging units that comprise a primary lobule. Several primary lobules are grouped together within connective tissue septa to form secondary lobules. A paucity of interlobular connective tissue prevents the definition of lobules in lungs of rodents, la-

gamorphs, carnivores, and nonhuman primates. Human lungs have several orders of branching respiratory bronchioles, as do lungs of dogs, cats, and monkeys. In contrast, respiratory bronchioles are rarely found in lungs of rats, hamsters, and mice; instead, the terminal bronchioles lead directly into alveolar ducts. Differences in structure of the terminal bronchioles of monkeys have also been reported.[21] *Macaca radiata* (bonnet) have long terminal bronchioles as found in man, while *M mulatta* (rhesus) and *M arctoides* (stumptail) have shorter terminal bronchioles. The terminal bronchioles of *M mulatta* also have been noted to have "transitional" areas with mixed epithelial types characteristic of both terminal and respiratory bronchioles and occasional alveoli. In the strictest sense, therefore, the term "centrilobular" could not be correctly applied to emphysematous lesions in lungs of animals (rodents and lagamorphs) not having respiratory bronchioles as found in man; thus, models for human centrilobular emphysema could only be developed in those species with well-defined respiratory bronchioles. A similar problem exists in using the term "paraseptal" in species without well-defined lobular septae. Panlobular and paracicatricial emphysema could be observed in all of the species mentioned.

Bronchial Asthma Models

We will have little to say about asthma models, because a naturally occurring disease characteristic of either the IgE-mediated (extrinsic) or non-IgE-mediated (intrinsic) type of asthma does not occur in animals, with the possible exception of certain dogs. Patterson and his co-workers [22,23] have substantially documented this canine disorder.

Dogs seem to be the only animals in which a defined hypersensitivity disease related to aeroallergens occurs. The clinical disease is most commonly ragweed pollenosis, although hypersensitivity to grass, tree, house dust, and cat antigens has been identified. The clinical manifestations include conjunctivitis, rhinitis, and an intensely pruritic dermatitis. In contrast to seasonal pollenosis in man, dermatitis, rather than ocular and respiratory symptoms, constitutes the major canine seasonal symptom. The mechanism of the dermatitis is not understood, since the same mechanism that occurs in production of rhinitis and conjunctivitis (mucosal absorption of antigen reacting with sensitized mast cells) appears unlikely. The only immune response defined has been the presence of reaginic antibody; the percutaneous absorption of pollen protein antigens has not been demonstrated.

A clinical syndrome in dogs similar to asthma does occur in pollen-sensitive dogs manifested by cough, dyspnea, and production of thick ropey

mucus. This is rare and, although its incidence has not been adequately documented, it appears to have a much lower rate of occurrence in allergic dogs than does asthma in allergic humans. In contrast to the rarity of lower respiratory tract response to environmental antigens, dogs with pollen allergy will almost always have a respiratory response to aerosolized antigen under controlled laboratory conditions.

Canine respiratory responses have been induced by immunologic stimuli in both the anesthetized and unanesthetized animal. The changes that occur in the unanesthetized dog may be observed by clinical evaluation or permanent records obtained by the recording of a pneumograph tracing. When the allergic animal is exposed to the appropriate aerosolized antigen in a chamber, acute dyspnea will develop. That is, increased frequency of respirations and labored breathing will occur. If the animal is removed from the test chamber, gradual recovery occurs, and if the dog is treated with epinephrine, there is more rapid resolution of these respiratory changes. Physical examination of these animals has not revealed wheezing. By using an endotracheal tube in anesthetized dogs and recording airflow after the controlled administration of an antigen aerosol by a respirator, the frequency of respirations (f), expiratory-inspiratory time ratio (E/I ratio), tidal volume (TV), and peak expiratory flow rate (PEFR) have been studied. The observed changes resemble those obtained in man and include increased f and E/I ratio and decreased TV and PEFR. In more recent studies, an increase in pulse rate in response to antigen challenge in ascaris-sensitive dogs has been documented. These changes are reversed by epinephrine. A summary of some of the characteristics of canine reagin-mediated respiratory responses is shown in Table 2.

Before leaving the subject of asthma models, we would like to briefly mention the occurrence in cattle of an eosinophilic bronchiolitis with many morphologic similarities to the lesions seen in long-standing human asthma. These lesions are characterized by marked smooth muscle proliferation in distal airways, glandular hyperplasia, eosinophil infiltrates in the hyperplastic mucosa, bronchospasm, and abundant plugging of smaller airways by mucoeosinophilic debris. The etiology and immunology of the condition are unknown at the present time, but the morphologic similarity to the changes of chronic asthma suggest that further exploration of this bovine disease is indicated.

Hyaline Membrane Disease Models

The syndrome of hyaline membrane disease (HMD) is believed to be an expression of a basic defect in elaboration and secretion of pulmonary surfactant, developing when the lung has not been exposed *in utero* to hor-

Table 2—Characteristics of Canine Reagin-Mediated Respiratory Responses*

Type of reactivity	Antigens used	Sensitization	Availability	Immunology	Respiratory response	Produced in normal animals	Limitations
Active pollen sensitivity	Ragweed and grass; others feasible	Results of environmental exposure	Not rare but very difficult to obtain for study	Reaginic antibody; probably IgE	Positive on repeated exposure; duration for many years	No	Rarely
Passive sensitivity	Ragweed; Others feasible	Passive sensitization with canine reagin	Requires serum donors	Canine reaginic antibody; probably IgE	Positive if sensitizing dose sufficient (volume and titer)	Yes	Requires repeated sensitization for each experiment
Active ascaris sensitivity	Purified *Ascaris suum* antigen	Result of infestation with *T canis*; experimental sensitization	Readily available	Reaginic antibody; probably IgE	Positive but varies in intensity	Occurs in most dogs after aerosol exposure	Variability of respiratory responses

* Modified from Patterson and Kelly.[23]

monal stimuli necessary for lung maturation, or because the surfactant-producing cells have been damaged, as may occur during episodes of hypotension or hypoxia. The 30 years or so that have passed since the first clinical description of infants with respiratory distress caused by HMD have been marked by extensive research. Not only is the pathophysiology carefully documented, but major advances have occurred in therapy, and prenatal prediction and prevention of the disorder are possible in some instances. Although considerable research effort has been directed at various experimentally induced models of HMD, little attention has been paid to the potential research applications of naturally occurring HMD in domestic animals. HMD has been reported and detailed largely in two species, the foal and the piglet.[24]

Foals born with HMD are usually carried to term and have been referred to as "barkers" because of a characteristic expiratory grunt.[25–28] Affected foals may be hypoxemic and have respiratory distress, as indicated by reduced tidal and maximal tidal volumes, increased respiratory rate, and low arterial blood oxygen saturation. Signs of cardiac dysfunction, as evidenced by a marked jugular pulse, rapid heart rate, and a hard peripheral pulse, may be present in some individuals.

Fractured ribs and myocardial damage may be found at postmortem examination; histologic changes in the lungs include uneven airway expansion, edema, and hemorrhage. A marked surfactant deficiency can usually be demonstrated in these foals.[26] Central nervous system signs, presumably related to growing anoxia, are sometimes seen; affected newborn foals may show signs of clonus, generalized convulsions, loss of the sucking reflex and affinity for the mare, apparent blindness, opisthotonos and extensor rigidity of hind and fore limbs, loss of righting reflexes, incessant chewing, sneezing, asymmetric pupillary apertures, muscular flaccidity and coma, and wandering or dummylike behavior.[29,30] The equine HMD syndrome is of only sporadic occurrence and appears to have a background in surfactant deficiency even though the foals are carried to term.[31–33]

An almost invariably fatal condition of newborn piglets, characterized by respiratory distress and, in some instances, subcutaneous edema and a short, fine haircoat, was first observed in England in 1973 but not reported until 1976.[34] These piglets had all been sired by the same large white boar, and analysis of breeding data suggested that the condition was inherited as an autosomal recessive trait. Affected piglets produced characteristic expiratory grunting sounds reminiscent of the so-called "barker" syndrome of foals, and consequently they were called "barker" piglets. Very soon after birth, affected piglets develop respiratory distress; at necropsy they have a distinctive array of lung lesions that includes uneven expansion of

the airways, epithelial necrosis, hyaline membrane formation, and hemorrhages. Lungs were also characterized by the presence of abnormal, probably immature alveolar epithelium, and by very marked deficiency of surfactant. Measurements of surface tension of alveolar washings have revealed little or no surfactant activity in any of the affected lungs; in fact, in many instances the surface tension was appreciably higher than that of isotonic saline.[35] Gross pulmonary lesions consisted of atelectasis and edema. Histologically the lungs showed an uneven expansion of alveoli; dilatation of bronchioles; subpleural, interlobular, peribronchial, and peribronchiolar edema and hemorrhage. Some alveoli had a glandular appearance or fetalization similar to that seen in the lungs of 90-day-old fetuses. The glandular appearance was due to the presence of large, rounded, or polygonal cells containing cytoplasmic PAS-positive material and lipid-free vacuoles. Osmiophilic inclusions were few or absent in alveolar epithelial cells of affected lungs. Hyaline membranes containing blood cells and cellular debris were common features of small respiratory passages. Subsequent electron microscopic studies confirmed that the pulmonary lesions are characterized by immaturity of the distal airways and of alveoli; by severe alveolar epithelial hyperplasia and hypertrophy; by increased width of the blood-to-air barrier; by alveolar and bronchiolar epithelial degeneration and separation; by the production of alveolar and bronchiolar hyaline membranes; and by alveolar hemorrhage and alveolar and peribronchial edema.[36] Many of the hyperplastic cells of the alveolar epithelium are pyramidal, rest on a basement membrane, have microvilli on their luminal surfaces, form tight junctions with their neighbors, and contain reduced numbers of lamellated electron-dense inclusions in the cytoplasm. These cells are dystrophic Type 1 or Type II pneumocytes. Both types contain large amounts of cytoplasmic carbohydrate material and are deficient in lamellated inclusions associated with Type II pneumocytes of normal piglets. Increase in thickness of the blood-to-air barrier from 0.22 to 0.55 μm in normal to 0.50 to 2.33 μm in moderately affected piglets, or to a maximum of 12 μm in severely affected piglets, was associated with increasing respiratory distress. Hyaline membranes were composed of epithelial cellular debris from saccular and bronchiolar epithelium. The reduction in size and number of the specific lamellated cytoplasmic inclusions of Type II pneumocytes in affected lungs was correlated with biochemical findings of increased surface tension and reduced phospholipid and lecithin contents of lung washings.

Hypersensitivity Pneumonitis Models

Hypersensitivity pneumonitis (extrinsic allergic alveolitis) has been identified as a naturally occurring disease in cattle and horses, although it

is presumed that other domestic species may also develop the disease. The bovine form of farmer's lung was, interestingly enough, first described in cattle from the same district of Britain in which the first human cases were recognized.[37,38] It quickly became apparent that the disease could be very prevalent in areas where moldy hay was used as feed and that clinical cases could be identified in large numbers in such regions.[39] Both acute and chronic forms have been identified.

Precipitating antibodies (precipitins) to *Micropolyspora faeni* can be demonstrated in the sera of cases of bovine "farmer's lung" by double diffusion tests.[40–42] Intradermal injection of *M faeni* antigen produces skin thickenings that reach maximum size after about 4 hours and slowly decline over the next 72 hours. Biopsy of the skin test site at 4 hours reveals that the swelling is mostly due to local edema in the dermis, and many neutrophils can be seen within small blood vessels, emigrating through their walls and accumulating around them; this histologic appearance is consistent with that of an Arthus reaction. At 72 hours, there is only slight edema, and the cell population around the small blood vessels consists of eosinophils, plasma cells, and lymphocytes, with a small number of neutrophils.

In acute cases the lungs are superficially normal, but closer inspection reveals the presence of a number of small gray spots on the pleural surface of many lobules. The peripheral acini of each lobule are overinflated, and this produces a raised pale edge around a darker-red central portion. On microscopic examination, however, widespread pulmonary lesions can be observed. There is diffuse infiltration of alveolar septa by lymphocytes, plasma cells, and interstitial cells, and intraseptal aggregates of lymphocytes without germinal centers are present. Where there has been recent experimental or natural exposure to *M faeni*, there are foci where the alveoli contain edema fluid, free erythrocytes, neutrophils, and macrophages, and neutrophils are frequently found in the alveolar septa, along with plasma cells and lymphocytes. Characteristic noncaseating epithelioid granulomata with multinucleated giant cells are often found. Studies of experimental hypersensitivity pneumonitis in cattle confirm its similarity to the human disease.[43–47]

The story with respect to hypersensitivity pneumonitis in horses is somewhat less complete. Cases of "heaves" attributed to extrinsic allergic alveolitis to *M faeni* have been found in Switzerland,[4] and an outbreak apparently involving chicken antigens was reported in the United States.[48] The functional defect is not described, but since the lesions are predominantly in the interalveolar septa a restrictive pattern of respiratory failure seems likely. The pulmonary lesions in the equine cases of farmer's lung resemble those of the human and bovine forms and are quite differ-

ent from those of other causes of equine chronic obstructive pulmonary disease. It thus seems unlikely that alveolar hypersensitivity as it occurs in farmer's lung is a cause of chronic obstructive pulmonary disease in horses, because the equine form of farmer's lung differs pathologically from chronic obstructive pulmonary disease.

Clinical and functional exacerbations of pulmonary disease can be produced by aerosol provocation tests using antigens derived from *M faeni*, *Aspergillus*, *Nocardia*, or other fungi. Immediate and delayed (4–6-hour) reactions may be obtained, which is an indication of pulmonary hypersensitivity to these antigens, although care must be taken to eliminate a nonspecific irritant effect. Antigens of *M faeni* are able to activate complement by the alternative pathway and produce inflammation without antigen-antibody interaction. Some antigens of *M faeni* are proteolytic enzymes with a trypsin or chymotrypsin-like effect. It is possible that these have both a direct inflammatory and an antigenic effect in the lung. An allergic reaction may take place in the small airways rather than in the alveoli as is typical of farmer's lung (extrinsic allergic alveolitis may also involve the bronchioles). The presence of pulmonary eosinophilia in some animals could be related to exposure or allergy to inhaled fungi, as in allergic bronchopulmonary aspergillosis or the pulmonary eosinophilias of man.

The exposure of horses to dusts derived from avian excreta can produce hypersensitivity pneumonitis. The evidence here favors an immunologic rather than an infectious cause for the disease in these horses. The horses become clinically normal when removed from their environment. The reaction of some horses to the chicken embryo-developed encephalomyelitis vaccine resembles an Arthus-type reaction. There are several factors that suggest that the disease in these horses is at least partially a Type 3 immune response. Biopsy specimens from the areas of intradermal tests to chicken serum resemble Arthus-type reactions induced experimentally in normal horses. There is no eosinophilia in the blood or bronchial secretions. Circulating precipitating antibody can be demonstrated and can be shown to increase and decrease relative to exposure to the specific antigen. The clinical signs can be reproduced some 4 hours after inhalation of chicken antigen. Other immunologic reactions, specifically the Type 1 and Type 4 immune reactions, have been related to hypersensitivity pneumonitis in man.

Interstitial Lung Disease Models

There is a need for spontaneously occurring examples of so-called primary or idiopathic pulmonary fibrosis and diffuse fibrosing alveolitis. Such a model may exist in a bovine condition described by Pirie and Selman.[49]

Diffuse fibrosing alveolitis in the bovine species seems to be a distinct clinical and pathologic entity. Clinically, these cases can be readily detected because, although the animals are usually bright, they have a persistently high respiratory rate, widespread adventitious sounds over both lung fields, and a cough. At rest hyperpnea is obvious, and even mild exercise is not readily tolerated.

Although they appear to eat readily, they are thin and there is a tendency to develop congestive cardiac failure. When the lungs are seen at postmortem examination, they may appear to be almost normal until inspected closely. This feature is particularly striking when the degree of clinical respiratory abnormality is known. The lesions seen microscopically are distributed widely in all the lobes of the lung, and the fibrosis is characteristically within the interstitium of the respiratory acinus.

The cellular thickening and fibrosis of the alveolar septa conform to the criteria suggested for diffuse fibrosing alveolitis. A second criterion of large mononuclear cells in the alveolar space was also fulfilled, although in most cows the interstitial reaction predominates.

Hyperplasia and metaplasia of the alveolar epithelium are not considered essential features of the disease in man, but they are present in many bovine cases. Although the predominant changes in the lungs are in the alveoli, there is also a reaction in the bronchial tree. The most common cause of bronchitis in cattle is the lungworm *Dictyocaulus viviparus*, but none are usually found in these animals, and the lesions do not conform to the usual bronchitis associated with a primary *D viviparus* infection. The lesions may be a hypersensitivity type of reaction to *D viviparus*, but whether they are related to the changes in the respiratory portion of the lung or are coincidental is not clear.

It has been suggested that one form of human fibrosing alveolitis could be due to farmer's lung, and it is possible that a similar pathogenesis is responsible for the disease in the cows with precipitins against *M faeni*, although there are some differences between the changes in these lungs and those in bovine farmer's lung.[50,51] This potential model obviously awaits further definition.

Summary

Most of the diseases, syndromes, lesions, and occurrences mentioned here are not well detailed from the standpoint that it is difficult to evaluate their potential usefulness as models for parallel human diseases of the respiratory tract. Nonetheless, all of them seem to have reasonably direct corollaries in diseases of human beings, they are naturally occurring diseases in animals of sufficient size to permit pathophysiologic detailing, and they are of common enough occurrence to be relatively available to the

research community. They are presented here in an attempt to attract potential investigators, for only through rigorous and well-controlled studies of the clinical, morphologic, and etiologic aspects of these diseases can their true potential as comparative pulmonary disease models be realized.

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