

EQUINE CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

Publications 1974 - 1985

Initiated and directed throughout

by

E.A. McPherson
M.Sc., M.R.C.V.S.

University of Edinburgh
Department of Veterinary Medicine

Diploma of Fellowship of the Royal College of Veterinary Surgeons
awarded for Meritorious Contributions to Learning.

February 1988

EQUINE CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Publications in Chronological Order

- Some Aspects of Chronic Pulmonary Diseases of Horses and Methods used in their Investigation. E.A. McPherson and G.H.K. Lawson, (1974) *Equine vet. J.*, 6, 1.
- Chronic Obstructive Pulmonary Disease (COPD): Identification of Affected Horses. E.A. McPherson, G.H.K. Lawson, Jill R. Murphy, Janet M. Nicholson, J.A. Fraser, R.G. Breeze and H.M. Pirie. *Equine vet. J.* (1978), 10 (1) 47-53.
- Pulmonary Artery Pressures in Normal Horses and in Horses affected with Chronic Obstructive Pulmonary Disease. P.M. Dixon. *Equine vet. J.* (1978), 10 (3) 195-198.
- A Pathological Study of Chronic Pulmonary Disease in the Horse. Julia M. Nicholls. Thesis (1978) University of Glasgow.
- Chronic Obstructive Pulmonary Disease (COPD) in Horses: Aetiological Studies: Responses to Intradermal and Inhalation Antigenic Challenge. E.A. McPherson, G.H.K. Lawson, Jill R. Murphy, Janet M. Nicholson, R.G. Breeze and H.M. Pirie. *Equine vet. J.* (1979), 11 (3), 159-166.
- Chronic Obstructive Pulmonary Disease (COPD): Factors influencing the occurrence. E.A. McPherson, G.H.K. Lawson, Jill R. Murphy, Janet M. Nicholson, R.G. Breeze and H.M. Pirie. *Equine vet. J.* (1979), 11 (3), 167-171.
- The Presence of Precipitating Antibodies in the Sera of Horses with Chronic Obstructive Pulmonary Disease (COPD). G.H.K. Lawson, E.A. McPherson, J.R. Murphy, J.M. Nicholson, P. Wooding, R.G. Breeze and H.M. Pirie. *Equine vet. J.* (1979), 11 (3), 172-176.
- Identification and Characterisation of the Major Antiproteases in Equine Serum and an Investigation of their role in the onset of Chronic Obstructive Pulmonary Disease (COPD). A.G. Matthews. *Equine vet. J.* (1979), 11 (3), 177-182.
- The nature of the prealbumin 'esterases' of horse serum. A.G. Matthews. *Anim. Blood Grps biochem. Genet.* 10 (1979) 181-184
- The effects of sodium cromoglycate on antigen inhalation challenge in two horses affected with chronic obstructive pulmonary disease (COPD). Jill R. Murphy, E.A. McPherson and G.H.K. Lawson (1979). *Vet. Immunol. Immunopathol.* 1, 89-95.
- Isoelectric focusing of horse acidic prealbumins on thin-layer polyacrylamide gels. Andrew G. Matthews. *Anim. Blood Grps biochem. Genet.* (1979) 10, 219-226.
- Some blood pressure studies in normal horses and in horses affected with chronic obstructive pulmonary disease. Padraic M. Dixon. Ph.D. Thesis (1979), University of Edinburgh.

- Chronic obstructive pulmonary disease (COPD): Effects of bronchodilator drugs on normal and affected horses. Jill R. Murphy, E.A. McPherson and P.M. Dixon. *Equine vet. J.* (1980), 12 (1), 10-14.
- Some serological studies in the horse in relation to the pathogenesis of chronic obstructive pulmonary disease. Andrew G. Matthews. Ph.D. Thesis (1981), University of Edinburgh.
- Chronic obstructive pulmonary disease anatomical cardiac studies. P.M. Dixon, J.R. Nicholls, E.A. McPherson, G.H.K. Lawson and J.R. Thomson. *Equine vet. J.* (1982) 14 (1), 80-82.
- Studies on the therapy of equine chronic obstructive pulmonary disease. Jill R. Thomson. Ph.D. Thesis (1982), University of Edinburgh.
- Serum protein electrophoresis in horses and ponies. A. G. Matthews (1982). *Equine vet. J.* 14 (4), 322-324.
- A reagin-like antibody in horse serum: 1. Occurrence and some biological properties. A. G. Matthews, P. Imlah and E.A. McPherson (1983). *Vet. Res. Comm.* 6, 13-23.
- A reagin-like antibody in horse serum: II. Anti-human IgE induced reversed cutaneous anaphylaxis-like responses in horse skin. A.G. Matthews, P. Imlah and E.A. McPherson (1983). *Vet. Res. Comm.* 6, 111-122
- A study on the possible role of chymotrypsin in the aetiology of equine chronic obstructive pulmonary disease (COPD). Jill R. Thomson, E.A. McPherson, G.H.K. Lawson, Penny Wooding and R. Brown (1983) 387-395.
- Chronic obstructive pulmonary disease in the horse: 1. Nature of the disease. E. A. McPherson and Jill R. Thomson. *Equine vet. J.* (1983) 15 (3), 203-206.
- Chronic obstructive pulmonary disease in the horse: 2. Therapy. *Equine vet. J.* (1983) 15 (3), 207-210.
- Effects of environmental control on pulmonary function of horses affected with chronic obstructive pulmonary disease. Jill R. Thomson and E.A. McPherson. *Equine vet. J.* (1984) 16 (1), 35-38.
- Tracheobronchial secretion from normal horses and horses with respiratory diseases. Susan L. Maxwell. M.Phil. Thesis (1984), University of Edinburgh.
- Chronic obstructive pulmonary disease in the horse - a review. Jill R. Thomson and E.A. McPherson (1985). *Irish Veterinary News*, August/September, 1985.

EQUINE CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)Summary

The condition now more frequently known as chronic obstructive pulmonary disease (COPD) has been recognised for centuries under its old title of "broken wind" or "heaves", a major cause of illness. It has been known as a hazard to the health of the horse since the time of Aristotle. Until the mid 1960s little effort had been made to separate "heaves" from other chronic respiratory conditions and possible causes of the condition remained shrouded in mystery apart from occasional observations of an association with dusty fodder and bedding.

Sasse (1971), working in Utrecht University, pioneered the work which clarified the physiopathological abnormalities of the condition. It was the realisation of this ability to define more clearly the clinical parameters of the disease that initiated these studies in the University of Edinburgh, under the direction of Mr. E.A. McPherson. A principally pathological component was undertaken jointly with the University of Glasgow Veterinary School, under the direction of Professor H.M. Pirie.

A reliable, objective, non-invasive method of distinguishing horses affected with COPD from those affected with other respiratory disease was developed. The aetiology was defined and the pathogenesis clarified. Methods of treatment and control were devised and it is now possible to render affected animals asymptomatic by environmental control, so that they can tolerate the hazard of normal surroundings when protected by a commercial product, the efficacy of which was researched here. Many aspects of diagnosis, treatment and prevention were surmounted in achieving these objectives but the problem of recognition of affected animals in clinical recession remains. As with other allergic conditions, this is often insoluble without resort to immunological challenge but future technological developments may overcome this hurdle and may enable veterinarians to recognise horses with a predisposition to the disease in time to take preventive measures.

Many stables have already adopted our environmental control measures for all animals, rather than for affected horses only, with resultant improvement in equine health and the economics of horse ownership.

EQUINE CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)Introduction

The techniques of Sasse (1971) made it possible to distinguish COPD-affected horses from other horses showing poor performance and chronic coughing from a variety of other causes and to study the disease in depth. Early work confirmed the methods chosen as practicable and satisfactory and were quickly employed to show an association between the disease and the use of poor quality hay or bedding and of poor ventilation. Attempts were then made to ascertain more specifically the causes and mechanisms involved in the adverse reactions seen following inhalation of the products of poor quality roughage. The important role of Micropolyspora faeni and Aspergillus fumigatus in Northern Britain was demonstrated although other agents were sometimes involved. More importantly, the horse's respiratory system was shown to be capable of developing hypersensitivity to antigens of this type; possibly in this country and elsewhere, other antigens are also of importance. Precipitating antibodies were shown to be prevalent and more common in horses affected with COPD than in healthy horses. In animals suffering from COPD, both precipitating and skin sensitising antibodies to M. faeni were good indicators of the antigen to which their respiratory systems would react; on their own, these tests were of no diagnostic value.

We have determined that pathologically the disease is manifested as an over-inflation of the alveoli with little true emphysema, contrary to earlier, reiterated statements. In addition, there is bronchiolitis of the small airways with goblet cell formation in the smaller airways, where they are normally absent, and there is an accompanying cellular response. A preponderance of neutrophils is present in the continuous, copious, mucopurulent, tracheal mucus. In vitro, it has been shown that the bronchus and bronchioles contract on exposure to acetone-extracted M. faeni cultures diluted in saline.

The disease process has been shown to be reversible if horses are kept in a minimal dust environment. Where this is not possible, the administration of the preventive drug, sodium cromoglycate, has rendered horses asymptomatic and broncho-dilator drugs have also been shown to be partially effective for amelioration of symptoms over the short term.

During these studies, it was shown that pulmonary hypertension caused by the hypoxaemia associated with the disease existed in COPD-affected horses but cor pulmonale did not ensue. The possible role of chymotrypsin and other antiproteases in the aetiology of COPD was investigated but these were not involved in the same way as they are in man. Investigations were carried out to determine the presence of serological factors which might be useful in indicating horses likely to develop COPD, but none was revealed. A preliminary investigation of the possible role of IgE was undertaken and this awaits further study.

From this it is clear that many of the objectives have been achieved. The detailed immunological mechanisms still have to be further explored and will undoubtedly yield much information as technology advances. It also remains to elucidate the mechanisms that initiate the condition, for in this secret lies the key to better prevention. The objective may be difficult to achieve as, in any condition in which initiation and expression of the disease are separated in time, the links are hard to trace.

The most important factors shown to affect animal health disadvantageously are poor ventilation and the use of hay as fodder and straw as bedding. The feeding of vacuum-packed silage (HorseHage) and/or Complete Diet Horse and Pony Cubes has been shown to be beneficial, and the use of peat moss, shredded paper or shavings as bedding helps to control and prevent the disease. These measures have been adopted by many stables for all horse feeding and bedding, as a desirable method of stable management and nutrition.

Some Aspects of Chronic Pulmonary Diseases of Horses and Methods used in their Investigation






E. A. McPHERSON

G. H. K. LAWSON

Royal (Dick) School of Veterinary Studies, University of Edinburgh

CHRONIC pulmonary dysfunction in the horse has in the recent past been described rather loosely by the clinical term "heaves", which itself has largely come to replace the older description, "broken wind". physiological abnormalities. The traditional description of respiratory symptoms in which crepitation together with a double expiratory movement predominate no longer suffices to include all

TABLE I
HEAVES IN HORSES

Pulmonary Lesions	Clinical Signs						
	Chronic or Intermittent	Dyspnoea	Cough	Nasal Discharge	Bronchial Sounds	Crepitation	Febrile
Broncho  Spasm Bronchiolar  (asthma)	+	+	—	Little (serous)	Wheezing	—	—
Bronchitis Bronchiolitis	+	+	+	Serous Mucoid Purulent	Dry Moist	—	?
Emphysema (Centrilobular) (Panlobular)	+	+	—	—	—	+	—
Interstitial Pneumonitis (including Allergic Alveolitis)	+	+	+	?	—	—	?
Pulmonary  Vascular  Disease  Spasms or obliteration of Bronchiolar arteries	+	+	?	?	?	—	—

Even the more modern term does not mean the same thing to all authors, and it seems likely that it embraces a variety of clinical and related pathological or

the chronic pulmonary conditions of which we are aware. Several candid writers have reported failure to hear crepitation at all in "heaves" cases, while others hear these sounds infrequently or only in limited parts of the lungs. Clearly there is an apparent conflict of observation, diagnosis or, alternatively, real differences

Presented at the annual congress of the British Equine Veterinary Association, Edinburgh 1972.

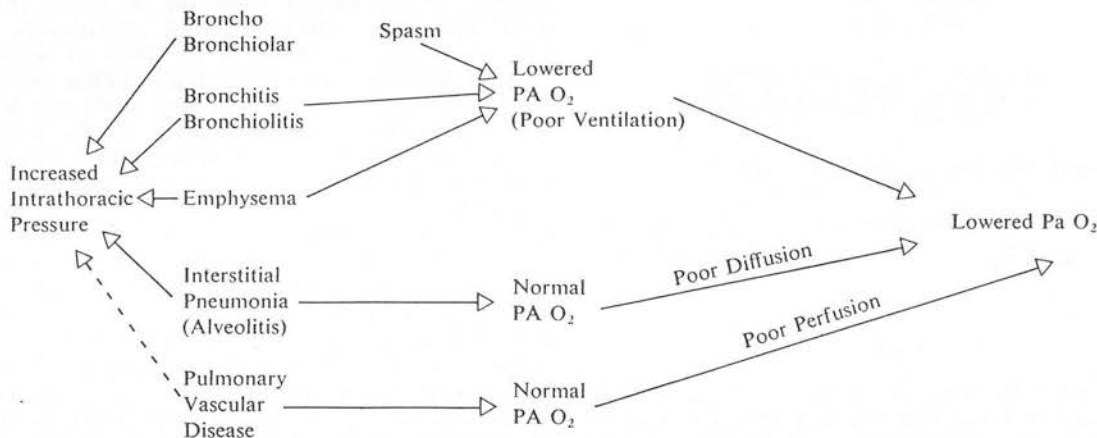
in the character of the entity occurring in different geographical areas or perhaps at different stages of development of the disease. In the present state of knowledge it is probably useful to consider "heaves" as "a complex of symptoms primarily originating in the lung" (Sasse, 1971); this, at least, ensures that we do not exclude conditions from consideration before being able to define them.

Before further progress can be made in investigating

CHANGES OF STRUCTURE AND FUNCTION IN "HEAVES"

In any consideration of clinical abnormality it is useful to examine the primary changes of tissue function and structure and then relate these to the development of particular disease symptoms. A variety of pulmonary dysfunctions have been described or postulated in "heaves" in horses. These abnormalities give rise to several prominent clinical signs and to alterations in

TABLE II
EFFECT OF PATHOLOGICAL CHANGES ON ALVEOLAR AND ARTERIAL OXYGEN AND INTRATHORACIC PRESSURE



PA O₂ and Pa O₂ = partial pressure of O₂ in alveoli and arterial blood respectively.

either the aetiology or pathogenesis of this complex of symptoms it seems important to try to define the conditions more accurately. This is our current, if optimistic, aim.

The pathological changes described in clinical "heaves" cases include chronic pulmonary emphysema, acute bronchiolar smooth muscle spasm, bronchitis, allergic asthma and a disease similar to farmer's lung in man (Gillespie, Tyler and Eberly, 1964). Jubb and Kennedy (1971) acknowledge the absence of emphysema in some cases and also remark on the confused state of knowledge, but regard obstructive obliterative bronchiolitis as the usual prelude to chronic emphysema, the distribution of the latter depending on the distribution of the bronchiolitis.

The complex of diseases under consideration causes symptoms which appear either suddenly or, more often, insidiously, and cases are seldom seen at a febrile stage, if indeed fever occurs. The condition is clinically chronic and is sometimes intermittent. A few of those cases which manifest themselves suddenly and acutely may, at that stage, have some similarity to acute infections of the respiratory tract. With the latter we are not concerned except to assess the part which such episodes may play as precursors of chronic pulmonary disease. Epidemiological considerations are the most useful guide to the presence of acute viral infections, but where horses are kept in small numbers this evidence may not be available.

the level of the blood gases which combine to decrease the animal's working capacity. These changes are set out in Tables I and II.

Broncho- and bronchiolar spasm, with a sticky mucus in the airways, appears to occur and, as this is essentially a functional change, the interpretation of this disturbance by conventional histological techniques is likely to prove difficult even where the problem of obtaining a satisfactory sample is overcome. The characteristic clinical effect is wheezing and scanty nasal discharge. There is dyspnoea but coughing is not necessarily a marked feature. Because of the restriction of the airways, the alveolar oxygen is below normal; consequently this may be expected to be reflected in a reduction of the oxygen content of the circulating blood. In man, bronchial spasm results in alveolar trapping of air which can pass inwards through a partially obstructed bronchus more readily than it can be expelled. Attempted forced expiration in such circumstances may cause breakdown of the alveoli and emphysema (CIBA Foundation, 1971). It seems reasonable to postulate a similar mechanism in the horse. The increased respiratory effort required to overcome the mechanical obstruction can be measured in the form of increased pressure changes in the pleural space as described below in bronchitis.

In bronchitis and bronchiolitis there is cellular proliferation and/or infiltration accompanied by varying degrees of exudation in the bronchial, bronchiolar and

peribronchial tissues. This gives rise to a variety of clinically observable changes. Coughing and dyspnoea are prominent, and there is nasal discharge which can vary from serous to mucoid and purulent. The passage of air along the bronchi, reduced in diameter as a result of an increase in the tissue within the cartilaginous rings, produces adventitious dry sounds which become moist, even bubbling when there is exudation or increased mucous secretion into the airways. This has the same functional effect as spasm and eventually may give rise to emphysema. Efforts to overcome this disability result in increased respiratory movements. These movements, both inspiratory and expiratory, may often be observed to occur in two stages, the abdominal muscle contractions and relaxations each being a two-phase action. As a consequence of the restriction of the airways and enhanced respiratory movement the difference in intra-pleural pressure between the end of inspiration and the end of expiration increase considerably. It has been shown by Sasse (1971) that measurement of the intra-pleural pressure at the end of inspiration and expiration by using a cannula in the pleural space and a recording device is a safe procedure in horses. He has shown also that the severity of the clinical state of "heaves" is related to the difference between the pressures existing in the pleural cavity at these times. The pressure is measured in either mm. of mercury or cm. of water and is usually a negative value, though in diseased lungs with forced expiration of air the pressure may exceed atmospheric pressure. For normal horses Sasse (1971) found the change in intra-pleural pressure during respiration to be 10.3 ± 2.9 cm. water, with pressure ranging from -17.0 cm. at inspiration to -1.4 cm. water on expiration. By contrast, one of his horses with chronic bronchitis, interstitial pneumonia and widespread emphysema had a maximum intra-pleural pressure change of 50.1 cm. water, the inspiratory pressure being -33.7 and the expiratory pressure being $+16.4$ cm. water.

Emphysema is usually considered to arise from overdistension of either the alveoli or the terminal bronchioles (centrilobular or panlobular emphysema), as a sequel to the changes described above. Some workers, however, have postulated vascular changes in the alveolar walls as a cause of the dilatation. Emphysema results in a reduced surface area for gaseous exchange

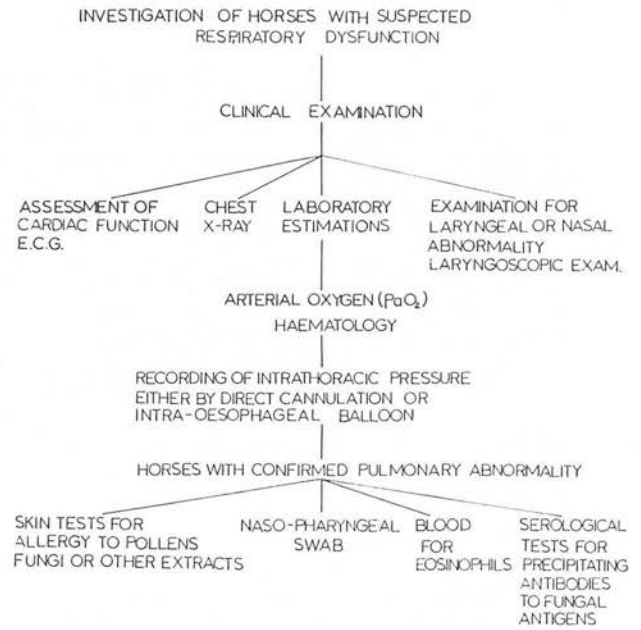


Fig. 1

and in poor ventilation of affected alveoli. These unfavourable sequelae are intensified as the damaged lungs are less able to collapse due to loss of elastin and the presence of additional collagen, with the result that the normal expiratory effort of the abdominal muscles is followed by a further contraction to compress the lungs; hence the clinical description "double lift". Emphysema becomes permanent only when breakdown of the walls occurs. On auscultation crepitation or crackling are detected. The reduced alveolar ventilation manifests itself by a lower alveolar oxygen concentration and a consequent lowering of blood oxygen.

Interstitial pneumonitis

Essentially, this involves cellular proliferation and/or infiltration and varying degrees of exudation in the interstitium of the lung, i.e. between the alveolar walls and in the support tissues of the lobules. There is confusion of terminology among authors. In man, marked cellularity of the alveolar walls and inter-

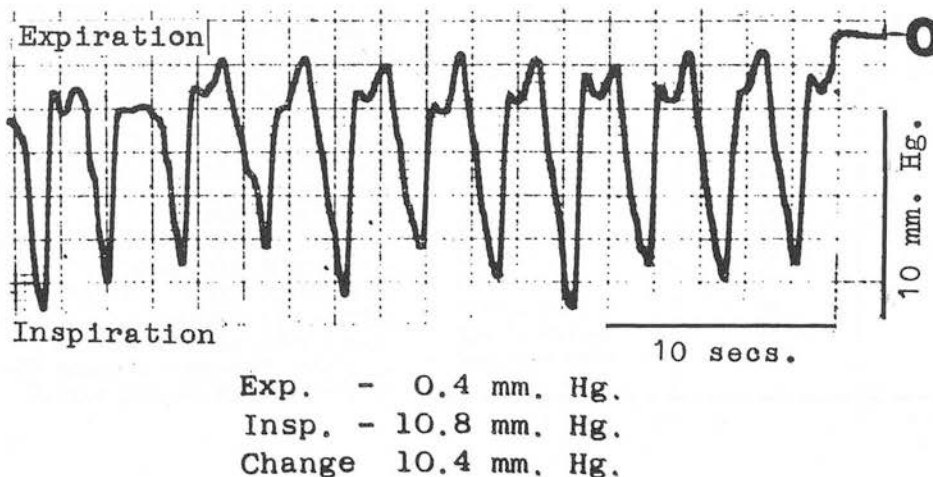


Fig. 2

TABLE III
INTRATHORACIC PRESSURE

Max. Change mm Hg.	
Cannula	Balloon
4.4	4.4
2.4	2.2
16.0	10.8
31.0	26.0
20.0	19.0

alveolar area is the essential feature of allergic diseases given clinical labels such as "farmer's lung", "bird fancier's lung", etc. Lymphoid cells and histiocytes are prominent; the exudative feature is minimal. Thurlbeck and Lowell (1964) have described similar findings in some cases of "heaves". We have observed, in one of the few cases so far examined at postmortem, similar changes with, in addition, granulomata containing giant cells resembling those recorded in farmer's lung. The airways are not obstructed unless there is concurrent bronchitis or bronchiolitis; so silent breathing on auscultation would be expected. This indeed happens, coughing and dyspnoea being the chief features of extrinsic allergic alveolitis in man, there being little exudation or secretory over-activity, and wheezing is absent. Similar findings have been reported in horses and we can confirm this clinical observation. The ventilation of the alveoli is not obstructed, but the increased distance between the alveolar space and the blood in the capillaries reduces the rate of diffusion of oxygen, with consequent lowering of the oxygen uptake in unit time and hence a reduction in the oxygen content of the arterial blood. To compensate, more alveoli become functional, i.e. distended by air and perfused by blood. This means more air in unit time has to traverse the main airways so hyperpnoea of varying severity occurs. The loss of pulmonary elasticity increases the animal's difficulties. To achieve adequate respiratory function, changes occur in intra-pleural pressure in the same way as those described in bronchitis. Double inspiratory abdominal movements can sometimes be observed in such cases.

Pulmonary vascular diseases

Gillespie *et al* (1964) have reported a reduced number of capillaries in the alveolar walls of emphysematous horses. Such vascular changes may well be secondary, as pressure on the distended alveolar walls could lead to alteration of the vascular network. Clearly, in gross cases, with large emphysematous spaces, atrophy must have occurred as vessels disappear without haemorrhage into the airways. On the other hand, recent observations by Eyre (1971) suggest the possibility of primary vascular involvement. Experimentally, he has shown that spasm of pulmonary blood vessels can be induced in calves and in horses, though it is interesting that venous strip preparations were more responsive than arterial ones. Spasm of pulmonary vessels, or central cardiovascular disease, will interfere with perfusion of the active alveoli of the lungs. This will result in a reduced uptake of oxygen in unit time. To compensate, further areas of

lung will become perfused and aerated so that dyspnoea with perhaps a moderate increase in intra-pleural pressure change can be expected. These changes would not be expected to give rise to any auscultatory changes except those attributable to an increased flow of air in the main airways.

Blood gases (O_2 and CO_2)

When the diffusion of O_2 from the alveoli into the blood stream or of CO_2 from the blood stream to the alveolar space is hindered by changes in the alveolar walls or in perfusion as described above, the alteration in O_2 levels is very much greater than the reduction, if any, in CO_2 exchange as the latter is a much more diffusible gas. Arterial O_2 is measured since the levels are uncomplicated by the metabolic processes of the body. The gas is not estimated chemically but is measured as the partial pressure exerted by the gas in solution in blood and is expressed as PaO_2 in mm. Hg. Values above 90 mm. Hg. were found to be normal by Sasse (1971).

From these considerations it is clear that the measurements of changes in intra-pleural pressure and of arterial oxygen (PaO_2) on their own do not permit differentiation of bronchial from alveolar abnormalities. In man, "forced expiratory effort" is measured, the patient making a maximum effort to exhale when requested by the observer. In asthma this is much reduced. The horse is not so co-operative, so we must search for other measurable means of differentiating the conditions described.

Primary causes of structural and functional changes

Among such causes, infections of the bronchi and bronchioles have their advocates but the evidence is unconvincing. The confusion arises possibly because clinicians are inclined to forget that fever may occur in the absence of infections. No bacteria or fungi have been identified consistently in these cases. Nevertheless, one must keep an open mind, and we have observed an aged Clydesdale mare which was believed to be normal until she became ill from influenza A/equi-2 virus infection complicated with *Streptococci zooepidemicus*. From then on she suffered from "heaves". In many "heaves" cases we have had a similar history. It may be that damage to the mucous membranes allows antigens to make contact with the immunologically

TABLE IV
ARTERIAL OXYGEN (PaO_2) (mm Hg.)

Clinical State	At Rest	Immediately Post Exercise
Normal	94	—
Normal	97	—
Normal	97	—
Normal	110	—
Heaves	75	70
Heaves	84	86
Heaves	79	75
Heaves	84	78
Systolic Murmur	90	88
Systolic Murmur	89	86
Systolic Murmur	100	91

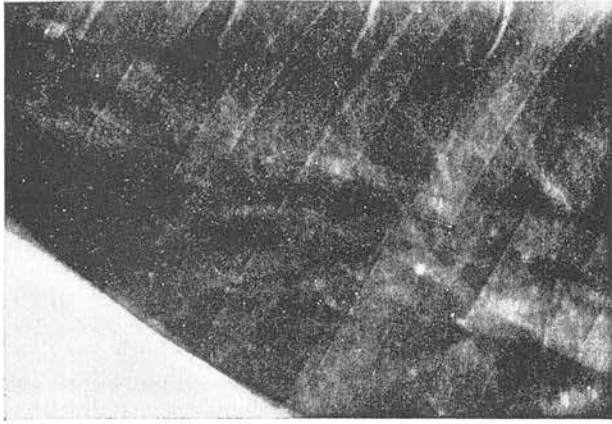


Fig. 3a

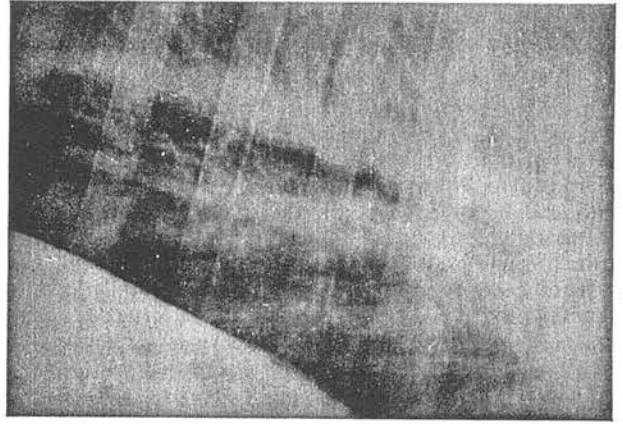


Fig. 3b

active tissue, and the subsequent development of allergy by respiratory tissues. Allergic pulmonary disease is now well recognised in other species. Several authors have reported strong evidence that dusty hay initiates or exacerbates "heaves" in a manner reminiscent of the response of man in similar circumstances. It is interesting to note that in dust diseases of man the particles are chiefly deposited in the respiratory bronchioles, the majority being too large to enter the alveoli. Since particles less than 6 to 9 μ are unlikely to pass into the alveoli, grass pollens, being over 30 μ , if introduced into the airways, are unlikely to reach the alveoli but will be deposited on tracheal or bronchial mucosae. Smaller antigens such as fungal spores or actinomycete spores are of the order of 1 μ , and when inhaled may therefore reach the alveoli and will be capable of setting up a different host response from that stimulated by larger particles such as pollen. There may, therefore, be a difference in the type of disease produced by different kinds of antigen for this simple physical reason. Equally it may be that the dynamics of particle deposition differ in the horse from those encountered in man.

METHODS OF INVESTIGATION

Most frequently, horses are presented because of poor performance or because of coughing in the stable or at work.

The first essential is a good history. Details not only of the animal itself but also of the habitat, food, bedding and ventilation may all be very important. The season of the year when attacks occur is noted as some observers have found a preponderance of cases in the winter and others in spring and summer. To eliminate cardiac inefficiency as a cause of poor work tolerance, the heart is examined clinically and by electrocardiography. The upper respiratory tract is examined for pathogens, bacterial and fungal, and by laryngoscope for other evidence of disease or anatomical abnormality. A radiograph of the chest is taken. The PaO_2 is estimated on carotid blood before and after exercise, and the changes in intra-pleural pressure are recorded. The procedure is summarised in fig. 1.

Changes in intra-pleural pressure

While we do use the same method of intra-pleural space monitoring as Sasse (1971), more frequently a balloon (a fine quality condom) on the distal end of a

medium-sized stomach tube† passed via the nasal passage into the mid-thoracic oesophagus is employed. The open end is connected to a recording system—a pressure transducer, amplifier and paper recorder*. The record made is illustrated in fig. 2 from which the following information is observable.

1. The rhythm and regularity of respiratory movements.
2. The rate of breathing: read on the horizontal scale, the paper moving at a known speed, usually 25 mm./10 sec.
3. The form of the respiratory excursions during expiration and inspiration: double efforts are recorded, not subjectively assessed.
4. The maximum intra-pleural pressure at the end of expiration and minimum at the end of inspiration, is read on the vertical scale and the difference between these two is the maximum change in intra-pleural pressure. The zero or atmospheric pressure is recorded and the deflection made by a pressure of 10 mm. Hg. noted.

The oesophageal method, which eliminates the temporary disfigurement associated with cannulation, has benefits for screening and for testing work requiring repeated recordings. We agree with Sasse (1971) that direct puncture gives the true reading in all cases, but the results by the balloon method have indicated abnormal pressure changes when they existed. Table III shows the comparative figures for five horses as an illustration.

CHANGES IN PARTIAL ARTERIAL OXYGEN PRESSURE (PaO_2)

Arterial blood is obtained by carotid puncture as described by Littlejohn (1969). Table IV shows some examples of PaO_2 figures obtained in the carotid blood from normal horses and from horses with "heaves" or circulatory inefficiency. These are comparable with Sasse's (1971) more extensive data from blood obtained from the *A. bronchialis*.

HAEMATOLOGY

It is necessary to exclude anaemia as a cause of poor oxygen transportation, and to monitor the effect of

†Portex, London.

*Devices Ltd., London.

splenic contraction during exercise; the Hb and PCV are determined each time blood is taken for gas analysis.

Radiographic changes in the lungs

In man, asthmatic subjects show either no radiographic changes or an increased air content in the lungs giving dark films; in patients with extrinsic allergic alveolitis films of greater density are obtained with nodular or honeycomb-like shadows. Figs. 3a and b illustrates the type of change we have observed in the horse. The first is from a normal horse, and the second is from one which has been seriously incapacitated by "heaves" for at least six months. The average exposure is 120 Kv. at about 60 MAS, the latter depending on the dimensions of the chest under examination.

ASSESSMENT OF PHYSICAL AND FUNCTIONAL CHANGES

By co-relating the data so obtained at this stage of the investigation a reasonable opinion can be given as to whether the horse is suffering from "heaves", but there are pitfalls. Occasionally a horse has shown a normal intra-thoracic pressure when first examined but has, when exposed to antigen by inhalation, shown a great increase with marked clinical signs. Inspection of the X-ray films is useful in trying to detect such cases when the disease is not clinically manifested though the history has suggested "heaves".

SUMMARY

The difficulty in defining "heaves" as a clinical or pathological entity is discussed. The structural changes which have been reported in "heaves" and the consequent functional changes are reviewed. Some of the possible primary causes are discussed in general terms and finally an outline of investigational work to be subsequently reported is given.

RÉSUMÉ

La difficulté de définir le syndrome "pousse" du point de vue pathologique et comme une entité clinique est évoquée. Les modifications tissulaires qui ont été constatées dans ce syndrome sont passées en revue avec leurs incidences fonctionnelles.

Un survol des causes initiales de ce syndrome est effectué avec l'esquisse de recherches qui seront exposées ultérieurement.

ZUSAMMENFASSUNG

Die Schwierigkeiten "Dämpfigkeit" als klinische oder pathologische Einheit zu definieren, werden diskutiert. Die strukturellen Veränderungen bei "Dämpfigkeit" und die daraus hervorgehenden funktionellen Beeinträchtigungen werden besprochen. Einige der möglichen primären Ursachen werden erwähnt. Ein Untersuchungsprogramm, dessen Resultate später veröffentlicht werden sollen, wird andeutungsweise wiedergegeben.

ACKNOWLEDGEMENTS

We wish to thank the Horserace Betting Levy Board for their financial support.

REFERENCES

- CIBA Foundation Study Group No. 38 (1971). Identification of Asthma. Churchill Livingstone, London.
- Eyre, P. (1971). Personal Communication.
- Gillespie, J. R., Tyler, W. S. and Eberly, V. Z. (1964). Blood pH, O₂ and CO₂ Tensions in Exercised Control and Emphysematous Horses. *Am. J. Physiol.* 207 (5), 1067.
- Jubb, K. V. F. and Kennedy, P. C. (1970). Pathology of Domestic Animals. 2nd Ed., Vol. I. Academic Press, New York and London.
- Thurlbeck, W. M. and Lowell, F. C. (1964). "Heaves" in Horses. *Am. Rev. Respirat. Diseases* 89, 82.
- Sasse, H. H. (1971). Some Pulmonary Function Tests in Horses. Ph.D. Thesis, State University, Utrecht.

Chronic Obstructive Pulmonary Disease (COPD): Identification of Affected Horses

J. A. McPHERSON, G. H. K. LAWSON, JILL R. MURPHY, JANET M. NICHOLSON and J. A. FRASER
University of Edinburgh Veterinary Field Station, Easter Bush, Roslin, Midlothian EH25 9RG

I. G. BREEZE and H. M. PIRIE

University of Glasgow Veterinary School, Department of Veterinary Pathology, Bearsden Road, Bearsden, Glasgow G61 1QH

SUMMARY

Mean normal values for PaO₂ and max Δ Ppl for horses were determined. Using 2 standard deviations below (PaO₂) and above (max Δ Ppl) the mean normal values as a guide, horses affected with COPD were satisfactorily distinguished from other horses in a series of 100 animals. The frequency of occurrence of 20 different clinical parameters in affected, not affected and possibly affected horses was examined statistically. Poor work performance and a history of previous febrile illness occurred more often in COPD horses than in others. The presence of a chronic cough, dyspnoea, double expiratory effort, increased breathing sounds, wheezing and crepitant breathing sounds occur more frequently in COPD horses than in others and the presence of prolonged coughing was highly indicative of COPD. Crepitant breathing sounds (observed only in horses in the affected group), wheezing and increased respiration sounds were observed in a high proportion of horses affected with COPD, but a diagnosis of COPD based solely on these parameters would lead to an unacceptably low number of cases being recognised. While radiological examination appeared to be helpful, x-ray films were in general difficult to interpret. Haematological examination was of no help in the diagnosis of COPD. The beneficial effect of removing affected horses from contact with hay and straw was recorded.

INTRODUCTION

McPHERSON and Lawson (1972) described a procedure for the investigation of horses in which pulmonary function was thought to be abnormal and in which it was believed that airway obstruction was a prominent feature. This report contains the results obtained by such an examination of a wide variety of horses and ponies some of which were suffering from obvious dyspnoea whilst others showed little or no evidence of clinical pulmonary abnormality. Subsequent papers will deal with pathological and aetiological studies on these horses.

MATERIALS AND METHODS

Animals

Eleven horses were selected on the basis of the absence of a cough, nasal discharge, upper respiratory diseases, dyspnoea and nasopharyngeal bacterial pathogens; in addition, none had a detectable cardiac abnormality or history of poor work performance and their sera had no precipitins against *Aspergillus fumigatus*, *Microspora faeni* or *Thermoactinomyces vulgaris*. One hundred horses and ponies were examined for evidence of respiratory malfunction; of these, 88 had been referred for examination of the respiratory or cardiovascular systems and 12 were

affected with a variety of other conditions (i.e. lameness, head shaking, cardiac atrioventricular murmurs, mesenteric abscessation or abdominal neoplasia). A data sheet was prepared for each horse at the time of admission. The animal's identity, age, breed, sex, colour, weight, the owner's evaluation of its work performance—racing, hunting/eventing, show-jumping, hacking or child's pony—were recorded. Details of the habitat were obtained from the owner, attendant and/or referring veterinary surgeon. These included whether the animal was kept indoors, outdoors or partly so, which kinds of fodder and bedding were used, whether these were considered to be mouldy or to contain pollen dust. Information on the adequacy of the ventilation was obtained, as well as any history of previous illness (e.g. strangles, infectious cough or pyrexia of unknown aetiology), and the age of the animal and the season of the year when disease was first observed. The nature and occurrence of coughing, nasal discharge, dyspnoea, wheezing and epistaxis were assessed from the history and by observation. Each horse was examined clinically, particular attention being paid to the respiratory and cardiovascular systems. The lungs and heart were auscultated at rest and after exercise. In the normal horse, breathing sounds are soft and low in volume and absent from the dorsal part of the chest when the animal is at rest; deviations such as harshness, increased volume

of sound and/or an increase in the area over which such abnormal sounds could be heard at rest were recorded as "increased breathing sounds". Crepitant sounds suggesting the presence of emphysema were noted, as were wheezing and other adventitious noises. Clinically abnormal cardiac size, sounds or rhythm were recorded. The horses were kept in well-ventilated looseboxes with a floor area of 13 m² and bedded with good quality wheat straw. The diet was good hay *ad lib* and oats appropriate to the needs of the animal. Horses with severe dyspnoea were given a bed of peat moss and fed proprietary Horse and Pony Cubes (Spillers Ltd., Liverpool) as a complete diet whenever it was necessary to relieve distress or eliminate the clinically abnormal respiration to permit aetiological studies.

Techniques

Electrocardiography: The standard leads were employed (i.e. I, II, III, Avr, Avl and Avf (after Boddie, 1969) with a conventional recording apparatus (Devices Ltd., London). Records were made before and after exercising for 10 min on a lungeing rein.

Examination of the upper respiratory system: This was carried out by the conventional clinical method and by direct inspection with the aid of a rhinolaryngoscope.

Respiratory function: Altered respiratory function was assessed by two measurements, namely partial pressure of arterial oxygen (PaO₂) and maximum intrathoracic pressure change during breathing (max Δ Ppl) (Sasse, 1971; McPherson and Lawson, 1972). Both were made while the horse was at rest. PaO₂ was also determined immediately after exercise on a lunging rein for 10 min at a pace appropriate for the type and condition of the horse.

PaO₂ determination: Blood was collected anaerobically from the carotid artery (using a 20 gauge, 4 cm needle) into a heparinised 10 ml glass syringe containing a brass washer to facilitate mixing and retained in iced water for not more than 2 hours before assaying, using the Clark oxygen electrode of an Astrup AME 1 Analyser (Radiometer Ltd., Copenhagen) (mmHg \div 7.5 = KPa (SI units)).

Max Δ Ppl measurement: This was measured in mmHg as outlined by McPherson and Lawson (1972). An M19 recorder (Devices Ltd., London) and an L22 transducer (Devices Ltd., London) were used to record the changes in pressure in the thorax during breathing; the transducer was connected by a hard walled plastic tube 260 cm long, of internal diameter 3 mm, to either a cannula in the pleural space or a balloon in the mid-thoracic region of the oesophagus.

In the cannula method a metal cannula with a hydraulic system was used initially (Sasse, 1971). Later, a 7.6 cm long metal human pneumothorax needle with 3 extra side holes near the tip and a gaseous system (air) was found to be easier to use and just as efficient. More recently, a 7.6 cm plastic pleural drain (2.5 mm diameter) (Vygon Ltd., France) with one lateral eye has proved efficient and minimises the risk of damaging the pulmonary pleura or parenchyma. The site for cannulation was in the 10th intercostal space on a line between the point of the shoulder and the tuber coxae. This method is not recommended for repeated use but is necessary for intractable horses which resent the passage of a stomach tube.

For the balloon method either a medium-sized horse stomach tube (Portex, London) or an equivalent length of nylon fuel piping (10 mm external, 4 mm internal diameter) was used. Four lateral openings were cut near one end; this end, including the eyes, was covered with a fine quality condom (Gossamer $\text{\textcircled{R}}$, LR Industries Ltd., London). This was bound with linen thread to a 2.5 length of rubber tubing slipped over and securely stitched to the plastic tubing. Horses were restrained by a nose twitch.

Haematology

The anticoagulant used was EDTA. The packed cell volume (PCV) was measured using a Hawksley microhaematocrit centrifuge (Gelman Hawksley Ltd., Lancing, Sussex); haemoglobin was estimated by the cyanmethaemoglobin method and a Coulter electronic haemoglobinometer (Coulter Electronics Ltd., Harpenden, Herts.); red and white cell counts were carried out on a ZF6 Coulter counter (Coulter Electronics Ltd., Harpenden, Herts.); smears were made and differential counts within one hour and stained by the Leishman method (Boddie, 1969).

Bacteriology

A sheathed cotton wool swab, 43 cm long, passed into the nostril into the nasopharyngeal region and removed to collect samples. These swabs were conventionally plated in duplicate in Hartley's blood agar (Cruickshank, 1965) and MacConkey (Oxoid, CM7). A particular search was made for colonies resembling the Group C *Streptococcus Bordetella bronchiseptica*. Other bacteria or fungi were identified only when the primary growth indicated that they might have significance. Bacteria were identified using standard methods (Cowan and Steel, 1965).

Radiography

Initially, lateral exposures at 100-120 kV and 300 mA were used, with a time of approximately 0.1 seconds on Kodak RP/R54 films (Kodak Ltd., London). A metal grid was used for preference, but, when horses would not stand close enough to it, a fixed grid was employed. Since faster films (Trimax XM) (3M Italia, S. Italy) became available they have been used routinely. For these, 110-120 kV and 300 mA with a time of approximately 0.03 to 0.04 seconds is satisfactory.

Statistical analysis

Where appropriate, chi-square and analysis of variance were employed. When the frequencies in the contingency tables were less than 10, the Pirie and Hamden correction factor was used. When analysis of variance revealed a significant difference between groups, Dunnett's multiple range test (Duncan, 1955) was used to identify the significantly different subsets. When assessing the duration of coughing, the standard deviations were so large that the Median Test (Downie and Heath, 1966) was used to compare the groups.

RESULTS

Determination of max Δ Ppl

Comparison of the max Δ Ppl values obtained from the cannula and oesophageal balloon methods contemporaneously produced the results in Table 1.

TABLE I

COMPARISON OF MAX Δ Ppl VALUES BY CANNULA AND BALLOON METHODS

No. of horses	Cannula (mmHg)	Balloon (mmHg)	Difference (mmHg)
11	111.8	104.4	-7.4

$$\text{test } t_{11} = \frac{d}{se} = \frac{-0.6167}{0.3810} = 1.619 \quad P < 0.20 > 0.10 \text{ NS}$$

There was, therefore, no significant difference between the methods. It should be emphasised, however, that the balloon must be carefully positioned in the oesophagus to obtain the maximum change, otherwise the results may not be comparable.

For this determination of normal values for PaO₂ and max Δ Ppl, the selected group of 11 horses was used and the results are summarised in Table II. For practical purposes it was considered that PaO₂ values ≤ 82 mmHg were abnormally low and max Δ Ppl values ≥ 6 mmHg were abnormally high.

TABLE II

NORMAL VALUES OF PaO₂ AND MAX Δ Ppl (mmHg) IN HORSES

	No.	Mean (\bar{x}) value (mmHg)	Standard Deviation (s.d.) (mmHg)
PaO ₂	11	90.54	4.21
Max Δ Ppl	11	3.726	1.088

Detection of horses affected with COPD

The horses presented for examination were classified as affected (**Group A**), possibly affected (**Group B**) and not affected (**Group C**). The basis was as follows:

Group A—PaO₂ ≤ 82 mmHg and max Δ Ppl ≥ 6 mmHg either on admission or in response to challenge with inhaled antigen.

Group B—All horses with PaO₂ and/or max Δ Ppl levels not meeting the requirements for **Group A** or **Group C**.

Group C—PaO₂ ≥ 86 mmHg and max Δ Ppl ≤ 5 mmHg (i.e. 1 s.d. below and above the mean value for normal animals respectively).

The PaO₂ and max Δ Ppl were determined on each horse soon after admission and the means and the standard deviations for these parameters in the three groups of horses are displayed in Table III. Classification was provisionally made at this time and finalised after consideration of the results obtained in response to respiratory challenge with antigens.

By analysis of variance there was a significant difference between the groups ($F_{X^2} = 28.46$; $P < 0.01$) and this was attributable to 2 subsets A and (B + C). The mean value of 77.03 mmHg in **Group A** was significantly less than in the other 2 groups. The mean of the lowest PaO₂ values for the individual horses in **Group A** at any time during the investigation was 72.49 ± 7.8 mmHg.

Exercise did not materially alter the PaO₂ values obtained in **Group A** horses. A mean of 77.75 ± 8.44 mmHg was obtained. For **Group B**, the mean was 87.75 ± 6.43 mmHg and for **Group C** it was 88.30 ± 7.70 mmHg.

Max Δ Ppl

Inspection of the data showed that the mean of **Group B** was reasonably close to that of **Group C** and that they were less than one-third that of **Group A**. The average of the highest values for **Group A** animals during the investigation was 22.06 ± 15.30 mmHg.

Clinical findings

Table IV gives the occurrence of the clinical signs and other data for each group of horses and in Table V the significance of the chi-square values is shown.

Poor work performance, antecedent febrile illness, coughing, dyspnoea, double expiratory effort in breathing, increased breathing sounds, the presence of wheezing and of crepitation were more common in **Group A** horses. Abnormalities of the upper respiratory tract, such as laryngeal hemiplegia or inflammatory conditions, occurred in only 16 per cent of **Group A** as opposed to 35 per cent of the other horses. Cardiac disorders, predominantly murmurs, were significantly more frequent in occurrence in **Groups B** and **C** than in **Group A**, a reflection of the fact that most horses had been referred because of suspect disease of the respiratory or cardiovascular systems.

The duration of coughing is shown in Table VI. Horses in **Group A**, when first examined, had on average been coughing for 15 months; 4 times as long as the other horses. Seventy-one per cent of **Group A** horses had coughed for more than 3 months, whereas only 34 per cent of **Groups B + C** had done so. Thirteen **Group A** horses had been coughing for a year or more. Only 2 horses in each of the **Groups B** and **C** had such a history.

Radiology

Films of increased density, with irregular, snowflake-type, opaque areas or with dark areas indicating bullae, were obtained from some horses in **Group A** which had been affected with COPD for many years. After stabling such horses on a peat bed and feeding them a diet of cubes for about 2 weeks, a marked change towards radiographic normality was evident; the lungs showed fewer opacities and bullae were not present. One or more abnormal features were observed in films from all the horses in **Group A**. The Trimax films used more recently are of superior quality but, due to the greater detail visible, require a new assessment of the normal animal. Despite the difficulties in interpretation, Tables IV and V indicate that significantly more horses in

TABLE III

MEAN VALUES (mmHg) OF PaO₂ AND MAX Δ Ppl OF 100 HORSES AT REST ON ADMISSION

	PaO ₂			Max Δ Ppl		
	n	\bar{x}	s.d.	n	\bar{x}	s.d.
Group A	38	77.03	9.06	38	14.12	8.56
Group B	28	87.34	6.61	28	4.08	1.98
Group C	34	91.456	8.82	34	3.46	0.955

TABLE IV
THE OCCURRENCE OF CLINICAL SIGNS AND OTHER DATA FOR THE 3 GROUPS OF HORSES EXAMINED

Observations	Group A (38)		Group B (28)		Group C (34)	
	+	-	+	-	+	-
Poor work performance	38	0	17	11	22	12
Antecedent febrile respiratory disease	25	13	10	18	3	31
Coughing	38	0	22	6	19	15
Dyspnoea	29	9	7	21	8	26
Double expiratory effort	35	3	5	23	6	28
Increased breathing sounds	22	16	4	24	0	34
Wheezing breathing sounds	20	18	2	26	0	34
Crepitant breathing sounds	7	31	0	28	0	34
Chest x-ray abnormal	33	5	17	11	9	25
Nasal discharge	32	6	18	10	15	19
Watery nasal discharge	21	17	11	17	7	27
Mucoid nasal discharge	7	31	3	25	6	28
Purulent nasal discharge	5	33	4	24	2	32
Epistaxis	1	37	1	27	3	31
Double inspiratory effort	8	30	10	18	7	27
Circulating eosinophilia	7	31	3	25	2	32
Nasal bacterial pathogens	1	37	3	25	2	32
Upper respiratory abnormalities	6	32	12	16	10	24
Cardiac abnormalities	2	36	11	17	11	23
Good bodily condition	36	2	25	3	32	2

(+ observation present; — observation absent)

Figures in brackets denote numbers in each group

TABLE V
PROBABILITY VALUES OF ASSOCIATION BETWEEN THE CLINICAL PARAMETERS FOR THE 3 GROUPS OF HORSES

Groups	A v B	A v C	A v (B + C)	B v C
Poor work performance	***	***	***	NS
Antecedent febrile respiratory disease	*	***	***	**
Coughing	**	***	***	NS
Dyspnoea	***	***	***	NS
Double expiratory effort	***	***	***	NS
Increased breathing sounds	***	***	***	*
Wheezing breathing sounds	***	***	***	NS
Crepitant breathing sounds	*	**	***	NS
Chest x-ray normal	*	***	***	**
Nasal discharge	NS	***	**	NS
Watery nasal discharge	NS	**	**	NS
Mucoid nasal discharge	NS	NS	NS	NS
Purulent nasal discharge	NS	NS	NS	NS
Epistaxis	NS	NS	NS	NS
Double inspiratory effort	NS	NS	NS	NS
Circulating eosinophilia	NS	NS	NS	NS
Nasal bacterial pathogens	NS	NS	NS	NS
Upper respiratory abnormalities	*	NS	*	NS
Cardiac abnormalities	***	**	***	NS
Good bodily condition	NS	NS	NS	NS

Probability (P): * = P < 0.05
** = P < 0.01
*** = P < 0.001

χ^2 calculated on 2 x 2 Contingency Tables

NS = Not Significant

TABLE VI

THE DURATION OF COUGHING AT ADMISSION IN THE 100 HORSES EXAMINED

	Group A	Group B	Group C
n	38	22	19
\bar{x} (months)	15.34	4.47	2.90
s.d. (months)	22.86	5.55	4.09
Median duration 3 months:			
No. above median	27	12	9
No. below median	11	16	25

$$\chi^2 = 14.7 \quad P < 0.001$$

Group A were judged to have radiologically abnormal lungs than all the other horses. More horses in **Group B** had radiological abnormalities than in **Group C**.

Haematology

Differences in the haematological findings between the groups were not significant, except in respect of the total white blood cell and the neutrophil counts (Table VII). **Group B** horses had a leucocytosis attributable to a neutrophilia. Inspection of the records of **Group B** horses revealed no explanation for this finding.

DISCUSSION

The term chronic obstructive pulmonary disease (COPD) was introduced to veterinary literature by Sasse (1971) as a more appropriate term for the complexity of symptoms variously termed "broken wind", "heaves" or chronic alveolar emphysema. COPD was then in current use to describe a similar complex in man. We have elected to continue to use this term despite its shortcomings (Thurlbeck, 1976). The latter currently prefers the term chronic airflow obstruction (CAO). The tests used to establish the latter diagnosis in man require patient participation, which clearly excludes their use in the majority of animals. The term CAO also has shortcomings (Thurlbeck, 1976). Our reliance on PaO₂ and max Δ Ppl values is based on deductions

from Sasse's data, from which it was apparent that changes in these 2 parameters were crucial.

The normal horses were specially selected and their mean PaO₂ was similar to that for horses in **Group C**. The standard deviation in the small normal group was about half that of **Group C** which contained horses with a variety of clinical disorders. Both means were comparable to those of de Moor (1968), Littlejohn (1969) and Bergsten (1974) who obtained 96, 96 and 94 mmHg respectively and were lower than those of other authors quoted by Bergsten (1974). However, it is accepted that minor variations in technique and equipment require normal values for certain tests to be established in each laboratory (Barnett, 1971).

The mean max Δ Ppl obtained for our normal horses was comparable to that obtained by Gillespie, Tyler and Eberley (1966) using a balloon method. It was, however, lower than that obtained by Sasse (1971) using a cannula. In our experience (Table I) either method was satisfactory, but the balloon method has the advantage of causing no trauma and we used it many times on individual animals. The lower normal level here reported may be attributed to the rigorous screening of the 11 horses measured. The value of the mean for **Group C** horses was even lower, most likely attributable to the removal of horses with high values to **Group B** in the classification.

Table VIII compares the differences between the mean values of normal and affected horses in respect of PaO₂ and max Δ Ppl levels in the horses here reported and those studied by Sasse (1971). The results are comparable considering that **Group A** (on admission) clinically most closely resembled Sasse's Group 3 and the latter's Group 2 horses probably paralleled our **Group A** when clinically at their worst. The high figure for max Δ Ppl in **Group A** at this time is probably a reflection of the efficiency of respiratory challenge with specific antigen to be reported later.

The immediate effect of exercise on PaO₂ levels was negligible and, in that, similar to Sasse's experience with samples taken 30 min after exercise. He found, however, that in COPD affected horses the level of PaO₂ one hour after exercise was significantly lower in his Group 2, in which COPD was confirmed at post mortem. The effect in his Group 3 horses, which did not go to post mortem, was small. The latter group presumably

TABLE VII

HAEMATOLOGICAL RESULTS OBTAINED FROM THE 100 HORSES EXAMINED

	Group A			Group B			Group C		
	n	\bar{x}	s.d.	n	\bar{x}	s.d.	n	\bar{x}	s.d.
RBC ($\times 10^{12}/l$)	37	8.23	1.17	27	8.25	1.265	31	8.232	1.147
PCV (l/l)	37	0.399	0.052	28	0.391	0.053	33	0.400	0.049
Hb (g/dl)	37	13.95	2.12	28	14.22	1.966	33	14.16	2.086
WBC ($\times 10^9/l$)	36	9.23	1.82	27	10.12	2.863	31	8.739	1.809
Neutrophils ($\times 10^9/l$)	36	5.361	1.557	27	6.061	1.088	31	5.020	1.115
Lymphocytes ($\times 10^9/l$)	36	3.071	1.023	27	3.324	1.029	31	3.102	1.080
Monocytes ($\times 10^9/l$)	36	0.366	0.213	27	0.323	0.206	31	0.328	0.238
Eosinophils ($\times 10^9/l$)	36	0.420	0.436	27	0.374	0.252	31	0.252	0.270
Basophils ($\times 10^9/l$)	36	0.043	0.071	27	0.035	0.067	31	0.034	0.055

Analysis of variance

WBCs F = 2.99 (NS); Neutrophils F = 4.72 (P < 0.05 > 0.01)

N.B. No other F values were significant

TABLE VIII
COMPARISON OF PaO₂ AND MAX Δ Ppl FOR NORMAL AND AFFECTED GROUPS (mmHg)
FOUND IN THIS STUDY AND BY SASSE (1971)

	Present Study					Sasse (1971)				
	Normal	Affected (Group A)				Normal	Affected			
		Admission		Maximum Severity			Group 2		Group 3	
	Mean	Mean	Difference	Mean	Difference	Mean	Mean	Difference	Mean	Difference
PaO ₂	90.5	76.5	-14	71.5	-19	100.0	80.0	-20	84.0	-16
Max Δ Ppl	3.7	13.7	+10	21.7	+18	7.6	18.6	+11	19.6	+12

SASSE (1971): Group 2—Confirmed COPD at post mortem. Group 3—No autopsies

resembled our **Group A** in that most had not reached a stage where slaughter was necessary.

The clinical signs traditionally regarded as characteristic of broken wind or COPD (Cook, 1976) were commonly present in **Group A** horses (PaO₂ \leq 82 mmHg, max Δ Ppl \geq 6 mmHg). With the exception of emphysema, they were not, however, exclusively a feature of horses of one class. The insignificant difference in the occurrence of these signs between **Group B** and **Group C** horses, compared with the highly significant difference when **Group A** was compared with **Group B** and **Group C**, justifies the use of PaO₂ and max Δ Ppl levels as a means of detecting horses with COPD, especially for our purpose which was to select horses for aetiological and pathological studies. These 2 measurements have the advantage of being free of observer error.

While measurement of PaO₂ and max Δ Ppl is a good method of confirming that a horse is or is not affected with COPD, in a few individuals the values will lie above and below respectively those required for **Group A** and it is likely that **Group B** contained such horses. Some will be early cases, often manifesting only a persistent cough with little or no nasal discharge. We encountered one pony initially in this category, which 3 years later was seriously affected with COPD, having become steadily worse over the intervening period. A few horses, when presented, were in a remission phase of the disease and it was only their response to respiratory challenge which revealed that they were affected; they were classified as **Group A** horses. A further few could have been compensating for the poor gas exchange in the lungs by hyperventilation or alteration in the ventilation: perfusion ratio so that, while the max Δ Ppl was high, the PaO₂ was normal. There are such horses in **Group B**. Long-term follow-up information on **Group B** horses may well indicate refinements in diagnostic criteria.

In these cases the presence of several of the classical clinical signs over a period of months should not be ignored and further consideration and tests are indicated. In this context, a horse which has been coughing for less than 3 months is much less likely to be affected with COPD than one which has done so. On the other hand, a cough of more than 3 months' duration is as likely to be associated with COPD as not, but if the cough has been present for a year the probability of the existence of COPD is high. Known affected horses removed from exposure to environmental dusts improve to such

an extent that the evidence of COPD practically appears, only to return if the feeding of hay and bedding on straw is resumed. This has been confirmed in times; indeed, all horses so treated have responded to the problem being to maintain a suitable environment throughout the year.

Reliable radiological evidence would be of considerable help in diagnosing COPD but interpretation is difficult even controversial. Radiologists found similar difficulties with some of Sasse's (1971) cases. Only lateral views with one lung superimposed on the other provide a sufficient area of lung for study. Neither of the experimental views—withers to chest entrance or ventro-dorsal angled view—has proved rewarding. In the former, the shoulder girdle reduced the field and required kV exceeded the maximum of the machine (120 kV). In the ventro-dorsal angled view, only a more dorsal peripheral area of a lung was visible. More recent work using the radiopaque Tantul powder blown into the airways in the conscious horse offers, on the basis of preliminary work, prospects of improved technique. Despite the admitted probability of considerable observer error, the lateral films taken in this series of horses indicate that radiographic changes occur more frequently in COPD horses than in others. Further work is being undertaken to try to clarify this situation.

Haematological examination was of no assistance in diagnosis of COPD. A raised neutrophil count was seen more often in the **Group B** horses and eosinophilia was observed in relatively few horses from all of the groups. Bacterial pathogens were only recovered from the nasopharynx of 6 per cent of horses distributed amongst all groups. Epistaxis, sometimes considered a feature of COPD, was not seen in this study.

Infestation with lungworms (*Dictyocaulus arnfieldi*) is reported to cause clinical signs resembling, in some respects, COPD (Cook, 1976). Faeces from all horses grazing with donkeys and a series of 20 horses from **Group A** were examined for lungworm larvae. None was recorded and when 12 of these 20 horses were examined at autopsy there was no evidence of lungworm infestation. Lungworms appeared to play no part in the disease process being studied.

ACKNOWLEDGEMENTS

This work was made possible by a grant from the Horse Betting Levy Board. Thanks are also due to the technical staff of the Department of Veterinary Medicine and to the School radiographers for their assistance.

REFERENCES

- Barnett, R. N. (1971). *Clinical Laboratory Statistics*, 1st ed., 63. Boston, Little, Brown & Co.
- Bergsten, G. (1974). Blood pressure, cardiac output and blood gas tensions in the horse at rest and during exercise. *Acta vet. scand.* Supplement 48, 1-88.
- Boddie, G. F. (1969). *Diagnostic Methods in Veterinary Medicine*, 6th ed., pp. 160-169 and 383. Edinburgh, Oliver and Boyd.
- Cook, W. R. (1976). Chronic bronchitis and alveolar emphysema in the horse. *Vet. Rec.* 99, 448-451.
- Cowan, S. T. and Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. Cambridge, University Press.
- Cruickshank, R. (1965). *Medical Microbiology*, 11th ed., Edinburgh and London, E. & S. Livingstone Ltd.
- De Moor, A. (1968). Influence of atropine on arterial oxygen tension, acid-base status and tissue hypoxia in the conscious horse. *Zentbl. VetMed.* 15, 494-498.
- Downie, N. W. and Heath, R. W. (1974). *Basic Statistical Methods*, 4th ed., 263-265. New York, Harper and Row.
- Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics* 11, 1-42.
- Gillespie, J. R., Tyler, W. D. and Eberley, V. E. (1966). Pulmonary ventilation and resistance in emphysematous and control horses. *J. Appl. Phys.* 21, 416-422.
- Littlejohn, A. (1969). Acid-base and blood gas studies in horses. *Res. vet. Sci.* 10, 263-266.
- McPherson, E. A. and Lawson, G. H. K. (1972). Some aspects of chronic pulmonary diseases of horses and methods used in their investigation. *Equine vet. J.* 6, 1-6.
- Pirie, W. R. and Hamden, M. A. (1972). Some revised continuity corrections for discrete data. *Biometrics* 28, 693-701.
- Sasse, H. H. L. (1971). Some pulmonary function tests in horses. Ph.D. thesis, State University of Utrecht.
- Thurlbeck, M. C. (1976). Chronic airflow obstruction in lung disease, p. 23 and 28. Philadelphia, Saunders.

RÉSUMÉ

Les valeurs moyennes normales de PaO₂ et les valeurs maximales de Ppl ont été déterminées chez le cheval. En employant les déviations standards au dessous pour PaO₂ et au dessus pour Ppl des valeurs moyennes, on peut distinguer les chevaux atteints dans une série de 100 chevaux.

La fréquence d'apparition de 20 paramètres cliniques différents fut déterminée de manière statistique pour les chevaux atteints, suspects et indemnes. Une aptitude physique insuffisante et les commémoratifs d'une maladie fébrile antérieure ont été constatés plus souvent chez les

chevaux atteints de MPCO. L'existence d'une toux chronique, de dyspnée, d'une expiration à deux temps et les bruits respiratoires crépitants ou sifflants est plus fréquente chez les chevaux atteints de MPCO que chez les autres.

Les bruits respiratoires crépitants (seulement observés chez les chevaux atteints de MPCO), les bruits respiratoires sifflants et l'intensité accrue des sons respiratoires furent constatés chez un grand nombre de chevaux à MPCO; toutefois le diagnostic de MPCO étayé par ces seuls paramètres serait par trop restrictif.

Les examens radiologiques furent utiles mais les clichés furent trop souvent difficiles à interpréter.

L'hématologie n'apporta aucune aide. On nota l'intérêt qu'il y avait de soustraire les animaux malades du contact des fourrages, paille ou foin.

ZUSAMMENFASSUNG

Normale Durchschnittswerte für PaO₂ und Δ Ppl max wurden bestimmt. Die Verwendung von zwei Standardabweichungen unter (PaO₂) oder über (Δ Ppl max) dem Mittelwert erlaubte die zufriedenstellende Unterscheidung von Pferden mit COPD und von anderen Pferden in einer Reihe von 100 Tieren. Die Frequenz des Auftretens von 20 verschiedenen klinischen Parameter bei befallenen nicht befallenen und möglicherweise befallenen Pferden wurde statistisch analysiert. Ungenügende Leistungsfähigkeit und eine Anamnese von durchgemachten febrilen Zuständen wurde bei COPD-Pferden öfter vermerkt als bei anderen. Das Vorliegen eines chronischen Hustens, von Dyspnoe, einer doppelschlägigen Expiration, vermehrter Atemgeräusche, Giemen und krepitierender Auskultationsgeräusche wird bei COPD Pferden häufiger festgestellt als bei anderen und ein langdauernder Husten ist ein guter Indikator für COPD. Krepitierende Auskultationsgeräusche (nur bei befallenen Pferden vorhanden), Giemen und verstärkte Atemgeräusche konnten bei der Mehrzahl von COPD Pferden festgestellt werden, aber eine Diagnose von COPD, die sich nur auf diese Parameter stützte, würde zu einer zu kleinen Anzahl erkannter Fälle führen. Wenn auch die Röntgenuntersuchung helfen mag, bleiben doch die Aufnahmen in der Regel schwierig zu interpretieren. Die haematologische Untersuchung war keine Hilfe für die Diagnose von COPD. Die günstigen Auswirkungen der Elimination von Heu und Stroh auf affizierte Pferde wurden festgehalten.

Accepted for publication 13.9.77

Pulmonary Artery Pressures in Normal Horses and in Horses affected with Chronic Obstructive Pulmonary Disease

P. M. DIXON

Department of Veterinary Medicine, Royal (Dick) School of Veterinary Studies, Easter Bush, Nr. Roslin, Midlothian EH25 9RG

SUMMARY

Horses clinically affected with chronic obstructive pulmonary disease (COPD) were found to have pulmonary artery hypertension which was associated with systemic arterial hypoxia. The pulmonary hypertension in symptomatic COPD-affected horses was partially reversible upon remission of clinical signs or by oxygen administration. The induction of acute hypoxaemia caused an increase in pulmonary artery pressure in both normal and COPD-affected horses.

INTRODUCTION

PULMONARY artery hypertension secondary to chronic pulmonary disease is of major importance in human medicine (World Health Organisation, 1963). The horse's pulmonary anatomy and pulmonary vascular distribution are similar to those of man (McLaughlin, *et al.*, 1965; Tyler, Gillespie and Newell, 1971) so that a similar pulmonary hypertension might be expected to develop in chronic pulmonary disease in the horse. The pulmonary artery pressure of normal horses has been described by many authors. In some reports (Gall, 1967; Beltran, 1973; Bergsten, 1974; Milne, Muir and Skarda, 1975; Milne, Gabel, Muir and Skarda, 1977; Orr, *et al.*, 1975; Buss and Bisgard, 1977), the values recorded were obtained in circumstances which fulfil the presently accepted criteria for normal resting pulmonary artery pressure, namely, the animals were standing and untranquillised and a suitable baseline was established for a fluid-filled manometric system. Pulmonary artery hypertension has been demonstrated in horses with a variety of chronic pulmonary diseases (Alexander, 1959; Sporri and Schlatter, 1959; Eberly, Tyler and Gillespie, 1966; Beltran, 1973; Bergsten, 1974). The mean pulmonary artery pressure (PAP) value derived from the work of these authors is 44.95 mmHg. Bisgard, Orr and Will (1975) have shown that pulmonary artery hypertension also occurs in ponies moved to high altitudes. Previously, the occurrence of pulmonary hypertension due to hypoxia caused by residing at high altitudes had been well recorded in cattle (brisket disease) and in other species, including man.

This paper describes some studies on the measurements of PAP and carotid arterial blood gases and pH in normal horses and in horses affected with chronic obstructive pulmonary disease (COPD) during different stages of

the disease. Observations are reported on the effect of oxygen administration and of acute hypoxaemia production in normal and COPD-affected horses.

MATERIALS AND METHODS

Horses were classified as COPD-affected, using the recently described criteria of McPherson, *et al.* (1978). The animals showed longstanding evidence of respiratory disease including many of the following clinical signs, coughing, dyspnoea, double expiratory effort, louder or wheezing chest sounds and in all cases a resting arterial oxygen partial pressure (PaO_2) of less than 82 mmHg and maximum intrapleural pressure changes of greater than 6 mmHg. The COPD cases referred to the veterinary hospital during a 3 year period, were all adults and consisted mainly of ponies and hunters with fewer Thoroughbred and draught horses. The controls included a higher proportion of ponies. COPD-affected animals were judged to be symptomatic or asymptomatic at the time of the recordings on the basis of clinical signs, their (PaO_2) and intrapleural pressures. Remission of clinical signs in COPD cases was obtained by using a peat bedding and feeding only a concentrated diet in cube form. These measures reduced exposure of the horses to the environmental aetiological agents. Clinical signs in COPD cases were induced by both natural and artificial exposure to the aetiological agents.

During all procedures, the animals were standing, untranquillised and were quietly handled to prevent pulmonary hypertension induced by excitement (Beltran, 1973). Prior to catheterisation, an area in the lower one-third of the jugular groove was anaesthetised with 1 ml lignocaine. A catheter was introduced into the jugular vein, passed through the right heart and advanced approximately 2 cm into the main trunk of the pulmonary

artery. Positioning was judged by the pulse contours and pressure values observed during manipulation of the catheter.

The catheter (Cardioflex 1150-09, Vygon U.K. Ltd.; Normocath 115-20, Vygon U.K. Ltd.) was connected by a tube (Lectocath 1150-20, Vygon U.K. Ltd.) to a strain gauge manometer (L-221-2-3, Bell & Howell, Basingstoke). This was attached to a pressure transducer (3552, Devices Instruments Ltd., Herts.). The manometer was positioned 2-3 cm above the point of the shoulder, at a site level with the right atrium (Beltran, 1973). The results were recorded on a multichannel recording system (M19, Devices Instruments Ltd., Herts.).

Intrapleural pressure measurements were made by the method of McPherson, *et al.* (1978) and standard lead I ECG tracings were also recorded.

Samples of carotid arterial blood were obtained as described by McPherson, *et al.* (1978). Acute hypoxaemia was produced by adding nitrogen to the inspired air through an open plastic face mask. Oxygen was similarly administered. The gases were administered at 20-50 l/min over a 3-10 min period until the heart rate and PAP reached a steady state.

Mid-inspiratory air samples were aspirated quickly into a 20 ml plastic syringe through a 14-gauge needle inserted interannularly into the trachea. The syringe was immediately sealed and the gas analysed within 5 min. Inspired air, blood gases and pH measurements were made using a gas analyser (Corning 161, Corning Ltd., Essex). The altitude of the hospital and laboratory is 200 m.

In the statistical analysis of results, the significance of differences between the means of groups was tested by Student's *t*-test. Paired data were analysed by Student's *t*-test as applied to paired observations.

RESULTS

Compared with the controls, horses showing clinical signs of COPD (Table I) had significantly raised PAP ($P < 0.001$) and significantly lowered PaO_2 ($P < 0.001$).

The arterial carbon dioxide partial pressures and pH values were within the normal range. During remission (asymptomatic) phase, 10 of the COPD cases were again investigated; they had PaO_2 levels within the normal range. Their PAP in this phase was significantly below ($P < 0.001$) that of the symptomatic phase but was still significantly greater ($P < 0.01$) than that of the controls.

Increasing the oxygen content of inspired air to 60 per cent, as determined by mid-inspiratory tracheal analysis, resulted in the clinically affected COPD group having a temporary partial remission of pulmonary hypertension until a few minutes after the oxygen administration was discontinued (Table II). The decrease in PAP was associated with PaO_2 levels, which were above normal.

Increasing the nitrogen content of the inspired air to 85-95 per cent in 8 normal and 8 COPD-affected horses caused a temporary hypoxaemia in all and, in addition, respiratory alkalosis in the control horses (Table II). In both groups a significant increase in PAP was induced. This was, however, more marked in the COPD-affected animals, although both groups showed a similar, induced reduction in PaO_2 .

DISCUSSION

The findings indicate the relationship of hypoxaemia to the pulmonary hypertension observed in symptomatic COPD-affected horses. Humans, when clinically affected with COPD, are usually hypoxic, hypercapnic and acidotic and the associated pulmonary hypertension is related to all 3 factors (Thurlbeck, 1976). Neither hypercapnia nor acidosis appears to play a part in the aetiology of pulmonary hypertension in horses clinically affected with COPD. The COPD syndrome of Sjöberg (1971) and, to a lesser extent, of Beltran (1973) and chronic alveolar emphysema of Bergsten (1974) appear to be functionally similar to the COPD syndrome of McPherson, *et al.* (1978) although they may differ aetiologically. The first 3 authors also found normocapnic hypoxaemia in their affected horses.

TABLE I
PULMONARY ARTERY PRESSURES AND CAROTID BLOOD GASES AND pH (MEAN VALUES AND S.D.) IN NORMAL AND COPD-AFFECTED HORSES

Group	No.	Pulmonary artery pressures (mm Hg)			Carotid arterial values		
		Maximum	Minimum	Mean	PaO_2 (mm Hg)	PaCO_2 (mm Hg)	pH
Control	20	33.77	15.08	23.54	91.15	36.28	7.389
		± 3.19	± 4.91	± 2.98	± 5.23	± 2.77	± 0.038
COPD cases symptomatic	25	65.45	31.01	44.56	66.13	39.38	7.423
		± 19.85	± 15.61	± 13.84	± 6.40	± 5.98	± 0.054
COPD cases asymptomatic	10	42.25	19.25	28.13	86.51	34.26	7.412
		± 4.60	± 4.64	± 4.37	± 6.39	± 2.75	± 0.033

PaO_2 : Arterial oxygen partial pressure

PaCO_2 : Arterial carbon dioxide partial pressure

TABLE II

EFFECT OF INHALATION OF AN OXYGEN-RICH MIXTURE ON CAROTID BLOOD GASES AND pH AND PULMONARY ARTERY PRESSURES (MEAN VALUES AND S.D.) IN 8 HORSES SYMPTOMATICALLY AFFECTED WITH COPD

Treatment	PaO ₂ (mm Hg)	PaCO ₂ (mm Hg)	pH	Mean PAP (mm Hg)
Pre-oxygenation	66.95 ± 6.22	38.54 ± 5.17	7.442 ± 0.040	38.8 ± 7.87
During oxygenation	108.03 ± 23.96***	40.77 ± 4.21	7.431 ± 0.032	33.4 ± 7.45**

*** P < 0.001

** P < 0.01

absence of hypercapnia in COPD-affected horses has been attributed by Sasse (1971) to the hypoxia-stimulated hyperventilation of normally functioning alveoli. However, because of the sigmoid shape of the haemoglobin oxygen dissociation curve, blood is unable to carry appreciably more oxygen from the overventilated areas and so is unable to compensate similarly for unsaturated blood leaving underventilated areas (Clark, Jones and Clark, 1977). The pathology of equine COPD is different from that of human COPD (Nicholls, 1978) and this may be a possible reason for the differences in CO₂ retention between human and equine COPD cases.

Systemic acidosis is also associated with a pulmonary vasoconstrictor response (Harvey, 1965; Rudolph and Yuan, 1966). There was no evidence of acidosis in the present horses while they were clinically affected with COPD and this is probably related to the absence of hypercapnia.

Euler and Liljestrand (1946) first showed the pulmonary hypertensive effect of hypoxia. Later work showed that that this was due, in part, to a direct pressor reflex by the capillary vessels (Lloyd, 1964) as well as to the more obvious hypoxia-induced increase in cardiac output caused by both tachycardia and increased myocardial contractility (Aviado, 1965).

In this work, hypoxaemia was shown to be the main factor in the aetiology of pulmonary hypertension in equine COPD. However, since oxygen administration only partially reversed the hypertension, the involvement of some factor or factors other than hypoxia is likely. Further support for this opinion is derived from the fact that in some asymptomatic cases the PAP remained elevated although PaO₂ was within the normal range. In some COPD horses the increased intrapleural pressure changes are mainly due to positive pressure changes

and in these cases the increased positive intrapleural pressure will cause an increase in the mean PAP. Likewise increased negative intrapleural pressure changes will tend to decrease the PAP. The reversibility, even partial, of the pulmonary hypertension of equine chronic respiratory disease does not appear to have been reported previously. That the hypertension can be reduced so readily implies that it is mainly due to a pulmonary vascular hypoxic response rather than to structural vascular changes.

A very wide variation in the degree of pulmonary hypertension in symptomatic COPD-affected horses was also observed (Tables I and II). This does not appear to be related to the degree of hypoxia as the symptomatic groups shown in the tables have similar PaO₂ values yet the group shown in Table I has a more marked pulmonary hypertension than the group shown in Table II. Acute hypoxaemia production caused a more pronounced pulmonary hypertensive response in the affected horses than in the control group. A similar marked pulmonary reaction to acute hypoxaemia was observed by Bisgard, *et al.* (1975) in ponies with existing high altitude-induced pulmonary hypertension.

ACKNOWLEDGEMENTS

I wish to thank Mr. E. A. McPherson for guidance and help, Mr. G. Keay and Mr. R. Brown for skilled technical assistance, Professor J. T. Baxter for help with the preparation of the manuscript and the Horserace Betting Levy Board for financially supporting part of this project.

REFERENCES

- Alexander, A. F. (1959). Chronic alveolar emphysema in the horse. *Am. Rev. Resp. Dis.* **80**, 141-146.
 Aviado, D. M. (1965). *The Lung Circulation*, Vol. I, Physiology and Pharmacology. Pergamon Press, Oxford, pp. 5-6.

TABLE III

EFFECT OF INDUCED ACUTE HYPOXIA ON CAROTID ARTERIAL GASES AND pH AND ON PULMONARY ARTERY PRESSURE (MEAN VALUES AND S.D.) IN 8 NORMAL AND 8 COPD-AFFECTED HORSES

	PaO ₂ (mm Hg)	PaCO ₂ (mm Hg)	pH	Mean PAP (mm Hg)
Normal horses				
resting	91.9 ± 7.33	36.7 ± 3.5	7.392 ± 0.032	24.1 ± 2.42
acutely hypoxic	50.5 ± 7.75***	29.1 ± 2.5	7.474 ± 0.060	33.7 ± 4.75***
COPD cases				
resting	71.5 ± 8.07	37.8 ± 3.86	7.411 ± 0.041	32.5 ± 2.20
acutely hypoxic	48.3 ± 6.37***	34.0 ± 3.08	7.442 ± 0.065	46.6 ± 4.60***

*** P < 0.001

- Beltran, L. E. (1973). A critical evaluation of right heart catheterisation in the horse. Ph.D. thesis, University of London.
- Bergsten, G. (1974). Blood pressure, cardiac output and blood-gas tension in the horse at rest and during exercise. *Acta vet. Scand.*, Supplement 48.
- Bisgard, G. E., Orr, J. A. and Will, J. A. (1975). Hypoxic pulmonary hypertension in the pony. *Am. J. vet. Res.* **36**, 49-52.
- Buss, D. D. and Bisgard, G. E. (1977). Haemodynamics and myocardial function during acute hypoxia in the pony. *Am. J. vet. Res.* **38**, 365-371.
- Clark, W. T., Jones, R. B. and Clark, J. (1977). Blood oxygen and carbon dioxide tensions in dogs with respiratory failure. *J. Small Anim. Prac.* **18**, 535-541.
- Eberly, V. E., Tyler, W. D. and Gillespie, J. R. (1966). Cardiovascular parameters in emphysematous and control horses. *J. Appl. Physiol.* **21**, 883-889.
- Euler, U. S. von and Liljestrand, G. (1946). Observations on the pulmonary arterial blood pressure in the cat. *Acta physiol. Scand.* **12**, 301-321.
- Gall, C. M. (1967). Intracarotid and right heart pressures of horses. M.S. thesis, Ohio State University.
- Harvey, R. J. (1965). The influence of hydrogen ion in the control of pulmonary artery pressures in patients with obstructive disease of the lungs. Proc. VII Conf. Res. in Emphysema, Aspen, 1964. *Med. thorac.* **22**, 108-117.
- Lloyd, T. C. (1964). Effect of alveolar hypoxia on pulmonary vascular resistance. *J. Appl. Physiol.* **19**, 1086-1094.
- McLaughlin, R. F., Tyler, W. D., Edwards, D. W., Crenshaw, D. L., Canada, R. O., Fowler, M. A., Parker, E. A. and Reifstein, G. H. (1965). Chlorpromazine-induced emphysema. *Am. Rev. Resp. Dis.* **92**, 597-608.
- McPherson, E. A., Lawson, G. H. K., Murphy, J. R., Nicholson, J. M., Fraser, J. A., Breeze, R. G. and Pirie, H. M. (1978). Chronic obstructive pulmonary disease (COPD): Identification of affected horses. *Equine vet. J.* **10**, 47-54.
- Milne, D. W., Muir, W. W. and Skarda, R. T. (1975). Pulmonary arterial wedge pressures: Blood gas tensions and pH in the resting horse. *Am. J. vet. Res.* **36**, 1431-1434.
- Milne, D. W., Gabel, A. A., Muir, W. W. and Skarda, R. T. (1977). Effects of training on heart rate, cardiac output and lactic acid in standardbred horses, using a standardised exercise test. *J. Equine med. Surg.* **1**, 131-135.
- Nicholls, Julia M. (1978). Personal Communications.
- Orr, J. A., Bisgard, G. E., Forster, H. V., Rawlings, C., Buss, D. D. and Will, J. A. (1975). Cardiopulmonary measurements in resting normal ponies. *Am. J. vet. Res.* **36**, 1667-1671.
- Rudolph, A. M. and Yuan, S. (1966). Response of the pulmonary vasculature to hypoxia and H⁺ concentration changes. *J. clin. Invest.* **45**, 399-411.
- Sasse, H. H. L. (1971). Some pulmonary function tests in the horse. Ph.D. thesis, State University of Utrecht.
- Sporri, H. and Schlatter, C. (1959). Blutdruckerhöhung im Lungenkreislauf. *Schweizer Arch. Tierheilk.* **101**, 525-541.
- Tyler, W. S., Gillespie, J. R. and Newell, J. A. (1971). Symposium on pulmonary and cardiac function. 1. Modern functional morphology of the equine lung. *Equine vet. J.* **3**, 84-94.
- Thurlbeck, W. M. (1976). Chronic Airflow Obstructive Lung Disease. Saunders, Philadelphia. pp. 324-326.
- World Health Organisation (1963). Chronic cor pulmonale. Tech. Rep. Series No. 213. WHO, Geneva.

RÉSUMÉ

Les chevaux atteints de maladie pulmonaire chronique obstructive présentent une hypertension pulmonaire artérielle associée avec une hypoxie artérielle systémique. L'hypertension pulmonaire chez les chevaux cliniquement atteints semble partiellement réversible lorsqu'il y a une rémission des signes cliniques ou administration d'oxygène. L'hypoxie provoquée engendre une élévation de la pression pulmonaire artérielle tant chez les chevaux sains que chez les chevaux atteints de maladie respiratoire chronique obstructive.

ZUSAMMENFASSUNG

Pferde mit einer klinisch feststellbaren chronischen obstruktiven Lungenkrankheit (COPD) wiesen eine Hypertension in der Pulmonalarterie auf zusammen mit einer systemischen, arteriellen Hypoxie. Der Lungenüberdruck bei kranken Pferden erwies sich als teilweise reversibel während der Remission von klinischen Krankheitszeichen oder nach O₂-Zufuhr. Die Provokation einer akuten Hypoxie erhöhte den Pulmonaldruck sowohl bei normalen als auch bei COPD-kranken Pferden.

Accepted for publication 10.4.78

A PATHOLOGICAL STUDY OF CHRONIC PULMONARY DISEASE
IN THE HORSE

Julia M. Nicholls

Thesis presented for the Degree of Doctor of Philosophy,
University of Glasgow (1978)

SUMMARY

The main part of this thesis describes the detailed pulmonary pathology in 25 cases of naturally occurring chronic pulmonary disease in the horse. Further sections deal with a survey of alpha-1 antitrypsin levels in the horse, the development of an experimental model of the disease and a study of the effects of ascarids on the equine lung.

The main pathological lesion was bronchiolitis, a lesion affecting all the small airways less than 2 mm in diameter and characterised by epithelial hyperplasia, goblet cell metaplasia, peribronchiolar cellular accumulations and an exudate of mucus and cells in the lumen. Rather more than half the cases had pulmonary eosinophilia. Emphysema was confined to small areas in the cranial lobe and periphery of the caudal lobe. The extent of emphysema was assessed by inflating one lung from each case with fixative, slicing it into thin slices and examining it under a microscope. The number and type of goblet cells in the bronchial epithelium of five normal horses were compared with ten horses with CPD; there was no significant difference and in addition there was no hyperplasia of the bronchial submucosal glands. The disease in the horse therefore bears no pathological resemblance to chronic bronchitis and emphysema of man. On the basis of these findings it is proposed that the disease be known as chronic bronchiolitis.

Quantitative measurements of the number of pulmonary mast cells at various sites in the lung showed that seven out of ten horses with CPD had significantly increased numbers of mast cells in all parts of the lung except peribronchially when compared to normal horses; the remaining three had markedly decreased numbers except peribronchially. It was not clear whether the mast cells were part of an allergic reaction or merely markers of inflammation.

Horses with CPD had similar alpha-1 antitrypsin values to normal horses. A deficiency of this in man predisposes to the early development of emphysema.

An experimental model of chronic bronchiolitis was produced in foals by the oral administration of 3-methyl indole and an eosinophilic bronchitis and bronchiolitis was produced by infecting foals with Parascaris equorum larvae.

frequency of right ventricular hypertrophy that was observed on post mortem examinations of horses affected with chronic pulmonary disease.

Chronic Obstructive Pulmonary Disease (COPD) in Horses: Aetiological Studies: Responses to Intradermal and Inhalation Antigenic Challenge

E. A. McPHERSON, G. H. K. LAWSON, JILL R. MURPHY and JANET M. NICHOLSON
University of Edinburgh Veterinary Field Station, Easter Bush, Roslin, Midlothian EH25 9RG

R. G. BREEZE* and H. M. PIRIE

University of Glasgow Veterinary School, Department of Veterinary Pathology, Bearsden Road, Bearsden, Glasgow G61 1QH

SUMMARY

Micropolyspora faeni and *Aspergillus fumigatus* were identified as common causes of respiratory hypersensitivity in horses affected with chronic obstructive pulmonary disease (COPD). Rye grass pollen and an *Actinomyces* evoked respiratory allergy in a few horses. Not infrequently, individual horses were found to have respiratory hypersensitivity to two or more antigens.

The methods used to examine for allergy were intradermal testing and inhalation challenge with environmental antigens. An intradermal test using an *M faeni* extract was demonstrated to be suitable for diagnostic use in horses previously accurately diagnosed as suffering from COPD. In contrast, the *A fumigatus* antigen used proved unsatisfactory for such a purpose. Skin reaction to *M faeni* and *A fumigatus* extracts by horses affected with COPD indicated that the hypersensitivity was a dual one—a weak response shortly after injection followed by an Arthus-like response 4 to 8 hours later.

As a parameter for monitoring responses to inhalation challenge, maximum intrathoracic pressure change (max Δ Ppl) proved satisfactory, whereas changes in partial pressure of arterial oxygen (PaO₂) did not.

INTRODUCTION

McPHERSON *et al* (1978) described the identification of horses affected with COPD by measurement of the partial pressure of arterial oxygen (PaO₂) and the maximum change in intrathoracic pressure (max Δ Ppl), the former being subnormal and the latter elevated in affected animals. From 100 horses referred to us principally because of poor work performance or chronic coughing, 38 were found to be affected (**Group A**), 34 were not affected (**Group C**) and 28 were in a doubtful category (**Group B**). These groups of horses were intradermally tested with environmental allergens believed to cause hypersensitive disease in other species (Austwick, 1966; Pepys, 1969a) and subsequently challenged by the inhalation of the same agents or extracts of them. The results are recorded and discussed in this paper.

MATERIALS AND METHODS

Animals

The 100 horses were assigned to **Groups A, B and C** and kept under the conditions described by McPherson *et al* (1978).

Techniques

The max Δ Ppl, PaO₂ and haematological examinations were carried out as described by McPherson *et al* (1978). Partial pressure of arterial carbon dioxide (PaCO₂) was determined on a Corning pH/blood gas 161 analyser (Corning Medical, Halstead, Essex).

Intradermal tests

Antigens

Bencard intradermal skin testing solutions (Bencard, Brentford, England) were used for grass pollens (0.02 per cent, B2), tree pollens (0.2 per cent, B3), mixed moulds (0.5 per cent, A13), mixed moulds (1 per cent, M5), *Cladosporium herbarum* (1 per cent, M3) and *A fumigatus*

* Present address: Washington State University, Department of Veterinary Microbiology and Pathology, Pullman, Washington 99164, USA.

(0.5 per cent, 2000). Bencard control solution was also used in conjunction with these antigens. Antigens from *M faeni* were prepared as described by Lawson *et al* (1979). The acetone precipitated *M faeni* antigen was diluted with 0.25 per cent phenol saline to give a solution containing 1.4 mg dry matter antigen/ml. The control was a nutrient broth (Oxoid CM1) diluted volume for volume with 0.5 per cent phenol saline. Extracts were also prepared from an uncharacterised *Actinomyces* (705/69) isolated from the nasopharynx of a horse. This organism was grown for 7 days at 37°C in glucose broth; after sonication for 30 min at 8 microns amplitude on ice, the extract was concentrated approximately 20 times with carbowax at 5°C and finally dialysed against phosphate buffered saline (0.01 M pH 7.2). The isolate was lost before it was identified. The solution used for skin testing contained approximately 25 mg/ml of dry matter in 0.25 per cent phenol saline.

Techniques

0.05 ml of the test and control solutions were injected intradermally into the closely clipped or shaven neck of the horse. The diameter of the wheal was measured (mm) horizontally at $\frac{1}{2}$, 1, 2, 4, 6, 8 and, if necessary, 10 hours after injection.

Inhalation challenge

In the case of fungal extracts, a final volume of 5 ml was administered as an aerosol over a period of 20 min. A Wright's nebuliser (Aerosol Products, London) converted the solution into an aerosol which was led by a plastic tube, 10 mm internal diameter, into a face mask constructed from a rigid plastic bucket and a flexible plastic sleeve. An aperture 9 cm in diameter was cut in the bottom of the bucket which was suspended by a strap over the horse's poll region. The plastic sleeve embraced the bucket tightly and was sealed to the face with plastic foam held in position by a self-adhesive nylon strip. The tube from the nebuliser passed through the face seal into the bucket. The attendant wore a mask and the administration was carried out in a well-ventilated room with a wide open door.

The resting max Δ Ppl and PaO₂ were determined on the morning of the test. After inhalations were completed, clinical observations were made hourly and monitoring of the foregoing parameters was repeated 4 to 5 hours after inhalation. When no changes had taken place, observation continued and, where necessary, further recordings were made up to 10 hours after completion of the inhalations.

M faeni, *A fumigatus* and *Actinomyces* 705/69. The first 2 antigens were prepared as described by Lawson *et al* (1979) and the last as described above. The challenge dose of *M faeni* and *A fumigatus* was approximately 12 mg of antigen extract (estimated as dry matter), whereas that of the *Actinomyces* was 50 mg (estimated as dry matter), suspended in all cases in 5 ml normal saline.

Mouldy hay. This material was used in the earlier part of the work and occasionally later, when horses failed to respond to the more specific antigens. Bales of poor quality fodder (mouldy or heated straw or hay as available) were shaken up in a loosebox in which the animal on test was confined for 1 hour before returning to its own loosebox for observation and monitoring as described.

Grass pollens. Rye grass pollen collected during harvesting of a hay crop, cut while the grass was in flowering and kept under dry conditions in the laboratory was used at a dose of approximately 1 gm. This was insufflated into a Cox's face mask (Arnolds, London) over a period of about 10 min. Horses were exposed when the history suggested pollen involvement or when no other challenge had produced a positive response.

Control inhalations. Where circumstances permitted each horse was made to inhale an antigen to which it had not reacted in the skin test, as well as those to which it was likely to respond, thus providing a control challenge. At the outset, horses were exposed to an aerosol of 5 ml of 0.25 per cent phenol saline to which none reacted.

Statistical methods

Chi-square tests and analysis of variance, followed when appropriate by Duncan's multiple range test were employed as described by McPherson *et al* (1979). Student's "t" test was employed in assessing response challenge and McNemar's χ^2 test corrected for continuity was employed in comparing the results of skin and intradermal tests.

RESULTS

Intradermal tests

Horses frequently responded by producing swelling to the control injections. This confused the interpretation of skin tests employing specific allergens. Of reactions to an antigen which exceeded the mean reaction of horses to the control plus two standard deviations ($\bar{x} + 2$ SD) were considered positive. The control means were calculated from the reactions of 75 horses to the Bencard control solution and 90 horses to the nutrient broth solution. These means, and the decision levels derived from them ($> \bar{x} + 2$ SD) for evaluation of response to antigens, are shown diagrammatically in Figs 1 and 3.

Using these criteria for interpretations, the response of all horses tested with a variety of antigens are set out in Table I and the statistical significance of differences in response given by Group A (affected) and Group B + C (doubtful or not affected) was determined. Significantly more of the horses affected with CO showed skin sensitivity to *A fumigatus* and *M faeni* than did horses in the other two classes.

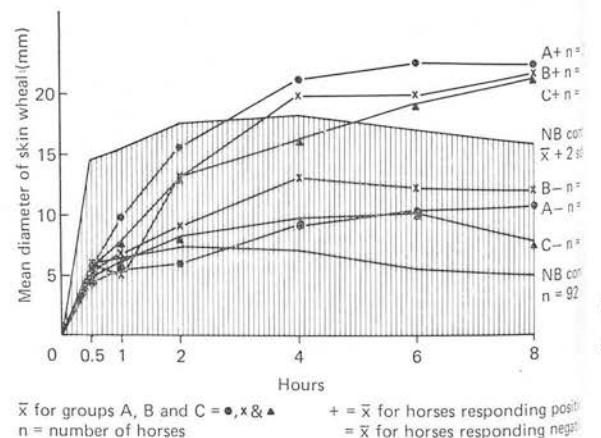


Fig 1. Skin response of horses to *M faeni* antigen (0.05% intradermally).

TABLE I
INTRADERMAL SKIN TEST RESPONSE OF HORSES TO ENVIRONMENTAL ANTIGENS

	Group A		Group B + C		Chi-square value	Probability (P)
	+	-	+	-		
*Grass pollen	2	31	5	34	0.931	> 0.05
*Tree pollen	3	30	6	33	0.647	> 0.05
*Mixed mould (A13)	25	9	27	14	0.515	> 0.05
*Mixed mould (A5)	13	21	19	22	0.499	> 0.05
* <i>Cladosporium herbarum</i>	15	19	18	20	0.076	> 0.05
<i>Actinomyces</i> spp	22	15	22	19	0.266	> 0.05
* <i>Aspergillus fumigatus</i>	17	17	5	34	11.926	< 0.001
<i>Micropolyspora faeni</i>	25	10	14	31	12.808	< 0.001

* Bencard antigen solutions

Descriptions of skin reactions

Each of the antigens employed failed to evoke a response in some horses. When horses responded, the wheals tended to be rounded and relatively circumscribed while developing and were dome-shaped, tense and painful to the touch. Later, the swellings softened and diffused into the surrounding tissue. The oedema then tended to move ventrally and, in severe reactions, the superficial lymphatics were visible. In most cases, the reactions were flattened and no longer clearly defined 4 to 6 hours after reaching their maximum but, in a few severe reactions, 12 to 24 hours passed before the swellings completely disappeared. No skin necrosis occurred. As descriptions are subjective, reliance was placed on measurement alone.

Response to *M faeni*

The mean diameter of the wheals at each time of measurement for horses in Groups A, B and C which gave positive (A+, B+, C+) and negative (A-, B-, C-) response to *M faeni* antigen were plotted in Fig 1.

The top of the hatched area is the calculated mean response ($\bar{x} + 2$ SD) for all animals to control nutrient

broth solution. There was, by 6 and 8 hours, a clear separation of the responses of horses which showed skin sensitivity to *M faeni* from those which did not. In horses belonging to Groups A and B this had occurred by 4 hours. In the reacting horses in Group C the response was late, at 6 and 8 hours, hence the mean wheal diameter for this group at 4 hours is within the hatched area. If interpretation of the test had been made only at 4 hours 12 per cent of positive reactions would have been missed; at 6 or at 8 hours, 10 per cent. If, however, readings were made at both 6 and 8 hours only 4 per cent would have been missed.

The times taken to reach a positive reaction and the maximum size of wheal are shown in Fig 2.

The timing of the maximum response to the *M faeni* skin test indicates that this response could involve an Arthus-like phenomenon. When those horses which clearly reached a positive response between 4 and 8 hours were then compared with animals failing to respond in such a manner in respect of their skin reactions early after injection, it was found that they differed. The mean response 1 hour post-injection of the A+ was significantly greater than that of the A- horses (Fig 1) and that of all horses to the control nutrient broth solution ($F = 4.89$; $P < 0.01$). Duncan's multiple range test indicated two overlapping subgroups: Control and A-; and A-, A+.

While the majority of animals (20/25) behaved in this manner a few showed no such primary response and a

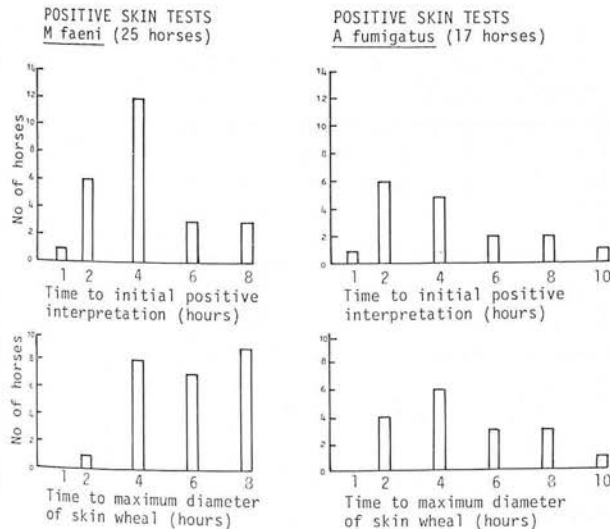


Fig 2. Horses affected with chronic obstructive pulmonary disease—Group A.

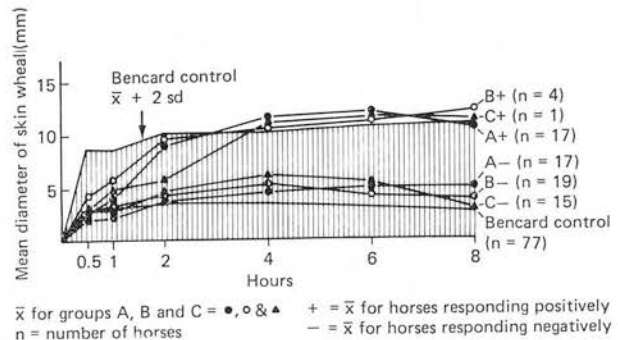


Fig 3. Skin response of horses to *A fumigatus* antigen (0.05 ml intradermally).

TABLE II
RESPONSE OF HORSES CHALLENGED BY INHALATION OF *M faeni* ANTIGEN
(post-challenge less pre-challenge max Δ Ppl)

	n	\bar{d}	SD	't' value	Probability (P)
POSITIVE					
All horses	29	6.0	4.3	7.582	<0.001
Group A	25	6.5	4.4	7.335	<0.001
Group B	4	3.15	1.39	4.532	<0.05
Group C	0	—	—	—	—
NEGATIVE					
All horses	23	0.38	1.14	1.826	>0.10
Group A	5	-0.5	0.6	1.826	>0.10
Group B	12	0.872	1.0	2.979	<0.05
Group C	6	0.125	1.31	0.234	>0.10

n = number of animals
 \bar{d} = mean difference (mmHg)

SD = standard deviation
't' = Student's 't' statistic

single animal reacted in a very positive manner shortly after injection.

Response to *A fumigatus*

The same procedure was adopted in assessing the *A fumigatus* skin test results. These are shown in Fig 3 where the top of the hatched area is $\bar{x} + 2$ SD for the Bencard control solution. The maximum response in the majority of horses occurred at or after 4 hours (Fig 2). Analysis of variance of the responses at 1 hour indicated no significant difference between A+, A- and control sites. At 2 hours, however, there was a difference ($F = 30.335$; $P < 0.01$) and Duncan's multiple range test indicated two significant subgroups, A- control solution and A+.

Inhalation challenge

Monitored by changes in maximum intrathoracic pressure

The criteria used in determining a positive response were as follows. Horses with a max Δ Ppl of <6 mmHg

which increased after challenge to >6 mmHg (the value delineating affected animals) were regarded as having made a positive response. Where the pre-exposure value of max Δ Ppl was already ≥ 6 mmHg, an increase of 15 per cent in this figure was considered to indicate a positive reaction. In the event, all horses in this latter category gave increases well above 15 per cent.

M faeni extract. The mean responses of all horses challenged are set out in Table II, together with a statistical evaluation indicating a clear response by a large proportion of **Group A** horses to a respiratory challenge with *M faeni*, compared with the minimal change induced in animals not hypersensitive to this organism.

In **Group A**, the difference in response between sensitive and non-sensitive horses is highly significant ($P < 0.001$). In **Group C**, only a few horses were challenged but all gave a negative response. **Group B**, as explained by McPherson *et al* (1978), contained a few animals which although showing some abnormality of PaO₂ or

TABLE III
RESPONSE OF HORSES CHALLENGED BY INHALATION OF *A fumigatus* ANTIGEN
(post-challenge less pre-challenge max Δ Ppl)

	n	\bar{d}	SD	't' value	Probability (P)
POSITIVE					
All horses	12	6.84	8.928	2.654	<0.05
Group A	12	6.84	8.928	2.654	<0.05
Gp B + C	0	—	—	—	—
NEGATIVE					
All horses	37	0.13	1.64	0.465	>0.10
Group A	15	0.47	1.845	0.980	>0.10
Group B	18	0.064	1.499	0.182	>0.10
Group C	4	0.25	1.636	0.306	>0.10

n = number of animals
 \bar{d} = mean difference (mmHg)

SD = standard deviation
't' = Student's 't' statistic

TABLE IV
RESPONSE OF HORSES TO INHALATION CHALLENGE BY HAY/STRAW MOULDS
AND GRASS POLLENS

	Group A		Group B + C		Chi-square value	Probability (P)
	+	-	+	-		
Mouldy hay/straw	6	3	0	11	10.318	<0.01
Rye grass pollen	5	6	0	6	3.736	>0.05

△ Ppl changes at rest, did not meet our requirements for classification in **Group A**. Four of the **Group B** horses gave a small but significant response to challenge ($P < 0.05$). Three of the 12 horses recorded as negative responded by an increase of 50 per cent, 74 per cent and 101 per cent in max △ Ppl over the pre-challenge level, but were even then below the threshold of 6 mmHg. This influenced the "t" test, giving a P value of <0.05. Analysis of variance, however, showed that there was a significant difference ($P < 0.01$) between the **Group B** horses reacting positively and those reacting negatively.

A fumigatus extract. The mean response of all horses and the groups is recorded in Table III with a statistical analysis of the results which indicates a highly significant difference ($P < 0.01$) between those responding and those failing to respond to this challenge. All 12 horses which responded were in **Group A**, the affected group.

Mouldy hay/straw dust. 20 horses were exposed to this crude challenge. From Table IV, it is clear that only horses in **Group A** responded in a positive fashion.

Rye grass pollen. 17 horses were challenged with pollen dust. Positive responses were only obtained from **Group A** horses, but the numbers were too small for analysis. It was noted that 3 of the 5 horses responding positively showed a clinical response within 90 min. They coughed, flared their nostrils when inspiring, showing tachypnoea and hyperpnoea and, on auscultation, wheezing sounds were detected. The only horse monitored at this stage had an increased max △ Ppl. All showed this increase at 4 to 5 hours, as in the responses to fungal antigens.

Response of the 38 Group A horses to respiratory challenge. Three horses were not challenged and 2 failed to respond to challenge with the antigens currently available—*M faeni* and grass pollen in one case and *M faeni*, *A fumigatus* and grass pollen in the other. The remaining 33 horses responded in a positive fashion to one or more agent. The responses to individual antigens are shown in Table V.

Since all horses were not exposed to all antigens, the percentage figures in Table V emphasise the importance of *M faeni* as a common, but not sole agent involved in COPD in our horses. Multiple hypersensitivity was not uncommon. Of the 25 hypersensitive to *M faeni*, 9 were also hypersensitive to *A fumigatus* and 2 of the latter also to rye grass pollen. Two horses hypersensitive to mouldy hay and to rye grass pollen did not respond to challenge with *M faeni* or *A fumigatus*. One horse gave a positive response to challenge with the *Actinomyces* extract. It also responded to *A fumigatus*.

Monitored by changes in PaO₂ levels

It was found that, following a single exposure to challenge by *M faeni* extract, the changes in PaO₂ levels at 4 to 6 hours after inhalation were apparently unpredictable and, as shown in Table VI, the mean changes were not significantly different from zero but the standard deviation was considerable.

Analysis of variance indicated that there was no significant difference between these groups of horses. For this reason, consideration of PaO₂ changes as a monitoring measurement was abandoned.

When, however, horses which had been coughing for less than 12 months were compared with horses which had been doing so for more than 12 months, in respect of whether the PaO₂ levels increased or decreased following challenge, the situation in Table VII was disclosed.

Thus, on average, in horses responding to *M faeni* the PaO₂ levels appeared to be elevated by challenge in horses affected for less than 1 year, whereas the levels were apparently decreased in those with disease of longer standing. By contrast, in horses not hypersensitive to *M faeni*, the PaO₂ levels did not show this tendency in relation to duration of the cough. The same was found to apply to horses challenged with *A fumigatus*.

The PaCO₂ levels of horses responding positively to *M faeni* challenge did not alter significantly ($P > 0.05$) as might be expected, since any tendency to rise is com-

TABLE V
INHALATION CHALLENGE OF 35 GROUP A HORSES

	<i>Micropolyspora faeni</i>	<i>Aspergillus fumigatus</i>	Mouldy hay	Rye grass pollen
Number exposed	32	29	8	12
Number positive	25	12	6	5
Percent positive	78	41	75	42

TABLE VI
CHANGES IN PaO₂ LEVELS AFTER *M faeni* INHALATION CHALLENGE

	n	\bar{d}	SD	't' value	Probability (P)
Animals +ve on max Δ Ppl challenge	28	2.5	7.04	1.333	> 0.05
Animals -ve on max Δ Ppl challenge	19	-0.24	6.04	1.386	> 0.05

n = number of animals
 \bar{d} = mean difference (mmHg)

SD = standard deviation of difference in PaO₂
't' = Student's 't' statistic

compensated by hyperventilation in normal animals and Dixon (1978) has shown that horses with COPD are capable of hyperventilating successfully. It seems, on the present evidence, that this is so in animals with relatively recent disease, whereas the older cases, while able to control their PaCO₂ levels, cannot maintain their PaO₂ levels.

Comparison of intradermal and inhalation testing on horses with *M faeni* and *A fumigatus*

The results of these tests are shown and compared in Table VIII.

M faeni extract. In Table VIII the results of the skin and inhalation tests are set out in contingency tables. It is likely that the response provoked by an inhalation test is a reliable indicator of respiratory hypersensitivity. The dose of *M faeni* extract used appeared to be satisfactory, although we have encountered an animal which only responded to a double dose. Inhalation challenge is a cumbersome test while a skin test is simple. The tables, therefore, compare the tests to see whether a skin test could be used instead. McNemar's χ^2 test (corrected for continuity) indicated that there were not significantly more positive responses to one or other test. There were, however, discrepancies between the tests. When only animals responding positively to the skin test are considered, the agreement between the tests was 92 per cent (22/24) in Group A horses, 22 per cent (2/9) in Group B and 73 per cent (24/30) in Group C. In all other comparisons between the tests the agreement was unacceptably low. Thus, in a horse affected with COPD a positive response to the *M faeni* skin test is a reasonably reliable indication that the animal has a respiratory hypersensitivity to an extract of the organism.

A fumigatus extract. There was no association between the two tests except in the Group B + C where the association was due to the relatively large number of animals which failed to react to either test. McNemar's χ^2 test (corrected for continuity) confirmed that where the results of the test disagreed there was no tendency for either test to evoke more positive reactions.

The agreement between the test results was poor except in the Group B + C, where a negative skin test was always matched by a negative inhalation challenge. Positive skin responses were as likely to occur in horses negative on inhalation challenge as in those which were positive to respiratory challenge.

DISCUSSION

The intradermal test showed that horses are sensitive to a number of antigens in their environment and that the development of skin sensitivity is not necessarily associated with the development of respiratory disease. This is paralleled in man (Schatz, Patterson and Finlay 1977). Nevertheless, the association of *M faeni* and *A fumigatus* skin sensitivity with equine COPD is brought out in Table I.

In the positive skin tests with *M faeni* antigen, the nature of the wheals, the absence of measurable reaction 1 hour post-injection in some horses later positive and the timing of the maximal response in the majority suggest that the chief reaction was Arthus-like. The differences in the mean responses of the subgroups A+ and A- at 1 hour post-injection suggests that in the majority of the horses the skin response to *M faeni* antigen was a dual one—an immediate followed by an Arthus-like reaction. This, however, requires more detailed

TABLE VII
EFFECT OF DURATION OF COUGH ON PaO₂ LEVELS IN HORSES CHALLENGED BY INHALATION OF *M faeni* EXTRACT

Duration of coughing	Animals +ve on max Δ Ppl challenge		Animals -ve on max Δ Ppl challenge	
	< 12 months	> 12 months	< 12 months	> 12 months
(raised PaO ₂ levels (18	3	14	1
(lowered	4	6	9	1

TABLE VIII
COMPARISON OF INTRADERMAL AND INHALATION TEST RESULTS ON HORSES AFFECTED
AND NOT AFFECTED WITH COPD

	All horses		Group A (COPD affected)		Group B + C (not affected)	
	Inhalation —	+	Inhalation —	+	Inhalation —	+
<i>Micropolyspora faeni</i>						
Intradermal —	14	5	3	3	11	2
Intradermal +	9	24	2	22	7	2
McNemar's χ^2 Probability (P)	0.6429 NS		0.000 NS		1.7778 NS	
<i>Aspergillus fumigatus</i>						
Intradermal —	27	9	9	9	18	0
Intradermal +	8	5	5	4	3	1
McNemar's χ^2 Probability (P)	0.000 NS		0.6429 NS		1.3333 NS	

NS = no significance

investigation. The same appears to be true of reaction to *A. fumigatus*.

The inhalation challenge tests were successful in demonstrating the involvement of *M. faeni* and *A. fumigatus* in COPD. That on occasion other agents are implicated has also been revealed. Two horses which responded to exposure to poor quality hay did not react to either *M. faeni* or *A. fumigatus*; 5 horses showed respiratory hypersensitivity to rye grass pollen; 1 horse was hypersensitive to inhalation of an unidentified *Actinomyces* extract.

The changes in PaO₂ following short challenge inhalation exposure tests were unexpected and their apparent random nature puzzling until retrospective analysis revealed the tendency for more recently affected horses to show a rise in PaO₂ and long-standing cases to show a fall. This merits further study.

The intradermal test as an indicator of the cause of respiratory hypersensitivity

When the *M. faeni* skin test was positive, the agreement between the responses to intradermal and inhalation challenge in our horses with COPD, accurately diagnosed by measurement of changes in PaO₂ and max Δ Ppl, was good. The same cannot be said of *A. fumigatus*, unfortunately, as the disagreement between the tests was considerable except in horses not affected with COPD where there was good agreement when the responses were negative.

There are several reasons which may account for the positive skin test against *A. fumigatus* being at variance with the inhalation test. First, the antigenic extracts used for intradermal and inhalation testing were obtained from different sources; antigens in different extracts of *A. fumigatus* are known to vary greatly (Pepys, 1969b). Secondly, the organism, unlike *M. faeni*, is

known to invade animal tissue on occasions. Rooney (1970) demonstrated *Aspergillus* hyphae in nodules in the lungs of horses examined at post mortem. He regarded these hyphae as opportunist invaders in nodules caused by parasitic larval migration through the lungs. That skin sensitisation takes place in some such instances following infection would appear a reasonable hypothesis. No such nodules were noted by Nicholls (1978) while examining the lungs of horses from our Group A (affected with COPD). It seems unlikely, therefore, that a satisfactory indirect method of demonstrating respiratory hypersensitivity to *A. fumigatus* will be found using a skin test.

The importance of *M. faeni* and *A. fumigatus* as aetiological agents in COPD in our sample of horses, drawn from Scotland and northern England, is unequivocal. That on occasion other dusts, eg, grass pollen and other *Actinomyces*, play a role seems likely. Our failure to demonstrate the antigen involved in 2 horses hypersensitive to mouldy hay suggests that antigens other than those investigated here are occasionally involved.

We should like to point out that, although sensitisation to *M. faeni* and *A. fumigatus* appears to be common, this does not preclude the possibility that many of our horses were also sensitive to other antigens. At the present time we have little indication that this is so, but the difficulty of evaluating all possible allergens is immense.

ACKNOWLEDGEMENTS

This work was made possible by a grant from the Horserace Betting Levy Board. Thanks are also due to Miss P. Wooding and Mr G. Keay, both of the Royal (Dick) School of Veterinary Studies, University of Edinburgh, for technical assistance.

REFERENCES

- Austwick, P. K. C. (1966). Fungal Diseases of Animals. p. 148. Commonwealth Agricultural Bureau, Slough, England.
- Dixon, P. M. (1978). Studies on pulmonary artery pressures in normal horses and horses affected with chronic obstructive pulmonary disease (COPD). *Equine vet. J.* **10**, 195-198.
- Lawson, G. H. K., McPherson, E. A., Murphy, J. R., Nicholson, J. M. and Wooding, P. (1979). The presence of precipitating antibodies in the sera of horses with chronic obstructive pulmonary disease (COPD). *Equine vet. J.* **11**, 172-176.
- McPherson, E. A., Lawson, G. H. K., Murphy, J. R., Nicholson, J. M., Fraser, J. A., Breeze, R. G. and Pirie, H. M. (1978). Chronic obstructive pulmonary disease (COPD): Identification of affected horses. *Equine vet. J.* **10**, 47-53.
- Nicholls, J. M. (1978). A pathological study of chronic pulmonary disease in the horse. PhD thesis, University of Glasgow.
- Pepys, J. (1969a). Hypersensitivity Diseases of the Lungs due to Fungi and Organic Dusts. p. 69. S. Karger, Basel (Switzerland).
- Pepys, J. (1969b). Hypersensitivity Diseases of the Lungs due to Fungi and Organic Dusts. pp 33 and 51. S. Karger, Basel (Switzerland).
- Rooney, J. R. (1970). Autopsy of the Horse. p. 117. Williams & Wilkins Co, Baltimore.
- Schatz, M., Patterson, R. and Fink, J. (1977). Immunopathogenesis of hypersensitivity pneumonitis. *J. Allergy and Clinical Immunology* **66**, 29.

RÉSUMÉ

Micropolyspora faeni et *Aspergillus fumigatus* ont été identifiés comme responsables d'une hypersensibilité respiratoire chez des chevaux atteints d'une maladie respiratoire obstructive chronique. Des pollens de graminées et un Actinomycète ont été tenus pour responsables d'allergies respiratoires chez quelques chevaux. Assez fréquemment des chevaux se révèlent individuellement hypersensibles à un ou deux, ou plusieurs antigènes au niveau de l'appareil respiratoire.

Les méthodes pour tester l'allergie furent l'injection intradermique et l'inhalation avec des antigènes trouvés dans l'environnement. Un test intradermique utilisant un extrait de *M. faeni* fut utilisé à des fins diagnostiques chez des chevaux atteints précédemment de maladie

respiratoire obstructive chronique. Par contre l'antigène préparé avec *Aspergillus fumigatus* ne fut pas satisfaisant. Les réactions cutanées aux extraits de *M. faeni* et *A. fumigatus* chez des chevaux atteints de maladie respiratoire obstructive chronique, montrèrent que l'hypersensibilité était double: d'abord une faible réponse suivant de près l'injection suivie par une réponse de type Arthus 4 à 8 heures plus tard.

La pression intrathoracique maxima se révéla être un indicateur satisfaisant de la réponse aux stimulations par inhalation. Au contraire les variations de la pression artérielle d'O₂ furent inexploitable.

ZUSAMMENFASSUNG

Micropolyspora faeni und *Aspergillus fumigatus* konnten als häufige Ursachen einer respiratorischen Hypersensibilität bei Pferden gefunden werden. Chronisch obstruktiven Lungenkrankheiten. Lolchpollen und ein Actinomycet verursachten eine respiratorische Allergie bei einigen Pferden. Recht häufig fand man bei individuellen Pferden auch eine respiratorische Ueberempfindlichkeit gegen zwei oder mehr Antigene.

Die Methoden zur Untersuchung der Allergie bestanden in intradermalen Tests und in der Inhalation von Umgebungsantigenen. Ein intradermaler Test mit *M. faeni*-Extrakt erwies sich als geeignet für die Diagnostik bei Pferden, die an chronisch obstruktiven Lungenkrankheiten litten. Das *A. fumigatus*-Antigen dagegen erwies sich nicht als zufriedenstellend. Die Hautreaktionen gegen *M. faeni* und *A. fumigatus*-Extrakte bei lungenleidenden Pferden, schienen darauf hinzuweisen, dass die Ueberempfindlichkeit eine Doppeltart ist: Eine schwache Reaktion kurz nach der Injektion war von einer Arthus-ähnlichen Reaktion 4 bis 8 Stunden später gefolgt.

Als geeigneter Parameter zur Ueberwachung der Reaktion auf Antigeninhalation, erwies sich die Messung der maximalen Schwankung des intratorakalen Druckes (max. Δ Ppl), während die Veränderungen des arteriellen Sauerstoffpartialdruckes sich als nicht geeignet herausstellten.

Accepted for publication 1.2.79

Chronic Obstructive Pulmonary Disease (COPD): Factors influencing the occurrence

E. A. McPHERSON, G. H. K. LAWSON, JILL R. MURPHY and JANET M. NICHOLSON
University of Edinburgh Veterinary Field Station, Easter Bush, Roslin, Midlothian EH25 9RG

R. G. BREEZE* and H. M. PIRIE

University of Glasgow Veterinary School, Department of Veterinary Pathology, Bearsden Road, Bearsden, Glasgow G61 1QH

SUMMARY

Breed, age, weight, type of work performed, seasonal onset, poor ventilation and exposure to moulds in the habitat were investigated in relation to the occurrence of chronic obstructive pulmonary disease (COPD).

COPD was most commonly detected in showjumping and hacking horses. The older a horse, the more likely it was to become affected although most were 6 to 10 years of age. Of the horses in this sample of the population, which was not a random one, thoroughbred horses were affected least and ponies most often. The high incidence in ponies was related to their more frequent exposure to poor quality fodder and bedding. Most horses are exposed to the hazard of moulds, but more affected horses were so exposed than those not affected with COPD. Poor ventilation of the stable increased the chance of a horse becoming affected. Sex, body weight and season of onset of coughing had no influence on the occurrence of the disease.

INTRODUCTION

McPHERSON *et al* (1978) identified 38 horses affected with chronic obstructive pulmonary disease (COPD) in a group of 100 horses mostly referred because of poor work performance and/or chronic coughing. Data concerning these horses and their habitat collected during that study are recorded and analysed.

MATERIALS AND METHODS

Animals

The same 100 horses described in our previous paper (McPherson *et al*, 1978) and classified as **Group A** (affected with COPD), **Group B** (possibly affected) and **Group C** (not affected) were used in this study.

The horses were referred chiefly because of poor work performance and/or chronic coughing and were therefore not a random sample. The classification of **Groups A, B** and **C** was by measurement of the partial pressure of arterial oxygen (PaO₂) and the maximum intrathoracic pressure changes (max Δ Ppl) the former being subnormal and the latter elevated in horses affected with COPD (**Group A**). The levels of these two parameters were normal in **Group C**. **Group B** horses did not fit into **Groups A** or **C**.

Collection of data

Details of breed, age, sex, weight and type of work performed were recorded when the horses were admitted. Information concerning the stables and the kind and quality of the hay and bedding was obtained by a combination of history taking and observation. Bedding or fodder that was visibly heavily contaminated by moulds, unusually dusty or showed marked evidence of dampness, or heating was considered to be of "poor quality". Assessment of fodder was only carried out on a subjective basis, as clearly it was not feasible to obtain a satisfactory retrospective sample of such material, even if the scientific basis for evaluating its quality were clear.

Statistical analysis

The data were assembled in multiple or 2 x 2 contingency tables as appropriate. Tests for significant differences were made by the chi-square technique using the Pirie and Hamden (1972) correction factor where appropriate.

RESULTS

Characteristics of the horses

Breed type. Each of the **Groups A, B** and **C** contained horses of three breed types. The affected **Group A** contained fewer Thoroughbreds but more ponies ($P < 0.05$) than the other groups.

* Present address: Washington State University, Department of Veterinary Microbiology and Pathology, Pullman, Washington 99164, USA.

TABLE I
INFLUENCE OF BREED TYPE, AGE AND WORK PERFORMED ON OCCURRENCE OF COPD IN HORSES

		Group A Affected COPD	Group B Probably not affected COPD	Group C Not affected COPD	Probability values of chi-square (P)
Breed	Thoroughbreds	7	11	16	<0.05
	Non-Thoroughbreds	16	9	13	
	Ponies	15	8	5	
Age	0—5 years	4	5	10	<0.05
	6—10 years	21	20	22	
	11—15 years	9	1	1	
	>15 years	4	2	1	
Work performed	Racing	3	6	13	<0.02
	Showjumping	14	7	5	
	Hunting/Eventing	6	9	11	
	Hacking	12	4	4	
	Child's pony	3	2	1	

Age. Animals from 2 years old upwards were found to be affected with COPD. The prevalence of the disease increased as horses aged.

Type of work performed. Fewer horses engaged in various forms of racing but more employed in showjumping were found to be affected ($P < 0.01$) than was the case with those engaged in other activities. Hacks also showed a higher prevalence ($P < 0.05$). When showjumpers and hacks, considered as a single class, were compared with other horses, the incidence of COPD was significantly higher in them ($P < 0.001$) (Table II).

To ascertain whether there was an association between showjumping/hacking and breed type, these were compared in Table III. A significantly higher proportion of the ponies and fewer Thoroughbreds were so employed ($P < 0.001$). A similar analysis revealed no association between age and breed type or age and type of work performed. These factors appeared to be independent in a chi-square test ($P > 0.05$), indicating that the difference attributable to breed type and type of work performed were independent of age.

TABLE II
INFLUENCE OF BREED TYPE, AGE AND TYPE OF WORK PERFORMED ON OCCURRENCE OF COPD IN HORSES

		A v. (B & C) Probability values of chi-square (P)
Breed	Thoroughbreds v. Ponies and Non-Thoroughbreds	<0.05
	Ponies v. Thoroughbreds and Non-Thoroughbreds	<0.05
	Non-Thoroughbreds v. Thoroughbreds and Ponies	>0.05
Age	0—10 years v. 11—15 years	<0.001
	0—5 years v. 6—10 years	<0.05
	6—10 years v. 11—15 years	<0.01
Work	Racing v. Other Types of Work	<0.01
	Showjumping v. Other Types of Work	<0.01
	Hunting/Eventing v. Other Types of Work	>0.05
	Hacking v. Other Types of Work	<0.05
	Child's pony v. Other Types of Work	>0.05
	Showjumping and Hacking v. Other Types of Work	<0.001

A = Group A (affected COPD)

(B & C) = Group B + Group C (not affected COPD)

TABLE III

ASSOCIATION BETWEEN BREED TYPE AND SHOWJUMPING/HACKING IN HORSES EXAMINED FOR COPD

	Thoroughbred	Non-Thoroughbred	Ponies
Showjumping/Hacking	7	20	19
Other work	27	19	8

$$\chi^2 = 15.733 \quad P < 0.001$$

Sex and body weight. There was no significant difference in the distribution of either the sexes or the body weights over the **Groups A, B and C** ($P > 0.05$).

Season of onset of coughing. There was no seasonal difference ($P > 0.05$) in the onset of coughing in the 79 horses in **Groups A, B and C** which had a cough.

Environmental conditions

Those factors on which data were collected are set out in Table IV. Keeping horses indoors when not at work did not, in itself, influence the incidence of COPD ($P > 0.05$) but poor ventilation of the stables did

shown to be associated with the incidence of COPD, and hay or straw moulds are known to be aetiological agents which induce the disease, exposure to poor quality hay and/or straw was examined in relation to breed type, age and type of work performed. The results for breed type shown in Table V indicate that ponies were exposed to moulds more often than other horses ($P < 0.05$). Horses of all ages and doing all kinds of work were not significantly differently exposed to the hazard of mould spores.

Since ponies were more often affected with COPD and were more frequently exposed to the hazard of

TABLE IV

COMPARISON OF ENVIRONMENTAL CONDITIONS OF HORSES EXAMINED FOR COPD

	Group A		Group B		Group C		Probability value of chi-square (P)
	Yes	No	Yes	No	Yes	No	
Indoors at all times	16	22	10	18	18	16	> 0.05
Ventilation inadequate	16	19	6	15	3	20	< 0.05
Straw bedding used	35	1	21	0	27	1	> 0.05
Hay fed	36	0	21	0	27	0	> 0.05
Mouldy straw used	25	11	11	10	20	7	> 0.05
Mouldy hay fed	29	7	14	7	17	10	> 0.05
Mouldy hay/straw used	34	2	15	6	22	5	< 0.05

Chi-square calculated in 3 x 2 contingency tables for each line in the table

($P < 0.05$). Poor ventilation was recorded for 46 per cent of the horses in **Group A**, but only for 20 per cent in the other two groups.

Contact with hay or straw of poor quality was significantly different in **Groups A, B and C** ($P < 0.05$). Of **Group A** horses, 94 per cent were exposed to this material compared with 77 per cent in **Groups B and C**.

Since breed type, age and type of work (showjumping or other types) apparently independently, have been

poor quality fodder, the ventilation of pony housing was compared with that for other horses (Table VI). There was no significant difference ($P > 0.05$).

DISCUSSION

Comparison with Gilmour and Jolly's (1974) population figures confirmed that our sample of 100 horses was not a random one so that care is needed in extrapolating for the population as a whole.

TABLE V

EXPOSURE TO MOULDY HAY/STRAW OF HORSES EXAMINED FOR COPD AND CLASSIFIED ACCORDING TO BREED

Exposed to mouldy hay/straw	Thoroughbred	Non-Thoroughbred	Ponies
Yes	19	27	25
No	6	7	0

$$\chi^2 = 6.645 \quad P < 0.05$$

TABLE VI
VENTILATION OF PREMISES HOUSING PONIES COMPARED WITH OTHER HORSES
EXAMINED FOR COPD

Ventilation	Ponies		Other horses		Probability value of chi-square (P)
	Adequate	Inadequate	Adequate	Inadequate	
All 79 horses	18	10	36	15	> 0.05
Group A only	7	9	12	7	> 0.05

Data available for only 79/100 horses

Horses and ponies of all breed types, ages, sexes, body weights and performing many different kinds of work were found to be suffering from COPD. The disease was more prevalent in ponies as a class than in other breed types.

Breed type, age and type of work performed influenced the occurrence of COPD but no association between them was detected. Fewer Thoroughbreds but more ponies than expected by chance were affected. Quite young animals were found to be affected but the prevalence increased progressively with age. Although most affected horses were in the 6 to 10 year age group this may be a reflection of the age grouping of the horse population as a whole. Showjumpers and hacks were the most frequent victims.

The prevalence of COPD in ponies may be related to their more frequent exposure to poor quality fodder and bedding. A larger proportion of the affected horses were exposed to poor quality fodder than of those not affected and a relationship exists between exposure to moulds from such material and the occurrence of COPD (McPherson *et al*, 1978). It was not surprising that poorly ventilated stabling was more commonly recorded in the history of horses affected with COPD. The absence of such a history in more than half of the affected horses suggests that we may not be aware of the proper ventilation requirements or that even in well-ventilated establishments animals exposed to poor quality fodder acquire the disease. This would be in keeping with our experience that clinically normal, susceptible horses kept out-of-doors can be induced to "heave" by feeding poor quality fodder. That poor ventilation worsens the situation is suggested by our observations in a large town stable where ventilation was very bad and over one-third of the horses were clinically affected and had serum precipitins to *Micropolyspora faeni* and/or *Aspergillus fumigatus*.

Thus the more frequent the exposure to moulds in fodder and bedding, especially if combined with inadequate ventilation of the stables, the more likely a horse is to develop COPD. The increase in the disease prevalence as horses age may be related to the summation of exposure to these factors. Pepys (1969a) remarks "that inhalation of increasing amounts of organic dust antigen by atopic subjects may be expected to produce disease in more subjects". Increased intensity of exposure produces more cases of acute onset while increased exposure over a long time would be expected to produce more cases of insidious onset if the parallel of respiratory allergies in man applies to horses. However, as large numbers of animals subjected to similar poor environmental conditions do not contract COPD,

it may be that some intrinsic factor, possibly related to the immunological reactivity of individuals, as Pepys (1969b) suggests for man, also plays a role.

It is interesting to compare the conclusions drawn from this work with the opinions expressed by the observant clinician Axe (1906). He suspected a hereditary predisposition, observed the disease more frequently in heavy or coarse-bred horses and in ponies and associated the exclusive use of damaged fodder with the aetiology of the disease, particularly in older animals.

ACKNOWLEDGEMENT

This work was supported by a grant from the Horserace Betting Levy Board.

REFERENCES

- Axe, J. W. (1906). The Horse and its Treatment in Health and Disease. Vol IV, p 12. Gresham Publishing Co, London.
- Gilmour, J. S. and Jolly, G. M. (1974). Some aspects of the epidemiology of equine grass sickness. *Vet. Rec.* **95**, 77-81.
- McPherson, E. A., Lawson, G. H. K., Murphy, J. R., Nicholson, J. M., Fraser, J. A., Breeze, R. G. and Pirie, H. M. (1978). Chronic obstructive pulmonary disease (COPD): Identification of affected horses. *Equine vet. J.* **10**, 47-53.
- Pepys, J. (1969a). Hypersensitivity Diseases of the Lungs due to Fungi and Organic Dusts, p 72. S. Karger, Basel (Switzerland).
- Pepys, J. (1969b). Hypersensitivity Diseases of the Lungs due to Fungi and Organic Dusts, p 2. S. Karger, Basel (Switzerland).
- Pirie, W. R. and Hamden, M. A. (1972). Some revised continuity corrections for discrete data. *Biometrics* **28**, 693-701.

RÉSUMÉ

La race, l'âge, le poids, le mode d'utilisation, l'appartenance saisonnière, le manque de ventilation et l'exposition à des moisissures ont été considérées pour leur rôle éventuel dans l'apparition de la maladie pulmonaire obstructive chronique.

On a constaté cette affection plus fréquemment chez les chevaux de concours hippique et chez les chevaux de selle. La fréquence d'installation est en relation avec l'âge de l'animal encore que pour la plupart, les chevaux apparaissent entre six et dix ans d'âge.

Parmi les chevaux de la population étudiée, il n'était point pris au hasard—les purs sangs paraissent être les moins touchés, et les poneys les plus souvent atteints. On pense que la plus grande fréquence rencontrée chez les poneys est en relation avec le fourrage de mauvaise qualité reçu et avec la qualité médiocre de litière.

La plupart des chevaux étaient exposés aux moisissures éventuelles mais les chevaux atteints étaient plus exposés que les chevaux indemnes. La mauvaise ventilation

locaux accroît les chances de maladie. Le sexe, le poids et le caractère saisonnier de la toux ont semblé n'avoir aucune influence sur l'apparition de la maladie.

ZUSAMMENFASSUNG

Rasse, Alter, Gewicht, Art der Arbeit, jahreszeitliche Schwankung des Auftretens, schlechte Belüftung und Exposition mit Pilzen in der Umgebung, wurden untersucht in Zusammenhang mit dem Auftreten von chronisch obstruktiven Lungenkrankheiten. Derartige Krankheiten wurden am häufigsten gefunden bei Spring- und Spazierpferden. Je älter das Pferd, desto wahrscheinlicher wird das Auftreten dieser Krankheiten,

obgleich die meisten Patienten 6 bis 10 jähig waren. Die untersuchte Population war keine zufällig ausgewählte; Vollblüter erkrankten am seltensten und Ponies am häufigsten. Die hohe Wahrscheinlichkeit des Auftretens bei Ponies liess sich in Zusammenhang bringen mit der häufigeren Exposition mit qualitativ schlechtem Futter und Streue. Die meisten Pferde sind pilzexponiert, aber lungenkranke Pferde häufiger als lungengesunde Tiere. Eine schlechte Stallbelüftung erhöhte die Möglichkeit einer Erkrankung. Das Geschlecht, das Körpergewicht und die Jahreszeit beeinflussten das Auftreten der Krankheit nicht.

Accepted for publication 1.2.79

The Presence of Precipitating Antibodies in the Sera of Horses with Chronic Obstructive Pulmonary Disease (COPD)

G. H. K. LAWSON, E. A. McPHERSON, J. R. MURPHY, J. M. NICHOLSON and P. WOODING
University of Edinburgh, Royal (Dick) School of Veterinary Studies, Veterinary Field Station, Easter Bush, Roslin, Midlothian EH25 9RG

R. G. BREEZE* and H. M. PIRIE

University of Glasgow Veterinary School, Department of Veterinary Pathology, Bearsden Road, Bearsden, Glasgow G61 1QH

SUMMARY

The sera of horses affected and not affected with chronic obstructive pulmonary disease (COPD) were examined for precipitins to *Micropolyspora faeni* and *Aspergillus fumigatus*. Precipitins to both antigens were not restricted to COPD cases but occurred more frequently in animals affected with COPD. Many animals without detectable precipitins responded clinically to inhalation challenge with these antigens.

INTRODUCTION

IN previous papers the authors have described the physiopathological changes that may be used diagnostically in horses affected with chronic obstructive pulmonary disease (COPD) and the response of these animals and others to exposure to inhalation of certain bacterial and fungal extracts (McPherson *et al*, 1978; McPherson *et al*, 1979). In man and cattle affected with extrinsic allergic alveolitis (Pepys *et al*, 1963; Wiseman, Selman, Dawson and Pirie, 1973) and in man with asthma with pulmonary eosinophilia (Longbottom and Pepys, 1964) precipitating antibodies are often found in the sera of affected individuals. The significance of such antibodies is sometimes disputed; however, although they may be confusing diagnostically there would seem to be agreement that they may be involved in the immunopathogenesis of the respective conditions (Pepys, 1969; Schatz, Patterson and Fink, 1977) and that they indicate exposure to the relevant antigen.

Horses with COPD are frequently hypersensitive to inhaled antigens derived from *Micropolyspora faeni*, *Aspergillus fumigatus* and possibly other organisms (McPherson *et al*, 1978). This paper is an account of the presence of precipitating antibody to these antigens found in those animals exposed by inhalation and also in certain other selected groups of horses.

MATERIALS AND METHODS

The strains of *M faeni* (1156) and *Thermoactinomyces vulgaris* (1150) were obtained from the Mycological Reference Laboratory, London School of Hygiene and Tropical Medicine. The three strains of *A fumigatus* employed were obtained as follows: 371/68 and 1330/72 from histologically confirmed cases of canine mycotic rhinitis and 294/71 from a case of bovine mycotic mammary granuloma. Antigens from all 3 strains reacted strongly in gel diffusion tests with the serum of the animal from which they had previously been isolated.

Preparation of antigens

M faeni. The method of preparing antigen was based on that described by the working party (Working Party, 1967). Stock cultures of *M faeni* maintained at 5°C were inoculated on to dextrose agar slopes which were incubated for 48 hours at 52°C. This culture was then used to seed 30 ml amounts of nutrient broth (Oxoid CM1) with 1 per cent dextrose in 8-ounce medical flats. Cultures were incubated for 3 or 6 weeks at 52°C.

The resultant growth was centrifuged and the deposit resuspended in distilled water. The cell suspension was sonicated in a 100-watt ultrasonic disintegrator (MSE Scientific Instruments, Crawley, Sussex) for 10 min at 8 microns amplitude. The sonicate was then held at +5°C for 48 hours before centrifuging to deposit the cell debris. The supernatant was precipitated with 2 volumes of acetone and a little sodium acetate and

* Present address: Washington State University, Department of Veterinary Microbiology and Pathology, Pullman, Washington 99164, USA.

allowed to stand at +5°C overnight. The precipitate was recovered by centrifugation and redissolved in the minimum quantity of normal saline and stored at -20°C. The supernatant from the 6-week broth culture was retained and 0.1 per cent w/v sodium azide added. The fluid was then dialysed against carbowax overnight to obtain a 10-fold reduction in volume. The dialysis tubing was tied off and the antigen dialysed against running tap water overnight. This antigen was stored at -20°C and referred to as concentrated broth extract.

A fumigatus. The method of preparing antigen was based on that described by Amos (1970). The selected strain of *A fumigatus* was grown on a Sabouraud's agar slope for 48 hours at 37°C, the culture was frozen and thawed and then inoculated into 8-ounce medical flats containing 30 ml Sabouraud's broth. The fungus was grown at bench temperature for 1 month, the fungal mat shaken to the bottom and sodium azide added to 0.2 per cent w/v. The culture was allowed to stand overnight before homogenising at maximum rpm for 2 min (MSE 77313, Crawley, Sussex). Two volumes of phosphate buffered saline (PBS, 0.1 M, pH 7.4) with 0.2 per cent sodium azide were added to the volume of bulked antigen and the suspension shaken daily for 1 hour for 14 days. The antigen was filtered through gauze and then centrifuged at 3,000 rpm for 30 min, the supernatant was dialysed against running tap water for 24 hours, concentrated approximately x 100 with carbowax at +5°C, the dialysis tube retied and the antigen solution once again dialysed against running tap water. The antigen was centrifuged to remove any remaining particulate matter and stored at -20°C.

T vulgaris. The method of preparing antigen was similar to that used with *M faeni*. Glucose broth cultures were incubated for 14 days at 42°C. The harvested mycelium was filtered, the growth resuspended in distilled water and homogenised (MSE 77313, Crawley, Sussex) for 10 min. Sonic disintegration, acetone precipitation and storage were as for *M faeni*.

Gel diffusion tests

Gel diffusion plates were prepared from borate buffered agar and citrate buffered agar (Proctor, 1967) using 20 ml agar in 87 mm diameter petri dishes. Wells were cut with the aid of a template. The central serum well was 14 mm in diameter and the 6 peripheral antigen wells were 5 mm in diameter and each was 8 mm from the central well. Sera were used undiluted and concentrated x 5, the latter being obtained by adding 2.5 ml serum to 0.4 g polyacrylamide gel (Lyophogel, Gelman Instrument Co). The central well held 0.5 ml of serum. Antigens were diluted with normal saline to contain 11-14 mg/ml on a dry matter basis and plates were incubated in a closed container for 7 days at 32°C. Representative tests carried out in borate buffered agar which developed precipitin lines were washed in 5 per cent sodium citrate for 45 min and the plates examined for persistence of the lines (Kaufman, 1976). Antigens were evaluated against control sera before use in tests.

Horses

The animals were divided into **Group A** (affected with COPD), **Group B** (possibly affected) and **Group C** (not affected) and were those described in a previous paper (McPherson *et al*, 1978). Sera from 4 additional groups

of animals were also examined. **Group A₂** were all COPD animals, **Group D** contained 10 animals affected with "contagious coughing", **Group F** comprised horses with no known respiratory dysfunction and **Group E** contained 18 animals with respiratory disorders and not showing the physiological defects of COPD. Many of the animals in this last group had laryngeal abnormalities.

The method of exposure of horses to aerosol challenge and the antigens utilised are described by McPherson *et al* (1979). Animals were exposed to approximately 12 mg of either *M faeni* or *A fumigatus* antigen administered as an aerosol by means of a face mask. Clinical observations were supported by measurements of intrathoracic pressure change, partial pressure of arterial oxygen and the changes of these values from resting values determined immediately before exposure.

RESULTS

Results of serological tests on admission (Table I)

Sera from 172 horses were examined on admission to the hospital for precipitins to *M faeni*, *A fumigatus* and *T vulgaris*. In the standard double gel diffusion test (SDGT), 12 horses were found with precipitins to *M faeni*; where replicate tests were carried out using concentrated sera (CDGT), a further 4 out of 123 animals examined in this manner were found to be positive. Only 1 animal showed precipitins to *T vulgaris* on admission, this being demonstrated using concentrated sera.

Many (10/15) of these reactions with *M faeni* acetone antigen in one or both tests consisted of a single precipitin line which was not always easily detected. The additional clarity of borate buffered agar made it easier to determine the presence of these lines. All representative tests done in citrate agar or washed in sodium citrate retained the lines observed in the borate buffered agar.

A few animals (4) did, however, have sera which developed well-defined multiple lines with one or other *M faeni* antigen. There was no evidence that these concentrated broth extracts were superior to conventional acetone precipitated antigen; indeed, the later detected precipitins in 5 animals in which precipitins were not detected using broth antigens, while broth antigens were detected 1 animal not found positive with acetone antigen.

Sera from the same horses tested for precipitins against *A fumigatus* showed 7 positive animals (7/172) in the SDGT, while an additional 5 animals were found positive in the 123 tested using the CDGT. Just as with *M faeni*, many (7/12) of the results were due to a single precipitin line and multiple lines were uncommon. In 5 cases, precipitins were present to all 3 strains of *A fumigatus*, but in 7, precipitins were only present against 1 or 2 strains. Precipitin lines were stable when carried out in citrate agar or upon washing with sodium citrate. Five animals out of the 172 showed precipitins to both *M faeni* and *A fumigatus*, 1 also possessed precipitins to *T vulgaris*.

Out of the 38 horses in the COPD **Group A**, 12 showed precipitins when first admitted (9 to *M faeni* and 5 to *A fumigatus*, 2 reacting with both antigens). Horses in **Groups B** and **C** together contained 1/50 and 2 animals with precipitins to *M faeni* and *A fumigatus* respectively. In **Groups D**, **E** and **F**, 44 animals were

TABLE I
PRESENCE OF PRECIPITINS IN SERA OF HORSES ON ADMISSION FOR EXAMINATION
OF RESPIRATORY FUNCTION

	Serum concentration†	Precipitins present to					
		<i>M faeni</i>		<i>A fumigatus</i>		<i>T vulgaris</i>	
All horses	1	12/172	6.9%	7/172	4.05%	0/172	0%
	5	16/123	13.0%	12/123	9.75%	1/123	0.81%
A (COPD)*	1	6/38	15.8%	2/38	5.25%	0/38	0%
	5	9/33	27.3%	5/33	15.4%	0/33	0%
A2 (COPD)	1	3/19	15.7%	2/19	10.5%	0/19	0%
	5	2/7	28.5%	2/7	28.5%	1/7	14.2%
B (COPD?)	1	0/24	0%	0/24	0%	0/24	0%
	5	0/22	0%	2/22	9.1%	0/22	0%
C (Normal)	1	1/26	3.85%	0/26	0%	0/26	0%
	5	1/23	4.35%	0/23	0%	0/23	0%
D (Contagious cough)	1	0/10	0%	0/10	0%	0/10	0%
	5	0/6	0%	0/6	0%	0/6	0%
E (Various respiratory—not COPD)	1	1/18	5.5%	1/18	5.5%	0/18	0%
	5	1/15	6.65%	1/15	6.65%	0/15	0%
F (Not respiratory—not COPD)	1	0/16	0%	1/16	6.25%	0/16	0%
	5	0/5	0%	1/5	20.0%	0/5	0%
B, C, D, E, F	1	2/94	2.13%	2/94	2.13%	0/94	0%
	5	2/71	2.8%	4/71	5.65%	0/71	0%

NB Numerators denote the number of animals with precipitins, denominators the numbers tested

* COPD horses in Group A relate specifically to the animals described in a previous paper (McPherson *et al*, 1979)

† 1 = unconcentrated serum 5 = serum concentrated x 5

examined and 3 were found to have precipitins, 1 to *M faeni* and 2 to *A fumigatus*.

Appearance of precipitins in response to respiratory challenge

Twenty-eight horses in **Group A** were exposed to specific antigen challenge, administered by mask using aerosol antigen. In 17 of these animals, precipitins had not been detected in sera tested prior to challenge; 8 horses developed precipitins subsequent to this provocation, 8 to *A fumigatus* and 1 to both *A fumigatus* and *M faeni*. The mean period between exposure and sampling for the demonstration of post-exposure precipitins was 11.7 and 10.8 days respectively for *A fumigatus* and *M faeni*; individual sera were found to have become positive 8 days after exposure.

These precipitins which developed post-exposure were present, with no exceptions, to more than 1 *A fumigatus* antigen and, indeed, in 4 out of the 7 cases were present to all 3 antigens used in the tests. In the 6 animals positive before exposure to *A fumigatus*, precipitin reactions increased in number or became present in unconcentrated sera which had previously been negative. In the 6 animals positive to *M faeni*, subsequent to challenge 4 either lost or showed fewer precipitin lines when compared with previous tests.

Taken overall, 18 of the 28 challenged **Group A** animals showed precipitins to one or other antigen either before and/or after exposure to inhalation challenge. These results are summarised in Table II.

Twelve **Group B** horses were exposed to respiratory challenge with both *M faeni* and *A fumigatus* and a further 4 animals challenged with one or other antigen.

TABLE II
PRESENCE OF PRECIPITINS IN 28 GROUP A HORSES
BEFORE AND AFTER RESPIRATORY CHALLENGE

Antigen	Precipitins present		
	Pre-challenge	Post-challenge	Total (pre- or post-challenge)
<i>M faeni</i>	8	6/27	9/28))18/28
<i>A fumigatus</i>	6	13/25	14/28)

Denominator = Number of animals challenged with each antigen

TABLE III
RELATIONSHIP OF CLINICAL RESPONSE FOLLOWING
INHALATION CHALLENGE TO PRESENCE OF
PRECIPITINS (*M faeni*)

		Precipitating antibody*	
		—	+
Clinical response to inhalation challenge	+	20	8
	—	18	2

$$\chi^2 = 2.440 \quad P > 0.05$$

* includes precipitins present on admission and post-exposure

Two animals only in **Group B** responded to challenge by producing precipitin lines, 1 against *M faeni* and the other to *A fumigatus*.

Relationship between clinical response to respiratory challenge and the presence of serum precipitins

In a comparison of the clinical response to respiratory challenge with *M faeni* and the presence of precipitins to this antigen, it was found that 20 of the 28 animals that responded to inhalation challenge did not have precipitins detectable by the methods employed and overall there was no statistical agreement between the tests ($P > 0.05$) (Table III). Eight animals with precipitins, however, responded to *M faeni* inhalation exposure; 2 animals could not, however, be provoked in this manner.

TABLE IV
RELATIONSHIP OF CLINICAL RESPONSE FOLLOWING
INHALATION CHALLENGE TO PRESENCE OF
PRECIPITINS (*A fumigatus*)

		Precipitating antibody*	
		—	+
Clinical response to inhalation challenge	+	5	5
	—	28	10

$$\chi^2 = 2.006 \quad P > 0.05$$

* includes precipitins present on admission and post-exposure

With *A fumigatus*, the situation is more confused and the relationship between the 2 tests is even less clear (Table IV). Five out of the 15 animals with precipitating antibody could be provoked by inhalation challenge and 5 out of the 33 animals without precipitating antibodies also responded to challenge.

DISCUSSION

In this study, horses with COPD more frequently had serum antibody to *M faeni* than horses not so affected. In the precipitin test, such antibodies were often only weakly demonstrated and sometimes the sera required concentration before they could be detected. The incidence of precipitins encountered in this work is considerably larger than that found by Schatzmann and Gerber (1972) and much more clearly indicates the

probability that *M faeni* acts as a major allergen involved in COPD in the horse. Clearly, such tests are so difficult to interpret diagnostically and many animals sensitive by inhalation challenge to *M faeni*, do not possess precipitating antibody demonstrable by the technique employed in this work and, as might be expected from studies in other species, individual animals may be exposed to antigen, develop antibodies and yet retain normal pulmonary functions.

The response of the horse to *A fumigatus* clearly differs from the response to *M faeni*; precipitating antibody is more readily detected when present, following exposure; the immunological response tends to follow an amnesic pattern and there is a poor clinical response to inhalation challenge in horses with antibodies. It seems likely that these differences reflect differences in the nature of the antigens and the behaviour of the micro-organisms following inhalation. Although it is tempting to discount the possible part played by *A fumigatus* in COPD, the positive responses to inhalation obtained with a number of animals and the increasing numbers of COPD-affected horses with precipitins against this fungus indicate that such a deduction could be erroneous. It may be that COPD-affected horses have precipitins to these fungi more frequently than non-affected animals either because pulmonary function is impaired, a parallel being present in man where, in some studies, 39 per cent of patients with pulmonary tuberculosis have precipitins to *A fumigatus* (Buechner *et al*, 1973) or because exposure to these antigens is greater.

ACKNOWLEDGEMENT

This work was made possible by a grant from the Horse Betting Levy Board.

REFERENCES

- Amos, W. M. G. (1970). The extraction of fungal antigens and their use in serological tests as an aid to the diagnosis of bronchial disorders. *J. med. Lab. Technol.* **27**, 18-32.
- Buechner, H. A., Seabury, J. H., Campbell, C. C., Georg, L. A., Kaufman, L. and Kaplan, W. (1973). The current status of serologic, immunologic and skin tests in the diagnosis of pulmonary mycoses. *Chest* **63**, 259-270.
- Kaufman, L. (1976). *Manual of Clinical Immunology*, pp. 381-382. Ed. by N. R. Rose and H. Friedman. American Society for Microbiology, Washington, DC.
- Longbottom, J. L. and Pepys, J. (1964). Pulmonary aspergillosis: diagnostic and immunological significance of antigens and C-substance in *Aspergillus fumigatus*. *J. Path. Bact.* **88**, 141-151.
- McPherson, E. A., Lawson, G. H. K., Murphy, J. R., Nicholas, J. M., Fraser, J. A., Breeze, R. G. and Pirie, H. M. (1977). Chronic obstructive pulmonary disease (COPD). Identification of affected horses. *Equine vet. J.* **10**, 47-53.
- McPherson, E. A., Lawson, G. H. K., Murphy, J. R., Nicholas, J. M., Breeze, R. G. and Pirie, H. M. (1979). Chronic obstructive pulmonary disease (COPD) in horses. Aetiological studies: responses to intradermal and inhalation antigen challenge. *Equine vet. J.* **11**, 137-144.
- Pepys, J. (1969). Hypersensitivity Diseases of the Lungs due to Fungi and Organic Dusts. *Monogr. Allergy* **4**. S. Karger, Basel (Switzerland).
- Pepys, J., Jenkins, P. A., Festenstein, G. N., Gregory, P. L., Lacey, M. E. and Skinner, F. A. (1963). Thermophilic *Actinomyces* as a source of farmer's lung antigen. *Lancet* **607-611**.
- Proctor, A. G. (1967). *Progress in Microbiological Techniques* pp 213-226. Ed by E. H. Collins. Butterworth, London.

- Schatz, M., Patterson, R. and Fink, J. (1977). Immunopathogenesis of hypersensitivity pneumonitis. *J. Allergy and Clin. Immunol.* **60**, 27-37.
- Schatzman, U. and Gerber, H. (1972). Studies on the aetiology of chronic lung disease of the horse. *Zentbl. VetMed* **19**, 83-101.
- Wiseman, A., Selman, I. E., Dawson, C. O. and Pirie, H. M. (1973). Bovine farmer's lung: a clinical syndrome in a herd of cattle. *Vet. Rec.* **93**, 410-417.
- Working Party (1967). The CFT in farmer's lung—a preliminary study. *Mon. Bull. Minist. Hlth.* **26**, 167-171.

RÉSUMÉ

Les serums de chevaux atteints de la maladie et ceux de chevaux indemnes ont été examinés pour y trouver des précipitines à *Micropolyspora faeni* et à *Aspergillus fumigatus*. La présence de précipitines n'était point limitée à la maladie respiratoire mais fut constatée plus

fréquemment chez les chevaux atteints de cette maladie. Beaucoup d'animaux chez lesquels les précipitines n'étaient point détectables réagirent cliniquement à l'inhalation d'antigènes.

ZUSAMMENFASSUNG

Die Seren von gesunden Pferden und diejenigen von Pferden mit chronisch obstruktiven Lungenkrankheiten wurden auf das Vorkommen von Praezipitinen gegen *Micropolyspora faeni* und *Aspergillus fumigatus* untersucht. Das Auftreten von Praezipitinen gegen beide Antigene erwies sich als nicht beschränkt auf die Fälle von Lungenkrankheiten, aber sie traten doch bei erkrankten Tieren häufiger auf. Viele Pferde ohne nachweisbare Praezipitine reagierten klinisch auf die Inhalation dieser Antigene.

Accepted for publication 4.4.79

Identification and Characterisation of the Major Antiproteases in Equine Serum and an Investigation of their role in the onset of Chronic Obstructive Pulmonary Disease (COPD)

A. G. MATTHEWS

Department of Veterinary Medicine, Royal (Dick) School of Veterinary Studies, Veterinary Field Station, Easter Bush, Roslin, Midlothian EH25 9RG

SUMMARY

Three major antiprotease components in equine serum were identified and characterised. These were the acidic prealbumin Pr, the homologue of human alpha-1 antitrypsin and 2 protease binding proteins, the acidic prealbumin Xc and alpha-2 macroglobulin, both capable of inhibiting the proteolytic activity of trypsin, but with only limited inhibitory effect on its esterolytic activity. The possible role of these serum antiproteases in the onset of chronic obstructive pulmonary disease (COPD), analogous to the hereditary dysproteinaemia of alpha-1 antitrypsin in man, was investigated. There was no evidence of a genetically determined variation in the protease binding proteins but an increased frequency of the electrophoretically slower Pr antitrypsin alleles was present in horses affected with COPD. However, because of both the mixed breeding of the animals investigated and the lack of correlation with low serum trypsin inhibitory capacity, measured by inhibition of DL-BAPA hydrolysis, the significance of this observation could not be critically assessed.

INTRODUCTION

TWO major protease inhibitory components have been demonstrated in equine serum, in the albumin and alpha-2 electrophoretic regions respectively (Nakamura and Wakeyama, 1961; Fossum, 1970; Erickson, 1975). The specific nature of the 2 antiprotease components of equine serum is at present unknown.

On both acidic starch gel electrophoresis (Fagerhol and Laurell, 1967) and on iso-electric focusing in the pH range 3-6 (Allen, Harley and Talamo, 1974), the major human serum antiprotease, alpha-1 antitrypsin, appears as a multi-allelic polymorphic protein system, the protease inhibitor (Pi) locus. Certain Pi phenotypes, particularly the homozygote of the electrophoretically slowest allele, result in a dysproteinaemia of serum alpha-1 antitrypsin. Such individuals show an increased susceptibility to early onset panacinar emphysema and chronic obstructive pulmonary disease (COPD) (Eriksson, 1964).

On acidic starch gel electrophoresis of equine serum, a number of protein systems have been described, designated prealbumin (Pr), Xc/Xd conglomerate, Xh and Xk in decreasing order of electrophoretic mobility (Braend, 1967). The genetics of the highly polymorphic Pr locus have been described by Braend (1970), Buis (1976) and Scott (1977). Ek (1977) demonstrated antitrypsin

activity associated with the more anodal protein bands of equine serum on acidic starch gel electrophoresis, attributing this activity to a single protein, the Pr locus.

This paper presents the results of a study undertaken to identify and characterise the major protease inhibitors of equine serum and to investigate their role as a possible intrinsic determinant of COPD in the horse (McPherson *et al*, 1978).

MATERIALS AND METHODS

Serum samples were obtained from 33 healthy Thoroughbred family groups and from 52 horses and ponies affected with COPD. The techniques used will be published in detail elsewhere. The methods were as follows.

Antiserum against the proteins anodal to the Xh protein on acidic starch gel electrophoresis was raised in experimental rabbits, using a method based upon that described by Martin, Vandeville, Martin and Ropartz (1974). The anti-anodal acidic prealbumin immunoglobulin fraction of the immune serum was extracted by ammonium sulphate precipitation (Stelos, 1967). Fibrinagar electrophoresis and immuno-electrophoresis was based upon the techniques of Heimburger and Schwick (1962) and Grabar and Williams (1953).

The antiprotease activity of the acidic prealbumin and the alpha-2 proteins on starch gels were determined using the chromogenic substrate staining technique described by Uriel and Berges (1968), using acetyl-DL-phenylalanine- β -naphyl ester as a trypsin substrate and by combining starch gel electrophoresis with fibrinagar plates according to the sandwich technique of Heimburger (1972). Immunofixation of the anodal acidic prealbumins Pr, Xc and Xd after electrophoresis in agarose (pH 8.6) was carried out using a modification of the techniques described by Johnson (1976) and by Ritchie and Smith (1976).

Gel filtration chromatography of serum samples was carried out on Sephadex G200 (Pharmacia, Uppsala, Sweden). Acidic starch gel electrophoresis of equine serum (pH 4.3–4.5) was carried out using a modification of the technique described by Fagerhol (1972). Isoelectric focusing of equine serum on polyacrylamide gel slabs, in the pH range 4–6, was carried out on an LKB 2117 Multiphor system, using a modification of the technique described by Karlsson, Davies, Ohman and Andersson (1973). Alkaline starch gel electrophoresis was carried out using modifications of the techniques described by Ashton (1958, 1960) and by Kristjansson and Hickman (1965).

Spectrophotometric determination of the serum trypsin inhibitory capacity of whole serum and the Sephadex G200 fractions was carried out as described by Troyer and Moskowitz (1968), using benzoyl-DL-arginine-P-nitroanilide (DL-BAPA) and casein as trypsin substrates.

RESULTS

There was a biphasic distribution of the antiprotease activity of equine serum about the alpha-2 and albumin electrophoretic zones (Fig 1a). The presence of 3 precipitin arcs on immuno-electrophoresis of equine serum against rabbit anti-horse anodal acidic prealbumin immunoglobulins (Fig 1b) indicated the presence of 3 antigenically distinct proteins anodal to the Xh protein on acidic starch gel electrophoresis. The most anodal precipitin arc corresponded to the albumin-zone antiprotease component.

Immunofixation of equine serum with rabbit anti-horse anodal acidic prealbumin serum demonstrated that the 3 most anodal acidic prealbumins migrate in the prealbumin/albumin, alpha-1 and alpha-2 zones respectively on agarose electrophoresis (pH 8.6).

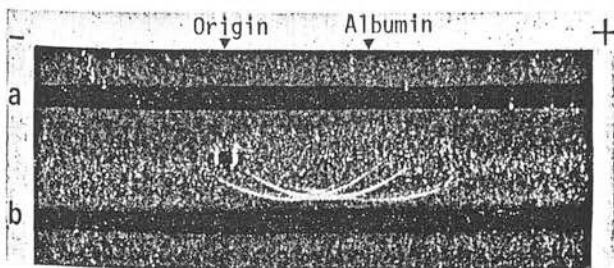


Fig 1. (a) Fibrinagar electrophoresis of pooled equine serum. The trough contains bovine pancreatic trypsin (320 μ g/ml in 0.0025 N HCl). Inhibition of the fibrinolytic activity of trypsin about the albumin and the alpha-2 regions is shown by inhibition of clearing of the fibrinagar gel.

(b) Immuno-electrophoresis of pooled equine serum against anti-horse anodal acidic prealbumin immunoglobulin.

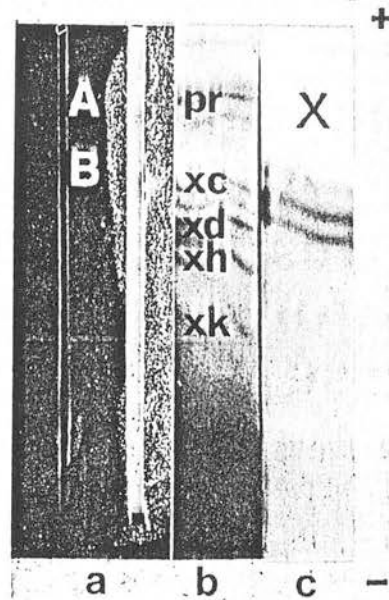


Fig 2. (a) Fibrinagar acidic starch gel sandwich technique. The right hand trough contains a section cut from the gel. The left hand trough contains bovine pancreatic trypsin (320 μ g/ml in 0.0025 N HCl).

(b) Acidic starch gel electrophoresis of equine serum of Pr phenotype LL. The acidic prealbumin nomenclature proposed by Braend (1967) is used.

(c) The same gel as (b) stained using the colorimetric substrate staining technique.

Using the fibrinagar-acidic starch gel sandwich technique, two peaks of inhibition of the fibrinolytic activity of trypsin (Fig 2a, A, B), indicated by inhibition of clearing of the fibrinagar, were detected in the more anodal prealbumin bands, corresponding to the Pr and Xc regions (Fig 2b).

Using the chromogenic substrate staining technique after acidic starch gel electrophoresis of equine serum (Fig 2c), inhibition of the esterolytic activity of trypsin, indicated by a zone of negative staining (X), was detected only in the most anodal group of bands, representing the Pr protein. The protein bands corresponding to the Xc protein stained positively against the diffusely stained background, indicative of only a limited inhibitory effect upon the esterolytic activity of trypsin. This staining was present without prior incubation of the gels with trypsin, indicating a natural esterase activity associated with the Xc antiprotease.

Fig 3 shows the distribution of the alpha-2 and albumin-zone antitrypsin components, determined by fibrinagar electrophoresis of the eluted fractions, after Sephadex G200 chromatography of equine serum. The alpha-2 antitrypsin activity was eluted with macroglobulins in the first (19S) peak and the leading edge of the second (7S) peak (Regions 1–2, fractions 9–13). The albumin zone of antitrypsin activity is eluted in the trailing edge of the second peak and in the third (4S) peak (Regions 3–4, fractions 15–19). There was some evidence of a minor, electrophoretically, distinct, interalpha-antitrypsin component eluted in the second peak (Region 2–3, fraction 14).

Immunofixation after agarose electrophoresis of the pooled alpha-2 and albumin-zone antitrypsin components

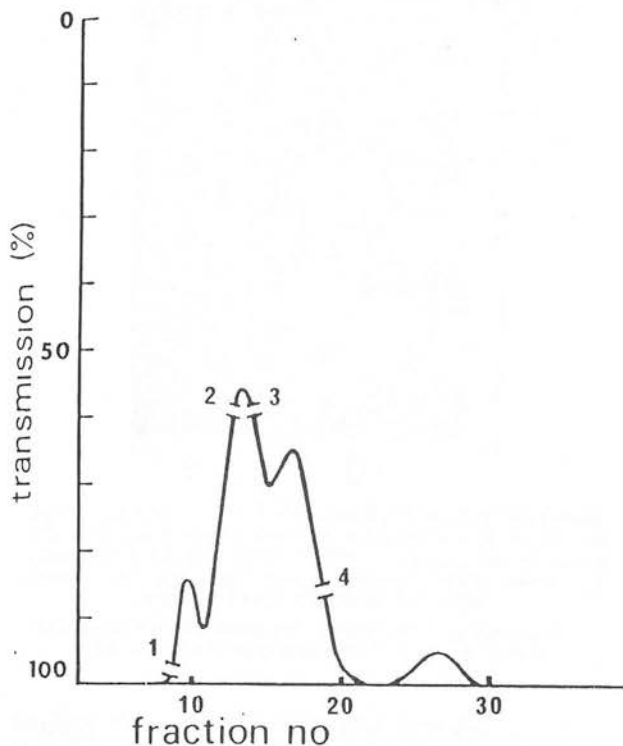


Fig 3. The elution pattern of pooled equine serum on Sephadex G200 gel filtration. Regions 1-2 and 3-4 are the major regions of antitrypsin activity.

after Sephadex G200 chromatography demonstrated that the Pr and Xc proteins are restricted to the albumin-zone component.

Alkaline starch gel electrophoresis, using a tris-citrate gel buffer, pH 7.6, of the Sephadex G200 fractions 10-16, showed the protein common to those fractions with alpha-2 antitrypsin activity (fractions 10-12) to be alpha-2 macroglobulin, appearing as a single, densely staining band. Fractions 14-16, with no alpha-2 antitrypsin activity, had no detectable alpha-2 macroglobulin (Fig 4).

Using a phosphate gel buffer, pH 7.35, the alpha-2 macroglobulin could be split into 2 components, both capable of inhibition of the fibrinolytic activity of trypsin shown using the fibrinagar starch gel sandwich technique (Fig 5). Using a tris-cacodylic acid gel buffer, pH 7.6-7.7, a third alpha-2 macroglobulin component, of intermediate electrophoretic mobility, could be detected. This component was found in some serum samples using the phosphate gel buffer technique.

Chromogenic substrate staining of the alkaline starch gel showed no inhibition of the esterolytic activity of trypsin by alpha-2 macroglobulin. However, natural esterase activity could be demonstrated in both electrophoretic components of alpha-2 macroglobulin.

The mean trypsin inhibitory capacity of whole serum and pooled Sephadex G200 fractions with alpha-2 antitrypsin activity is shown in Table I. There was a substrate-related variation in the trypsin inhibitory capacity, particularly marked in the alpha-2 antitrypsin component, casein, resulting in higher levels than DL-BAPA.

The alpha-2 antitrypsin fraction comprised 48 per cent of the total serum antitrypsin activity, measured by casein hydrolysis, but only 8.5 per cent measured by DL-BAPA esterolysis, the remaining antitrypsin activity being made up of the Pr and Xc proteins, the interalpha trypsin and, possibly, other as yet unidentified antiproteases.

The frequency of occurrence of the Pr alleles in the Thoroughbred, using iso-electric focusing and acidic starch gel electrophoresis, was similar to those previously described by Scott (1977). The Pr gene frequencies determined by iso-electric focusing are shown in Table II. Using iso-electric focusing in contrast to acidic starch gel electrophoresis, the bands corresponding to the Xc protein appeared markedly heterogeneous. There was no evidence that this iso-electric heterogeneity of the Xc protein in the Thoroughbred was the result of a genetically determined biochemical polymorphism.

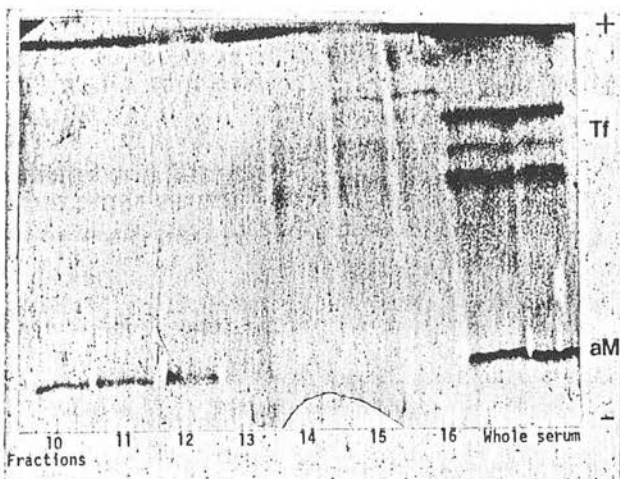


Fig 4. Alkaline starch gel electrophoresis, tris-citrate gel buffer, pH 7.6, of Sephadex G200 fractions 10-16 and of whole equine serum. aM = alpha-2 macroglobulin Tf = transferrin

TABLE I

MEAN TOTAL TRYPSIN INHIBITORY CAPACITY (mg/ml) OF WHOLE SERUM AND OF THE SEPHADEX G200 CHROMATOGRAPHY FRACTIONS WITH ALPHA-2 ANTITRYPSIN ACTIVITY

Substrate	Whole serum (n = 8)	G200 fractions containing Alpha-2 antitrypsin activity (n = 8)
Casein	1.75 ± 0.25	0.84 ± 0.16
DL-BAPA	1.28 ± 0.38	0.11 ± 0.07

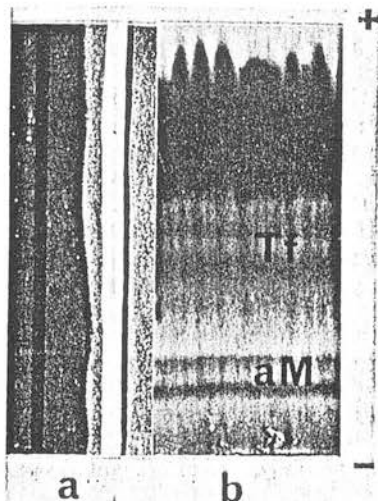


Fig 5. (a) Fibrinagar-alkaline starch gel sandwich technique. The right hand trough contains a section cut from the gel (phosphate gel buffer, pH 7.35) after electrophoresis of equine serum. The left hand trough contains bovine pancreatic trypsin (320 µg/ml in 0.0025 N HCl).

(b) A section of the same gel stained with 1% nigrosine.
aM = alpha-2 macroglobulin Tf = transferrin

Similarly, there was no evidence to show that the electrophoretic heterogeneity of alpha-2 macroglobulin in the Thoroughbred represents a genetically determined biochemical polymorphism.

Horses of mixed breeding affected with COPD showed no significant difference in the mean serum trypsin inhibitory capacity, measured by DL-BAPA

hydrolysis inhibition, compared to the healthy Thoroughbreds. However, some individual animals in the COPD-affected population had a serum trypsin inhibitory capacity below 2 standard deviations of the mean of the population as a whole. These low levels did not correspond to any one particular Pr phenotype.

The gene frequencies of the Pr alleles in the COPD-affected population as determined by iso-electric focusing are shown in Table II. The gene frequencies for a number of specific horse and pony breeds described in the literature are given for comparison. There was a tendency towards an increased frequency of the electrophoretically slower U and W alleles in the COPD-affected population.

DISCUSSION

The results of this study have established the existence of 3 functionally and immunologically distinct prealbumin proteins, migrating anodally to the Xh protein on acidic starch gel electrophoresis of equine serum. Braend (1967) described 3 protein zones in this region on acidic starch gel electrophoresis, but only the Pr protein has been definitively identified, due to its well-defined genetically polymorphic nature. The existence of 2 distinct proteins, Xc and Xd, between the Pr and Xh groups on acidic starch gel electrophoresis has not, until now, been established. In a study of the alpha-1 antitrypsin homologue of equine serum, Ek (1977) suggested that the extension of the trypsin inhibitory area cathodally from the Pr protein zone on acidic starch gel electrophoresis, shown here to be due to the Xc protein, might be the result of slower, as yet unrecognised, Pr alleles.

TABLE II

THE GENE FREQUENCIES OF THE Pr SYSTEM OF A NUMBER OF HORSE AND PONY BREEDS AND OF A MIXED POPULATION OF HORSES AFFECTED WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Breed Reference No in study	Thoroughbred ¹ Scott (1977) 1500	Arab ¹ Scott (1976) 70	Shetland Pony ¹ Buis (1976) 280	Dole Pony ¹ Braend (1970) 122	Trotter ¹ Braend (1970) 111	Thoroughbred ² Matthews 63	COPD Population ² Matthews 52
F	0.057	0.170	0.014	0.102	0.032	0.063	0.096
A	G	0.030	0.097	—	—	—	—
L	I	0.077	—	0.046	0.004	0.045	0.019
L	L	0.447	0.351	0.395	0.070	0.207	0.317
E	N	0.177	0.009	0.070	0.635	0.203	0.048
L	S	0.079	0.135	0.345	0.156	0.374	0.115
E	T	—	—	—	0.008	0.063	—
S	U	0.133	0.170	—	—	0.151	0.211
	W	—	—	0.130	0.025	0.077	0.192

¹ Determined using acidic starch gel electrophoresis

² Determined using iso-electric focusing (pH range 4—6)

The major antiprotease components of equine serum are shown to be the Pr and Xc acidic prealbumins and the alpha-2 macroglobulin. The mechanism of action of the Xc and alpha-2 macroglobulin antiproteases is different from the Pr component shown by the substrate-related variation in trypsin inhibitory activity. The alpha-2 macroglobulin and Xc protein appear to combine with trypsin to form an inhibitor-trypsin complex, able to inhibit the fibrinolytic activity of the enzyme yet still retain the ability to hydrolyse low molecular weight esters. In human serum, linkage of trypsin to the alpha-2 macroglobulin to form the trypsin-protein esterase complex (Haverback *et al*, 1962) similarly results in the loss of protease activity, but esterolytic activity is retained (Haverback, *et al* 1962; Ganrot, 1966; Troyer and Moskowitz, 1968; Barrett and Starkey, 1973).

In the dog (Ohlsson, 1971) and in the rabbit (Berthillier, Got and Bertagnolio, 1968), 2 trypsin binding alpha macroglobulins have been reported, both forming esterolytically active complexes with trypsin. However, a search of the literature has failed to reveal any report of a trypsin binding protein in any other species analogous to the Xc protein of equine serum. The multiple band form of the Xc protein of equine serum on iso-electric focusing between pH 4 and 6 could arise due to different degrees of saturation of the carrier protein with the protease enzyme, a mechanism which is recognised as giving rise to iso-electric heterogeneity of a particular protein (Vesterberg, 1973). The naturally occurring esterase activity of the Xc protein could arise due to steric inhibition of the proteolytic activity but not the esterolytic activity of the protein bound enzyme.

The Pr protein of equine serum, like the alpha-1 antitrypsin of human serum, combines with trypsin resulting in a loss of both proteolytic and esterolytic activity. Hence it is suggested that the present, non-descriptive terms Pr and Xc be dropped in favour of prealbumin protease inhibitor and alpha-1 protease binding protein respectively.

On alkaline starch gel electrophoresis of equine serum, 2 or 3 alpha-2 macroglobulin components could be demonstrated. In the absence of a genetically determined polymorphism, this multiple band form could represent varying degrees of saturation of the carrier protein. In man, electrophoretic heterogeneity of alpha-2 macroglobulin has been suggested to be due to the protease interaction mechanism and secondary conformational change of molecular structure (Saunders, Dyce, Vannier and Haverback, 1971; Barrett and Starkey, 1973). Natural esterase activity has been demonstrated in human alpha-2 macroglobulin preparations (Saunders *et al*, 1971).

Alpha-1 antitrypsin deficiency and its role in the onset of pulmonary disease in man has been reviewed by Kueppers and Black (1974), Lieberman (1976), Fagerhol (1976) and Heidelberger (1976). In the horse, Breeze, *et al* (1977) found no significant correlation between the mean serum trypsin inhibitory capacity, measured by DL-BAPA hydrolysis, the electrophoretically measured mean serum alphaglobulin component and the occurrence of COPD in a population of horses and ponies. Corbella, Ottonello and Ubaldi (1977) found an increase in the serum trypsin inhibitory capacity in 3 horses with acute pulmonary emphysema, although no increase in electrophoretically measured alpha-1

globulin was found. These authors assumed the serum protease homeostatic mechanism in the horse to be similar to that in man, although a basic difference in the relative importance of the albumin and alpha-2 components had been described in the literature (Nakamura and Wakeyama, 1961; Fossum, 1970).

This study details a number of major differences in the serum protease homeostatic mechanism in man and in the horse. Firstly, the relative magnitude of the alpha-2 macroglobulin component differs; in man it produces only 15 per cent of the serum antitrypsin activity. Secondly, there is no evidence of a trypsin binding protein in man analogous to the Xc protein in the horse. Thirdly, the distribution of the "alpha-1 antitrypsin" (Pr) alleles in the horse population is very wide compared to man, where the large majority of individuals are homozygous for the one allele. There is evidence of variation in the serum trypsin inhibitory capacity, measured, by DL-BAPA hydrolysis inhibition, with certain Pr phenotypes in normal horses, but this is not associated with the electrophoretically slower alleles (Matthews, unpublished data).

The apparent increased frequency of the electrophoretically slower Pr alleles in the COPD-affected population is difficult to assess critically, due to the very mixed genetic types in the population. Until a population of COPD cases of a single breed is available for the study, this tendency towards the slower allele phenotypes in the COPD-affected population, not correlated with abnormally low serum antiprotease activity, can remain no more than an interesting observation.

ACKNOWLEDGEMENTS

I wish to thank Drs P. Imlah and G. Blundell for their advice and use of facilities, Miss S. Thomson and Mr G. Keay for technical assistance and Mr R. Munro for photography. I also wish to thank Mr J. P. Thorne for help with the collection of serum samples.

REFERENCES

- Allen, R. C., Harley, R. A. and Talamo, R. C. (1974). A new method for determination of alpha-antitrypsin phenotypes. Use of iso-electric focusing on polyacrylamide gel slabs. *Am. J. Clin. Path.* 62, 732-739.
- Ashton, G. C. (1958). Serum protein variation in horses. *Nature* 182, 1029-1030.
- Ashton, G. C. (1960). Beta-globulin polymorphism and economic factors in dairy cattle. *J. agric. Sci* 54, 321-328.
- Barrett, A. J. and Starkey, P. M. (1973). Interaction of alpha-2 macroglobulin with proteases. Characteristics and specificity of the reaction and a hypothesis concerning its molecular mechanism. *Biochem. J.* 133, 709-724.
- Berthillier, G., Got, R. and Bertagnolio, G. (1968). Biochimie de l'alpha-1 macroglobuline de lapin. IV. Effet sur l'activité estérasiq ue de la trypsi ne et de la chymotrypsine. *Biochim. biophys. Acta* 170, 140-151.
- Braend, M. (1967). Variation of horse prealbumins in acidic starch gels. *Acta vet. scand.* 8, 193-194.
- Braend, M. (1970). Genetics of horse acidic prealbumins. *Genetics* 65, 495-503.
- Breeze, R. G., Nicholls, J. M., Veitch, J., Selman, I. E., McPherson, E. A. and Lawson, G. H. K. (1977). Serum antitrypsin activity in horses with chronic pulmonary disease. *Vet. Rec.* 101, 146-149.
- Buis, R. C. (1976). Genetic polymorphism of blood proteins in a population of Shetland ponies. H. Veenman and Zonen, B. V., Wageningen.
- Corbella, E., Ottonello, S. and Ubaldi, A. (1977). Serum antiproteases and respiratory diseases of the horse. *Folia vet. Lat.* 7, 1-15.

- Ek, N. (1977). Identification of the Pr albumins in horse serum. *Acta vet. scand.* 78, 458-470.
- Erickson, E. D. (1975). Alpha globulins of equine serum. *Proc. 1st Int. Symp. on Equ. Haematology.* pp 152-157.
- Eriksson, S. (1964). Pulmonary emphysema and alpha-1 antitrypsin deficiency. *Acta medica scand.* 175, 197-205.
- Fagerhol, M. K. (1972). Acid starch gel electrophoresis for detection of alpha-antitrypsin variants (Pi types): outline of techniques employed currently. In: *Pulmonary Emphysema and Proteolysis*, pp 145-149. Ed. Mittman, C. Academic Press, New York and London.
- Fagerhol, M. K. (1976). The genetics of alpha-1 antitrypsin and its implications. *Postgrad. med. J.* 52, Supplement 2, 73-79.
- Fagerhol, M. K. and Laurell, C. B. (1967). The polymorphism of "prealbumins" and alpha-1 antitrypsin in human sera. *Clin. Chim. Acta* 16, 199-203.
- Fossum, K. (1970). Proteolytic enzymes and biological inhibitors. II. Naturally occurring inhibitors in sera from different species and their effect on proteolytic enzymes of various origins. *Acta path. Microbiol. Scand., Sect. B* 78, 605-618.
- Ganrot, P. O. (1966). Alpha-2 antitrypsin activity and different trypsin substrates. *Clin. Chim. Acta* 13, 518-521.
- Grabar, P. and Williams, C. S. (1953). Methode permettant l'étude conjuguée des propriétés électrophorétique et immuno-chimiques d'un mélange de protéines. Application au serum sanguin. *Biochim. biophys. Acta* 10, 193-194.
- Haverback, B. J., Dyce, B., Bundy, H. T., Wertschaster, S. K. and Edmundson, H. A. (1962). Protein binding of pancreatic proteolytic enzymes. *J. Clin. Invest.* 41, 972-980.
- Heidelberger, K. L. (1976). Alpha-1 antitrypsin deficiency. A review 1963-1975. *Ann. Clin. Lab. Sci.* 6, 110-117.
- Heimburger, N. (1972). Proteinase inhibition in human serum: identification, concentration, chemical properties, enzymatic specificities. In: *Pulmonary Emphysema and Proteolysis*, pp 307-310. Ed. Mittman, C. Academic Press, New York and London.
- Heimburger, N. and Schwick, G. (1962). Die Fibrinagar-Elektrophorese. *Thromb. Diath. Haemorrh.* 7, 432-443.
- Johnson, A. M. (1976). Genetic typing of alpha-1 antitrypsin by immunofixation electrophoresis. Identification of subtypes of PiM. *J. Lab. Clin. Med.* 87, 152-163.
- Karlsson, C., Davies, H., Ohman, J. and Andersson, U. B. (1973). LKB (2117) Multiphor. I. Analytical thin layer gel electrofocusing in polyacrylamide gel. LKB Application Note 75 LKB Produktor A.B., Brommal, Sweden.
- Kristjansson, F. K. and Hickman, C. G. (1965). Subdivision of the allele TfD for transferrins in Holstein and Ayrshire cattle. *Genetics* 52, 627-630.
- Kueppers, G. and Black, L. F. (1974). Alpha-1 antitrypsin and its deficiency. *Am. Rev. Resp. Dis.* 110, 176-194.
- Leiberman, J. (1976). Alpha-1 antitrypsin deficiency and chronic pulmonary disease. *N. Y. St. J. Med.* 76, 181-186.
- Martin, J. P., Vandeville, D., Martin, C. and Ropartz, C. (1974). Identification des phenotypes du systeme Pi et comparaison des methodes de depistage des deficits en alpha-1 antitrypsine. *Ann. J. Biol. Clin.* 32, 197-207.
- Matthews, A. G. Unpublished data.
- McPherson, E. A., Lawson, G. H. K., Murphy, J. R., Nicholson, J. M., Fraser, J. A., Breeze, R. G. and Pirie, H. M. (1978). Chronic obstructive pulmonary disease (COPD): Identification of affected horses. *Equine vet. J.* 10, 47-53.
- Nakamura, S. and Wakeyama, T. (1961). Distribution of trypsin inhibitors in the sera of various animals. *Nature (London)* 192, 1077.
- Ohlsson, K. (1971). Comparison of affinity of trypsin for two alpha macroglobulin fractions and for alpha-1 antitrypsin in dog serum. *Clin. Chim. Acta* 32, 215-220.
- Ritchie, R. F. and Smith, R. (1976). Immunofixation. II. Application to typing of alpha-1 antitrypsin at acid pH. *Clin. Chem.* 22, 1735-1737.
- Saunders, R., Dyce, B. J., Vannier, W. E. and Haverback, B. J. (1971). The separation of alpha-2 macroglobulin into five components with differing electrophoretic and enzyme binding properties. *J. Clin. Invest.* 50, 2376-2383.
- Scott, A. M. (1976). Immunogenetic analysis as a means of identification in horses. In: *Proc. 4th Int. Conf. Equ. Infect. Dis.* Lyons, 1976. Ed. Bryans, J. T. and Gerber, H. Veterinary Publications Inc., New Jersey, pp 259-268.
- Scott, A. M. (1977). Prealbumins: the single most useful system in Thoroughbred horse blood typing. Abstract 15th International Conference Animal Blood Groups and Biochemical Polymorphisms, Dublin, 1976. *Anim. Blood Grps. Biochem. Genetics* 8, Supplement 1, p 19.
- Stelos, P. (1967). Isolation of immunoglobulin (Chapter 1): Salt fractionation. In: *Handbook of Experimental Immunology*, pp 1-9. Ed. Weir, D. M. Blackwell Scientific Publications, Oxford and Edinburgh.
- Troyer, H. and Moskowitz, R. W. (1968). Serum trypsin inhibitors: a comparison of assay methods using amide and casein substrates. *Enzym. Biol. Clin.* 9, 1-9.
- Uriel, J. and Berges, J. (1968). Characterisation of natural inhibitors of trypsin and chymotrypsin by electrophoresis in acrylamide agarose gels. *Nature (London)* 218, 578-580.
- Vesterberg, O. (1973). Separation and characterisation of proteins by iso-electric focusing, pp 81-98. VII Symposium on Chromatography and Electrophoresis, Brussels.

RÉSUMÉ

Trois antiproteases majeures ont été identifiées dans le serum du cheval, et caractérisées. Il s'agit de la Pr préalbumine acide, homologue de l'alpha-1 antitrypsine humaine, et de deux protéases, la préalbumine Xc acide et la macroglobuline alpha 2, toutes deux capables d'inhiber l'activité protéolytique de la trypsine, mais avec seulement un effet inhibiteur limité sur l'activité estérolytique de cette enzyme. Le rôle éventuel de ces antiproteases dans l'apparition de la maladie pulmonaire chronique obstructive, analogue à la dysprotéinémie héréditaire de l'antitrypsine alpha 1 chez l'Homme, a été recherché.

On n'a pas constaté une variation liée à des facteurs génétiques chez ces protéases à l'exception d'une fréquence accrue d'allèles correspondants à des antitrypsines Pr. Cependant, en raison de l'imprécision génétique des animaux étudiés et du manque de corrélation avec la capacité inhibitrice trypsique du serum, la signification de cette observation paraît difficile à exploiter.

ZUSAMMENFASSUNG

Drei wichtige Antiproteasen konnten im Pferdeserum identifiziert und charakterisiert werden. Es handelte sich um das saure Prealbumin Pr, ein Homolog des menschlichen alpha-1 Antitrypsins und um zwei Proteasebindenden-Proteine, das saure Praalbumin Xc und alpha-2 Macroglobulin, beide befähigt, die proteolytische Aktivität von Trypsin zu hemmen aber mit nur eingeschränkter, hemmender Wirkung auf dessen esterolytische Aktivität. Die mögliche Bedeutung dieser Serum-Antiproteasen für das Auftreten chronisch obstruktiver Lungenkrankheiten—analog der hereditären Dysproteinaemie von alpha-1 Antitrypsin beim Menschen—wurde untersucht. Es fanden sich keine Hinweise auf eine genetisch fixierte Variation bei den proteasebindenden Proteinen, aber die elektrophoretisch langsameren Pr Antitrypsinallele kamen bei Pferden mit chronisch obstruktiven Lungenleiden häufiger vor. Die Signifikanz dieser Beobachtung konnte indessen nicht kritisch beurteilt werden, weil die Pferde nicht aus einer Reinzucht stammten und weil sich keine Korrelation mit der tiefen Serumtrypsin-Hemmkapazität (gemessen durch die Hemmung der DL-BAPA Hydrolyse) ergeben hatte.

*Full With Compliments
Andy*

The nature of the prealbumin 'esterases' of horse serum

A. G. Matthews

Department of Veterinary Medicine, Royal (Dick) School of Veterinary Studies,
Veterinary Field Station, Easter Bush, Roslin, Midlothian EH25 9RG, Scotland

Received 20 March 1979; accepted 24 April 1979

Key-words: horse prealbumin, protease-inhibitory protein, esterase

Summary

Evidence is presented to suggest that the acidic prealbumin esterases in horse serum represent a protease-inhibitory protein. The esterase activity may arise from residual enzymic activity of the bound protease.

Introduction

On acidic starch gel electrophoresis two functionally and antigenically distinct protease-inhibitor protein systems have been demonstrated in the anodal prealbumin region of horse serum (Matthews, 1979), corresponding to the Pr and Xc prealbumin protein systems described by Braend (1967). The Pr protein interacts with trypsin to inhibit the total hydrolytic activity of the bound enzyme, and appears analogous to the alpha-1 antitrypsin of human serum. However, the Xc protein interacts with trypsin to inhibit the proteolytic, but not the esterolytic, activity of the enzyme (Matthews, 1979). Naturally occurring aliesterase activity has been demonstrated in the prealbumin region of horse serum on acidic starch gel electrophoresis at both pH 5.4 (Gahne, 1966) and pH 4.05 (Scott, 1970). This brief communication presents evidence to suggest that the Xc protease-inhibitory protein and the prealbumin esterase are one and the same, and proposes an explanation of the origin of the natural esterase activity.

Methods

Acidic starch gel electrophoresis was performed using a modification of the discontinuous horizontal system described by Fagherol (1972), the acidic component of the gel buffer being increased, giving a final pH of 4.3 - 4.5. Protein staining was carried out with 1 % nigrosine.

Inhibition of the proteolytic activity of trypsin by the acidic prealbumins of horse

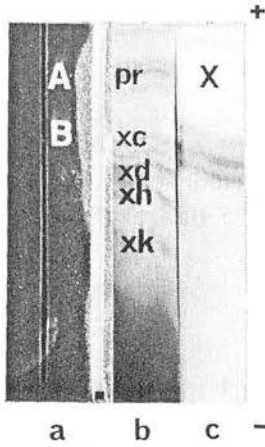


Fig. 1a. Fibrinagar-acidic starch gel sandwich technique. The left hand trough contains bovine pancreatic trypsin (320 μ g/ml in 0.0025 N HCl), the left hand trough contains a section cut from the starch gel parallel to the direction of the run. Inhibition of fibrinolysis is indicated by inhibition of clearing of the fibrin agar.

Fig. 1b. Acidic starch gel electrophoresis of horse serum of Pr type LL, stained with 1% nigrosine.

Fig. 1c. Acidic starch gel electrophoresis of the adjacent section of gel to 1b stained by the chromogenic, ester substrate technique of Uriel & Berges (1968).

serum after starch gel electrophoresis was demonstrated using a fibrin agar-starch gel sandwich technique after Heimbürger (1972).

Inhibition of the esterolytic activity of trypsin by the acidic prealbumins of horse serum after starch gel electrophoresis was demonstrated using the chromogenic, ester substrate, staining technique described by Uriel & Berges (1968) after prior incubation of the gel with trypsin. The same technique, without prior incubation of the gel with trypsin, was used to demonstrate naturally occurring esterase activity in the acidic prealbumin region.

Results

Fig. 1b shows the distribution of the acidic prealbumin protein zones after acidic starch gel electrophoresis. The nomenclature proposed by Braend (1967) is used.

Inhibition of the proteolytic activity of trypsin by the acidic prealbumins is demonstrated in Fig. 1a by inhibition of clearing of the fibrin agar by trypsin applied to the left hand trough. Two peaks of inhibitory activity are apparent in the anodal prealbumin region, labelled A and B, and correspond to the Pr and Xc proteins respectively.

Fig. 1c shows the adjacent section of the same gel as 1b stained with the chromogenic, ester substrate, staining method after incubation of the gel with trypsin. The gel background stains diffusely as a result of trypsin diffusion into the gel surface during the incubation process. However, a zone of negative staining, labelled X, indicating inhibition of the esterolytic activity of trypsin is apparent, and is restricted to the Pr protein region. Three distinct positively staining bands, indicating zones of intense esterolytic activity, appear cathodally to the region X and correspond to the Xc protein. When another section of the same gel is stained with chromogenic, ester substrate technique without prior incubation in trypsin, similar bands to those

observed
protein

Discussion

The inhibitory activity of such associated natural substances (e.g. a simple compound) have been observed at the alpha

Acknowledgements

I am indebted to Dr. Manfred Jourdain

References

Barre J. 193 Faghe (Pi) pro: Gahn: pre: Heiml

Anim.

observed in 1c are seen. These bands stain less intensely, but correspond to the Xc protease inhibitory protein and indicate a natural esterase activity.

Discussion

The evidence presented in this paper strongly suggests that the Xc protease-inhibitory protein and the acidic prealbumin esterase zones are one and the same thing. The Xc protein is shown to combine with trypsin to inhibit the proteolytic activity of the enzyme, but the bound trypsin retains a marked esterolytic activity. Such a mechanism would suggest that the naturally occurring esterase activity associated with the Xc protease-inhibitory protein may be the result of the steric nature of the inhibitor-enzyme binding mechanism. This prevents access of substrates of high molecular weight (e.g. proteins) to the active site of the bound endogenous enzymes (e.g. leucoproteases), although substrates of low molecular weight (e.g. esters) may still gain access to the site. Barrett & Starkey (1973) have proposed a similar mechanism to explain the residual esterase activity of the inhibitor-enzyme complex formed by human alpha-2 macroglobulin on interaction with proteolytic enzymes. Isolated human alpha-2 macroglobulin preparations have been shown to have naturally occurring esterase activity (Saunders et al., 1971; Szewczuk & Szczeklik, 1973).

On starch gel electrophoresis pH 8.5 the acidic prealbumin esterase activity appears in the post albumin region (Gahne, 1966), while on agarose electrophoresis at pH 8.6 the Xc protein has been shown, by immunofixation, to appear in the alpha-1 region (Matthews, 1979).

Acknowledgments

I am grateful to Dr P. Imlah for his advice and encouragement with this work, and to Dr G. H. K. Lawson and Mr E. A. McPherson for constructive criticism of the manuscript. The figure is reproduced by kind permission of the Equine Veterinary Journal.

References

- Barrett, A. J. & P. M. Starkey, 1973. Interaction of α_2 -macroglobulin with proteinases. *Biochem. J.* 133: 709-724.
- Braend, M., 1967. Variations of horse prealbumins on acidic starch gels. *Acta vet. scand.* 8: 193-194.
- Fagherol, M., 1972. Acid starch gel electrophoresis for detection of alpha-antitrypsin variants (Pi types). Techniques employed currently. In: C. Mittman (Ed.), Pulmonary emphysema and proteolysis. Academic Press, New York & London, pp. 145-149.
- Gahne, B., 1966. Studies on the inheritance of electrophoretic forms of transferrins, albumins, prealbumins and plasma esterase of horses. *Genetics* 54: 681-694.
- Heimbürger, N., 1972. Proteinase inhibition in human serum: identification, concentration, *Anim. Blood Grps biochem. Genet.* 10 (1979)

- chemical properties, enzymatic specificities. In: C. Mittman (Ed.), Pulmonary emphysema and proteolysis. Academic Press, New York & London, pp. 370-310.
- Matthews, A. G., 1979. Identification and characterisation of the major protease inhibitors in equine serum and a study of their role in the onset of chronic obstructive pulmonary disease (COPD). *Equine vet. J.* (in press).
- Saunders, R., B. J. Dyce, W. E. Vannier & B. J. Haverback, 1971. The separation of alpha-2 macroglobulin into five components with differing electrophoretic and enzyme binding properties. *J. clin. Invest.* 50: 2376-2383.
- Scott, A. M., 1972. Improved separation of polymorphic esterases in horses. *Proc. 12th Eur. Conf. Anim. Blood Grps biochem. Polymorphism* (Budapest) 551-553.
- Szewczuk, A. & A. Szczeklik, 1973. Amidase activity of human α macroglobulin. *Arch. Imm. Ther. Exp.* 21: 839-847.
- Uriel, J. & J. Berges, 1968. Characterisation of natural inhibitors of trypsin and chymotrypsin by electrophoresis in acrylamide agarose gels. *Nature* 218: 578-580.

Short Communication

THE EFFECTS OF SODIUM CROMOGLYCATE ON ANTIGEN INHALATION CHALLENGE IN TWO HORSES
AFFECTED WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

JILL R. MURPHY, E.A. McPHERSON and G.H.K. LAWSON

Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh
(Great Britain)

(Accepted 9 August 1979)

ABSTRACT

Murphy, J.R., McPherson, E.A. and Lawson, G.H.K., 1979. The effects of sodium cromoglycate on antigen inhalation challenge in two horses affected with chronic obstructive pulmonary disease (COPD). *Vet. Immunol. Immunopathol.*, 1: 89-95.

80 mg sodium cromoglycate (SCG) was administered by inhalation to two COPD-affected animals known to have respiratory hypersensitivity to Micropolyspora faeni. SCG treatment 20-30 minutes prior to inhalation challenge with M. faeni prevented exacerbation of respiratory disease, usually seen 4-8 hours after challenge. The duration of protection against antigen challenge after a single SCG treatment was 4-5 days. The duration of protection was not prolonged by reducing the frequency of antigen challenge. Multiple antigen challenge, using M. faeni and Aspergillus fumigatus, shortened the protective period of SCG to 3 days.

INTRODUCTION

Horses affected with COPD (heaves) show respiratory hypersensitivity to inhaled antigens, e.g. Micropolyspora faeni and Aspergillus fumigatus, in the form of a delayed response at 4-8 hours after challenge (McPherson et al., 1979). Inhaled sodium cromoglycate (SCG) is widely used in the prophylaxis of human bronchial asthma and it inhibits both immediate and delayed asthmatic reactions to inhaled allergens in allergic subjects (Altounyan, 1967; Pepys, 1968). It is believed to act by maintaining membrane stability, so inhibiting degranulation of sensitised pulmonary mast cells after exposure to the antigen and thus preventing release of the pharmacological mediators which cause constriction of smooth muscle in the airways.

The pathogenesis of COPD in the horse is not completely understood but airway spasm is known to be involved (Murphy et al., 1979). The role of mast cells in this disease has not been established, but mast cell hyperplasia has been noted in COPD-affected animals (Nicholls, 1978).

This paper describes the preliminary findings of prophylactic SCG administration to COPD-affected horses.

MATERIALS AND METHODS

Horses

Two animals, horse A, an 8-year-old hunter gelding, and horse B, a 14-year-old hunter mare, were diagnosed as being affected with COPD according to the criteria of McPherson et al. (1978). Horse A showed respiratory hypersensitivity to M. faeni and A. fumigatus, whilst horse B was hypersensitive to M. faeni only. To minimise exposure to organic dust antigens, the horses were bedded on peat moss and fed only on proprietary Horse and Pony Cubes (Spillers Ltd., Liverpool, U.K.).

Techniques

Treatment. Nebulised Sodium Cromoglycate B.P. 1% w/v solution (Fisons Ltd., Loughborough, U.K.) was administered via a mask which covered the horse's mouth and nose. 80 mg SCG was administered as a single dose.

Antigen inhalation challenge. M. faeni and A. fumigatus antigens were prepared according to the technique of Lawson et al. (1979). The challenge dose of both antigens was 12 mg suspended in 5 ml normal saline. Nebulised antigens were administered over a period of 20 minutes.

The horses' maximum change in intrathoracic pressure (max. Δ Ppl), arterial oxygen partial pressure (PaO_2) and clinical state were determined prior to antigen inhalation. Clinical observations were made hourly thereafter and monitoring of the max. Δ Ppl and PaO_2 were repeated 5 hours after inhalation. The following max. Δ Ppl values were taken as indicating a positive response; where pre-exposure value was <6 mmHg and increased after antigen challenge to >6 mmHg (the value delineating affected animals) or where pre-exposure max. Δ Ppl was already >6 mmHg, an increase of 15% in this figure was considered to indicate a positive reaction (McPherson et al., 1979). Max. Δ Ppl was measured using an intra-oesophageal balloon (McPherson et al., 1978) and PaO_2 was determined on a Corning pH/blood gas 161 analyser (Corning Medical, Halstead, Essex) from carotid samples.

To establish the mean response of each horse to antigen inhalation, horses A and B were challenged with M. faeni on three occasions and horse A was also challenged with A. fumigatus on three occasions prior to commencing trials.

Trials

The horses were in the remission stage of this disease (showing no signs of COPD, max. Δ Ppl <6 mmHg and $\text{PaO}_2 >82$ mmHg) at the start of all trials unless otherwise stated.

Trial 1: 80 mg SCG was administered 20-30 minutes prior to the M. faeni challenge of both horses on four occasions and the response monitored as previously described. Both horses were in the remission stage at the start of three

treatments and were symptomatic on the remaining occasion.

Trial 2: The duration of protection provided by a single SCG treatment in horses A and B against repeated M. faeni challenge was tested as follows:

- a) The horses were treated and challenged as in Trial 1 and thereafter were subject to daily antigen challenge until a positive response was recorded.
- b) Horses received SCG treatment on day 1 and were challenged on days 2 and 4.
- c) Antigen challenge was applied only at the end of the protective period as established in (a), on day 4 for horse A and days 4 and 5 for horse B.

Trial 3: The effects of multiple antigen challenge on the duration of protection provided by a single SCG treatment was tested in horse A, using M. faeni and A. fumigatus challenges on alternate days.

- a) SCG was administered on day 1 and the horse challenged with M. faeni. On day 2, the horse was challenged with A. fumigatus and on day 3 with M. faeni.
- b) The trial took the same form as (a) except that A. fumigatus was administered on days 1 and 3 and M. faeni on day 2.

RESULTS

The effect on the max. Δ Ppl and PaO_2 in horses A and B challenged by M. faeni inhalation and in horse A challenged by A. fumigatus is shown in Table I. The horses were not always in an identical disease state at the time of antigen challenge and this accounts for the wide range of max. Δ Ppl values for horse A before and after A. fumigatus challenge. There was a mean increase of 7 mmHg in max. Δ Ppl after antigen challenge, which constituted a good positive response being well in excess of the required 15% increase.

Changes in PaO_2 values were less consistent. Mean PaO_2 levels decreased after antigen challenge, but wide variation occurred. Both animals showed a double expiratory effort and increased harsh, inspiratory chest sounds at 5 hours after antigen challenge, in contrast with the absence of these signs before challenge.

Trial 1: SCG inhalation did not induce any clinical max. Δ Ppl or PaO_2 changes in either animal within 30 minutes of treatment, whether animals were symptomatic or in remission. Table II shows the mean max. Δ Ppl and PaO_2 values at rest (after SCG treatment and prior to antigen challenge) and 5 hours after antigen challenge, in the trials where animals were asymptomatic prior to challenge. In contrast to the findings in Table I, little change was recorded in max. Δ Ppl values in either horse, with the mean values at 5 hours after challenge being <6 mmHg. PaO_2 changes recorded after challenge were very slight. The horses were not dyspnoeic after challenge and there was no evidence of double expiratory effort or increased breathing sounds.

When symptomatic at the commencement of the trial, horse A showed no increase in max. Δ Ppl after antigen challenge and a PaO_2 decrease of 4.2 mmHg, whilst in

TABLE I

Maximum change in intrathoracic pressures (max. Δ Ppl) and arterial oxygen partial pressures (PaO_2). Mean values and standard deviation before and 5 hours after M. faeni and A. fumigatus inhalation challenge in two COPD-affected horses

Horse		A	A	B
Antigen		M. faeni	A. fumigatus	M. faeni
No. of exposures		3	3	3
Rest	max. Δ Ppl (mmHg)	6.83 \pm 2.84	8.00 \pm 4.50	4.67 \pm 1.16
	PaO_2 (mmHg)	82.93 \pm 2.15	77.70 \pm 4.29	84.87 \pm 5.26
5 hours post-challenge	max. Δ Ppl (mmHg)	13.67 \pm 2.30	16.00 \pm 4.27	12.50 \pm 2.00
	PaO_2 (mmHg)	76.80 \pm 4.25	74.47 \pm 9.17	80.23 \pm 2.15
\bar{d}	max. Δ Ppl (mmHg)	7.17 \pm 1.15	7.67 \pm 0.29	7.83 \pm 0.76
	PaO_2 (mmHg)	-6.13 \pm 5.64	-3.43 \pm 5.75	-4.63 \pm 4.71

\bar{d} = mean difference between pre- and post-challenge values

TABLE II

Maximum change in intrathoracic pressures (max. Δ Ppl) and arterial oxygen partial pressures (PaO_2). Mean values and standard deviation before and 5 hours after M. faeni inhalation challenge in two COPD-affected horses pre-treated with sodium cromoglycate (SCG)

Horse		A	B
No. of exposures		3	3
Rest	max. Δ Ppl (mmHg)	4.33 \pm 1.89	4.67 \pm 1.61
	PaO_2 (mmHg)	81.97 \pm 3.61	85.43 \pm 2.64
5 hours post-challenge	max. Δ Ppl (mmHg)	4.66 \pm 2.08	4.50 \pm 1.50
	PaO_2 (mmHg)	85.30 \pm 4.85	86.90 \pm 4.35
\bar{d}	max. Δ Ppl (mmHg)	0.33 \pm 0.29	3.33 \pm 5.12
	PaO_2 (mmHg)	-0.17 \pm 0.58	1.47 \pm 3.92

\bar{d} = mean difference between pre- and post-challenge values

horse B max. Δ Ppl increased from 6 mmHg to 7 mmHg with a PaO_2 increase of 3.2 mmHg.

Trial 2: Horses A and B, challenged daily with *M. faeni* antigen inhalation did not show a positive response until days 4 and 5 respectively after SCG treatment. Table III shows the daily pre- and post-challenge max. Δ Ppl and PaO_2 values. In horse A on day 5, the pre-exposure max. Δ Ppl value was elevated, PaO_2 depressed and the horse showing clinical signs of COPD. This was possibly a result of the positive response to challenge on day 4. This reaction was intensified by antigen challenge on day 5.

TABLE III

Maximum change in intrathoracic pressures (max. Δ Ppl) and arterial oxygen partial pressures (PaO_2) in two COPD-affected horses treated with sodium cromoglycate (SCG) followed by daily *M. faeni* inhalation challenge. Mean values and standard deviation before and 5 hours after *M. faeni* inhalation on days 1-3

Horse		A	B	
No. of exposures		5	5	
Rest	max. Δ Ppl (mmHg)	Days 1-3	3.67 \pm 1.55	3.50 \pm 0.5
		Day 4	3.5	3.5
		Day 5	9.0	4.0
	PaO_2 (mmHg)	Days 1-3	85.50 \pm 1.55	87.37 \pm 1.07
		Day 4	86.7	88.9
		Day 5	72.4	84.9
5 hours post-challenge	max. Δ Ppl (mmHg)	Days 1-3	3.83 \pm 0.58	3.16 \pm 0.29
		Day 4	10.0	4.5
		Day 5	14.5	9.5
	PaO_2 (mmHg)	Days 1-3	85.77 \pm 3.93	89.27 \pm 3.89
		Day 4	80.4	90.4
		Day 5	70.6	80.6

When the frequency of *M. faeni* inhalation challenge was reduced to days 2 and 4 after SCG treatment, both horses showed a positive response to challenge on day 4.

A single antigen challenge on day 4 after SCG treatment induced a positive response in horse A, but not in horse B. A second challenge on day 5 produced a positive response in horse B.

Trial 3: The duration of protection after SCG treatment in horse A was shorter in the face of multiple antigen challenge. In trials 3 (a) and (b) this animal showed an increase in max. Δ Ppl of 2 and 2.5 mmHg respectively on day 2; the post-challenge value, however, was <6 mmHg. On day 3, max. Δ Ppl increased by 5.5 and 7.5 mmHg respectively and showed clinical signs of COPD.

DISCUSSION

These antigen inhalation studies in two COPD-affected animals showed that the usual response to challenge can be prevented by prior treatment with SCG. This

protection was most efficient when horses were in the remission stage of the disease, but when horses were symptomatic SCG prevented the intensification of the disease after challenge. ^{A single} SCG inhalation in symptomatic animals did not produce any apparent clinical improvement, this being similar to the findings in man (Cox, 1969).

The findings showed that the duration of protection after a single SCG dose was much longer than in human asthmatics where SCG inhibits antigen-induced bronchoconstriction at 5 hours but not at 24 hours after treatment (Kolotkin et al., 1973). The reason for the prolonged protection in horses may be due to differences in the pathogenesis of these diseases or to differences in the cellular physiology in the horse.

The duration of SCG protection in the horse does not appear to be related to the frequency of challenge with a single antigen but multiple antigen challenge does shorten the protective period. The response is different in man, where protection occurs when the same antigen is used sequentially and not when a dissimilar antigen is introduced 5 hours after SCG treatment (Kolotkin et al., 1973).

Although these trials were performed on only two horses, they suggest that SCG may prove effective in controlling COPD in horses. A clinical trial to evaluate the efficacy of SCG on a larger number of affected horses is currently in progress.

ACKNOWLEDGEMENTS

Thanks are due to Miss P. Wooding of the Department of Veterinary Pathology for antigen preparation, the staff of the Department of Veterinary Medicine for technical assistance and Fisons Ltd. for the supply of materials and financial support.

REFERENCES

- Altounyan, R.E.C., 1967. Inhibition of experimental asthma by a new compound - disodium cromoglycate (Intal). *Acta Allergol.*, 22: 487-489.
- Cox, J.S.G., 1969. Review of chemistry, pharmacology, toxicity, metabolism, specific side-effects, anti-allergic properties in vitro and in vivo of disodium cromoglycate. In J. Pepys and A.W. Frankland (Eds.), *Disodium Cromoglycate in Allergic Airways Disease*. Butterworths, London, pp. 13-25.
- Cox, J.S.G., 1976. Disodium cromoglycate (Cromolyn Sodium) in bronchial asthma. In E.B. Weiss and M.S. Segal (Eds.), *Bronchial Asthma: Mechanisms and Therapeutics*. Little, Brown and Co., Boston, pp. 805-836.
- Kolotkin, B.M., Lee, C.K., and Townley, R.G., 1973. Duration and specificity of sodium cromolyn on allergen inhalation challenges in asthmatic patients. *J. Allergy Clin. Immunology*, 51: 87-88.
- Lawson, G.H.K., McPherson, E.A., Murphy, Jill R., Nicholson, Janet M. and Wooding, P. 1979. The presence of precipitating antibodies in the sera of horses with chronic obstructive pulmonary disease (COPD). *Equine vet. J.* (in press).
- McPherson, E.A., Lawson, G.H.K., Murphy, Jill R., Nicholson, Janet M., Fraser, J.A., Breeze, R.G. and Pirie, H.M., 1978. Chronic obstructive pulmonary disease (COPD): Identification of affected horses. *Equine vet. J.*, 10: 47-53.
- McPherson, E.A., Lawson, G.H.K., Murphy, Jill R., Nicholson, Janet M., Breeze, R.G. and Pirie, H.M., 1979. Chronic obstructive pulmonary disease (COPD) in horses: Aetiological studies: Responses to intradermal and inhalation antigenic challenge. *Equine vet. J.* (in press).

Isoelectric focusing of horse acidic prealbumins on thin-layer polyacrylamide gels

Andrew G. Matthews

Department of Veterinary Medicine, Royal (Dick) School of Veterinary Studies, Veterinary Field Station, Easter Bush, Roslin, Midlothian EH25 9RG, Scotland

Received 15 December 1978; accepted 23 August 1979

Key words: isoelectric focusing, horse, acidic prealbumins, Pr protein

Summary

The paper describes a technique of thin-layer polyacrylamide gel isoelectric focusing of horse serum, within a pH range of 4.0–6.0, which permits the improved resolution of the acidic prealbumin protein bands. The increased heterogeneity of the Pr prealbumin antiprotease allele products apparent using this technique is described and discussed in detail, and the potential use of the technique in routine Pr phenotyping is considered.

Introduction

On acidic starch gel electrophoresis of equine serum, a large number of prealbumin protein bands become apparent (Braend, 1967). The more anodal and cathodal bands are grouped into two genetically determined polymorphic protein systems, Pr and Xk (Braend, 1970). Between the Pr and Xk systems lie, in order of increasing electrophoretic mobility, a discrete group of bands designated Xh (Braend, 1967) and two antigenically and functionally distinct proteins, corresponding to the Xc/Xd group of bands suggested by Braend (1967) (Matthews, 1979). The Pr protein has been identified as the homologue of human alpha-1 antitrypsin and the 'Xc' protein as an alpha-1 protease-inhibitory protein (Matthews, 1979).

Difficulties encountered in the technique of acidic starch gels in relation to Pr phenotyping were mentioned by Scott (1976) and are reflected in the results of both the 1975 and 1977 horse blood typing comparison tests (Buis, 1976; Storset & Braend, personal communication). In man, isoelectric focusing on thin-layer polyacrylamide within a limited acidic pH range has superseded acidic starch gel electrophoresis in the study of genetic variants of the acidic prealbumin, alpha-1 antitrypsin (Allen et al., 1974). For these reasons, isoelectric focusing was used to

study the multiple molecular forms of the acidic prealbumins as part of an investigation into the possible role of serum antiproteases in the onset of chronic obstructive pulmonary disease in the horse. This paper details the isoelectric focusing technique used and describes and discusses the resulting isoelectric heterogeneity of the Pr protein.

Materials and methods

Serum samples were obtained from 33 Thoroughbred sire-dam-offspring groups and a number of horses and ponies of undetermined breeding. Serum was stored at -20°C for up to two years until used. All Thoroughbred serum samples had been Pr phenotyped by a modification of the discontinuous acidic starch gel electrophoretic technique described by Fagherol (1972). This modification involved the adjustment of the relative concentrations of the gel buffer stock solutions to give a final pH of 4.3–4.5. Thirty sera from the population of animals of undetermined breeding were Pr phenotyped by the horse blood typing laboratory in Oslo according to the technique of Braend (1970).

Thin-layer polyacrylamide gel electrofocusing in the pH range 4.0–6.0 was carried out using the LKB 2117 Multiphor system.

The gel frames (250 mm \times 125 mm) were made up of a thick glass plate (LKB 2117-105), a thin glass plate (LKB 2117-104), a rubber gasket (LKB 93 90 6010) and a second thick glass plate held together with bulldog clips. The gel contact surfaces were thoroughly cleaned with detergent, dried and wiped with ethanol before use.

Three gel stock solutions were used:

- *acrylamide*: 58.1 g acrylamide was dissolved in 200 ml distilled water, passed through a 0.22 μm -pore size filter and stored at 4°C ;
- *NN'-methylenebisacrylamide*: 1.8 g bisacrylamide was dissolved in 200 ml distilled water, passed through a 0.22 μm -pore size filter and stored at 4°C ;
- *riboflavine*: a saturated solution in distilled water was prepared and stored at 4°C .

The gel was prepared by mixing 13 ml acrylamide and 13 ml bisacrylamide with 30 ml of 25% (w/v) sucrose solution, to which 3.5 ml ampholine, pH 4.0–6.0 (LKB 1809-116), withdrawn under sterile conditions, was added. To this mixture 0.4 ml riboflavine solution was added as a polymerising agent.

The mixture was degassed by strong suction into a 50 ml plastic syringe through a 19-gauge needle, the needle was removed and the mixture poured into the frame. Care was taken to exclude air from the frame during pouring.

The gel was allowed to polymerise at room temperature under ultraviolet light. Polymerisation was usually completed within 2–3 hours, indicated by a change in refractive index at the periphery of the gel. The gels may be stored overnight at 4°C with no effect on their subsequent use.

After careful removal of the two thick glass plates, serum samples (approximately 5 μ l) on rectangular strips of LKB inserts (2117-106) were applied along the length of the gel, 1 cm from the cathodal wick, using a paper template as a guide. The anodal and cathodal wicks were applied to the gel after soaking in 1 M phosphoric acid and 1 M sodium hydroxide respectively, giving an electrode distance of 100 mm.

The gel on the thin glass plate was placed upon a layer of water laid over the cooling plate of the Multiphor. The focusing lid allowing voltage application across the breadth of the tank was fitted, ensuring good electrode contact with the wicks on the gel surface. After switching on the cooling system, the power pack was set to deliver a maximum of 1000 V and 30 W. The initial potential difference of 400 V increased to maximum during the run and the initial current of 30 mA fell as the pH gradient formed. Electrofocusing was completed after a 6-hour run.

The gels were stained for 30 minutes at 60 °C with Coomassie Brilliant Blue (Vesterberg, 1972). Destaining takes place overnight using an 8:3:1 water/ethanol/acetic acid solution. The gels may be mounted for prolonged storage in a glycerine/destaining solution (1:4) mixture.

The stained protein bands on the gels are best visualised with transmitted light.

Results

Thin-layer polyacrylamide gel electrofocusing of equine serum in the pH range 4.0 – 6.0 is a highly reproducible technique, permitting the resolution of a large number of acidic protein components of differing isoelectric points (pI). The separation of these components into distinct groups within the lower range of the pH gradient appears essentially similar to that of the prealbumin proteins after acidic starch gel electrophoresis (Fig. 2). The Pr and Xk proteins may be identified by their polymorphic nature. Between the Pr and Xk proteins, a number of bands may be appreciated. These appear distributed into three discrete groups (Fig. 2) similar to the distribution on acidic starch gels (Braend, 1967).

A 6-hour electrofocusing time resulted in optimal resolution of the proteins. By this technique, a 2-hour period as recommended for the electrofocusing of human alpha-1 antitrypsin variants resulted in incomplete isoelectric separation which was not comparable with acidic starch gel electrophoresis.

The Pr protein

With an effectively linear pH gradient, the pI of the Pr protein will lie approximately between pH 4.1 and 4.5.

On acidic starch gel electrophoresis, the Pr phenotypes are determined by a minimum of 10 codominant alleles, designated *F*, *G*, *I*, *L*, *N*, *S*, *T*, *U*, *W* and *Z* in order of decreasing electrophoretic mobility (Braend, 1970; Scott, 1976, 1977). By this electrofocusing technique, the appearance of the Pr phenotypes (Fig. 1) is

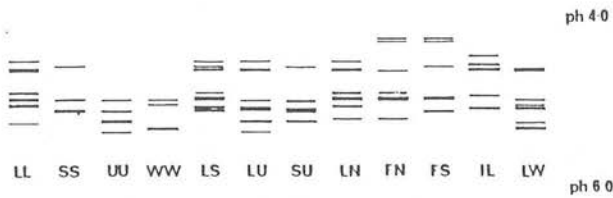


Fig. 1. Schematic appearance of some Pr phenotypes after isoelectric focusing within the pH range 4.0 - 6.0.

basically similar to that on starch gels. However, the number and resolution of the bands in the Pr system is increased, each allele controlling a multiple band pattern of one or two intensely stained bands, with a variable number of lightly stained bands.

After isoelectric focusing, the PrF allele products, recognized only in heterozygotes in this series of animals, appear as indistinct double bands near the lower pI limit of the protein, with a third band of pI indistinguishable from the major PrN allele product. These bands are shown in the FN (Fig. 2 Nos 9 and 10), FL (Fig. 3 No 5) and FS (Fig. 3 No 9) phenotypes. The PrI allele products, again recognized only in heterozygotes in this series, appear as two zones with pI distributed about that of the most acidic PrL allele products, with an additional band of pI slightly more acidic than the common PrN and PrF allele product. These bands are shown in the IL phenotype (Fig. 3 Nos 12 and 21). In cases where the PrI allele products are weakly expressed, the two more acidic zones may be difficult to visualise.

The PrL allele product shows the three bands characteristic of the homozygote on acidic starch gel electrophoresis (Braend, 1970). However, two additional bands of intermediate pI are apparent in the homozygote (Fig. 2 Nos 1 and 2), the pI of the least acidic of these two bands being indistinguishable from the apparently common PrN and PrF bands. In a number of PrL homozygotes, one or two additional bands are apparent, of lower pI than those already described (Fig. 2 Nos 15, 16 and 17). The more intensely staining of the more acidic PrL bands may, in some animals, be separated into two bands of close pI, as shown in an LL type in Fig. 3 No 3. The less acidic of these two 'sub' bands has a pI close to a band, considered to be a PrS allele product, shown in the adjacent LS phenotype (Fig. 3 No 2) and in an SS phenotype (Fig. 2 No 4). An LS type with only the more acidic of these 'sub' bands is shown in Fig. 3 No 13.

The PrS allele product appears heterogeneous. In addition to the acidic band already described in Fig. 3 No 2, the allele product appears to have a major band of varying pI. In some animals, the pI of this band is similar to that of the least acidic of the major PrL bands as seen in the SS type (Fig. 2 Nos 4 and 5), while in others the pI of the S band is distinctly less acidic pI than the PrL band, as seen in the SU (Fig. 2 Nos 13 and 14) and LS types (Fig. 3 Nos 2, 7, 8, 13 and 17). The

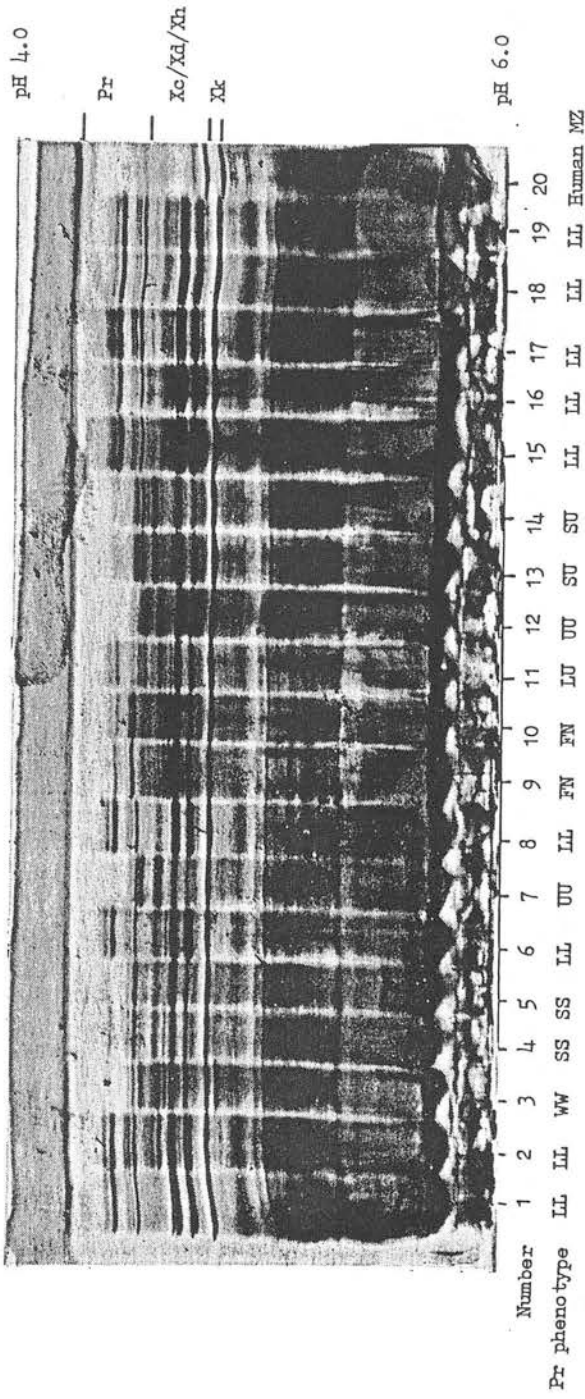


Fig. 2. Isoelectric focusing of horse serum on thin-layer polyacrylamide gels within the pH range 4.0 - 6.0. The presumed limits of the acidic prealbumins are shown and the Pr phenotypes of the individual samples given. A human MZ alpha-1 antitrypsin phenotype is shown for comparison (sample 20).

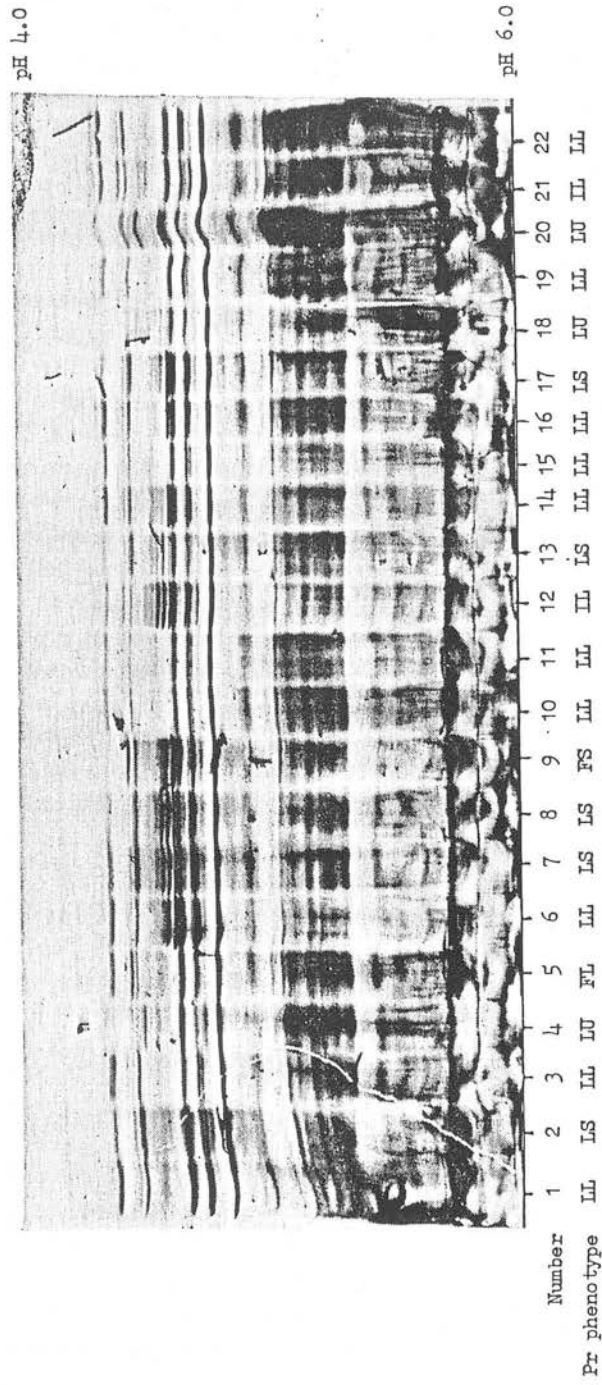


Fig. 3. Isoelectric focusing of horse serum on thin-layer polyacrylamide gels within the pH range 4.0 - 6.0.

PrS product has an additional band of pI similar to the apparently common PrF, PrN and PrL bands.

The PrU allele product (Fig. 2 Nos 7 and 12) appears as two distinct bands, the more acidic band having a pI similar to the major S band. In some cases, two additional bands of more and less acidic pI's respectively are apparent.

The protein bands considered to represent the PrW allele products are shown in Fig. 2 No 3 in a sample taken from a non-Thoroughbred animal.

Discussion

Isoelectric focusing provides a readily reproducible method of examining the Pr phenotypes of relatively large numbers of samples simultaneously. Although the highly polymorphic Pr locus is reported to be the single most effective system in the detection of falsely assigned parentage in the horse (Scott, 1976), only 7 of the 22 laboratories included in the 1977 horse blood comparison test (Storset & Braend, personal communication) reported Pr phenotypes. Amongst those 7 laboratories, however, there was only limited agreement as to the correct identity of the Pr allele products. Similar disagreement on Pr phenotypes was reported by Buis (1976) after the 1975 horse blood comparison test.

In comparison to acidic starch gel electrophoresis, this isoelectric focusing technique results in an increased number of bands in the Pr region. Recently, Ek (1979), using antigen-antibody crossed electrophoresis with monospecific anti-Pr antiserum, has identified a number of additional bands in the Pr system after starch gel electrophoresis. Some of these bands are weak or inapparent after routine glycoprotein staining of acidic starch gel, and some lie cathodally to the Pr bands originally described by Braend (1970). The results of Ek (1979) are compatible with the previously undescribed Pr bands apparent after isoelectric focusing.

Although the PrF allele products are easily identified after starch gel electrophoresis, after isoelectric focusing they are relatively inapparent, possibly due to the pI of the more acidic bands approaching the lower limit of the pH gradient. However, extension of the pH gradient towards pH 3.5 may correct this anomaly.

The bifid appearance of the more acidic of the major PrL bands may represent the genetically determined variation in the L zones alluded to by Braend and Storset (1979). The identification of this variant after isoelectric focusing would permit investigation of its possible genetic control. Due to their variable appearance, the additional bands of lower pI observed in only some PrL homozygotes may belong to a separate protein system or represent Pr isoelectric variants independent of the gene products. The heterogeneity of the PrS allele product may be due to two separate alleles, the more acidic variant of pI similar to the L band being the PrT allele product as described by Braend (1970).

The protein bands considered to represent the PrW allele product was not observed in any of the Thoroughbred horses in this series. It differs from the allele

product on starch gels, as described by Braend (1970), in that on isoelectric focusing the stronger of the two bands of more acidic pI lies much closer to the major band than on acidic starch gels. It is possible that these bands, here considered to be PrW allele products, may represent a subdivision of the PrU allele product as indicated by Braend & Storset (1979). Further investigation, using this technique, will be necessary to establish this.

Despite the complexities of the Pr phenotypes after isoelectric focusing, resulting from the multiplicity and part identity of the allele products, the technique could provide a standard method for routine Pr phenotyping and an accurate tool in the investigation of new variants at the locus.

Acknowledgments

The author extends his thanks to Dr P. Imlah, Dr Gillian Blundell and Dr M. Braend for their help and encouragement in this work, and to Dr D. L. Doxey and Mr E. A. McPherson for constructive criticism of the manuscript. Also, to Mr R. Munro for photography and Mr J. P. Thorne for help in the collection of serum samples. The work was carried out with the support of the Horserace Betting Levy Board and the Department of Clinical Chemistry, Western General Hospital, Edinburgh.

References

- Allen, R. C., R. A. Harley & R. C. Talamo, 1974. A new method for determination of alpha-1 AT phenotypes. Use of isoelectric focusing on polyacrylamide gel slabs. *Am. J. clin. Path.* 62: 732-739.
- Braend, M., 1967. Variation of horse prealbumins in acidic gel slabs. *Acta vet. scand.* 8: 193-194.
- Braend, M., 1970. Genetics of horse acidic prealbumins. *Genetics* 65: 495-503.
- Braend, M. & A. Storset, 1979. Blood polymorphisms of trotter horses in Norway. *Proc. 16th int. Conf. Anim. Blood Grps biochem. Polymorphisms* (Leningrad, 1977) (to be published).
- Buis, R. C., 1976. Horse Blood Typing Comparison Test 1975. *Anim. Blood Grps biochem. Genet* 7: 187-188.
- Ek, N., 1979. The characterization of equine prealbumin (Pr) proteins by antigen-antibody crossed electrophoresis. *Acta vet. scand.* 20: 100-190.
- Fagerhol, M. K., 1972. Acid starch gel electrophoresis for detection of alpha-antitrypsin variants (Pi types): outline of techniques employed currently. In: C. Mittman (Ed.), *Pulmonary emphysema and proteolysis*. Academic Press, London and New York, pp. 145-149.
- Matthews, A. G., 1979. The identification and characterization of the major antiproteases in equine serum and an investigation of their role in the onset of chronic obstructive pulmonary disease (COPD). *Equine vet. J.* (in press).
- Scott, A. M., 1976. Immunogenetic analysis as a means of identification in horses. *Proc. 4th int. Conf. equine Infectious Dis.* (Lyons, 1976). Veterinary Publishers, New Jersey, pp. 259-268.
- Scott, A. M., 1977. Prealbumins: the single most useful system in Thoroughbred horse blood typing. *Abstr. 15th int. Conf. Anim. Blood Grps biochem. Polymorphisms* (Dublin, 1976). *Anim. Blood Grps biochem. Genet.* 8 (Suppl. 1) 19. (Abstract.)
- Vesterberg, O., 1972. Isoelectric focusing in polyacrylamide gels. *Biochem. biophys. Acta* 275: 11-19.

Some blood pressure studies in normal horses
and in horses affected with chronic obstructive
pulmonary disease.

by

PADRAIC MARTIN DIXON

M.V.B. (National University of Ireland) M.R.C.V.S.

This thesis is presented for the degree of Doctor of
Philosophy of the University of Edinburgh 1979.

SUMMARY

The literature indicated that the main obstacle to indirect peripheral blood pressure measurements (sphygmomanometry) in horses, is the lack of a large superficial artery capable of being temporarily occluded. Consequently, the standard human sphygmomanometric techniques employing palpatory and auscultatory methods are unsatisfactory in horses. Some other sphygmomanometric techniques including the xylol bead modified palpatory, the photo-electric and the modified auscultatory methods were assessed by trials on horses. The latter method was shown to be the only potentially useful technique.

Blood pressure measurements using this technique showed that the blood pressure of resting horses shows continuous short term cyclic variations, an observation which was supported by direct peripheral blood pressure measurements. Peripheral blood pressure was shown to significantly increase in horses during excitement and also following submaximal exercise. During longer term studies, many technical difficulties were encountered with the modified auscultatory technique and it was concluded that it would be unlikely to become acceptable for general clinical use.

The literature concerning right heart blood pressure measurements in horses indicated that very little information was available concerning the right heart blood pressure alterations that occur in chronic pulmonary disease. Angiographic studies indicated that the use of a single hydrostatic

baseline for all right heart blood pressure measurements, as is currently used by all authors, causes an under-estimation of right ventricular pressure. A separate hydrostatic baseline was therefore established for right ventricular blood pressure measurements.

It was shown that horses clinically affected with chronic obstructive pulmonary disease (COPD), had pulmonary and systolic right ventricular hypertension and that this hypertension became reversed during remission stages of the disease. Further studies showed that a close relationship existed between carotid arterial hypoxaemia and pulmonary hypertension in COPD affected horses.

This relationship between arterial hypoxaemia and pulmonary hypertension in COPD was substantiated by inducing partial remission of pulmonary hypertension in clinically affected horses, by oxygen administration. In contrast, normal pulmonary hypertension was induced by rendering horses temporarily hypoxaemic, by administration of nitrogen enriched air.

Marked pulmonary hypertension was also induced during experimental hypercapnia or acidosis production. Bicarbonate, atropine or furosemide administered intravenously had no significant short term effects on pulmonary arterial pressure.

No clinical or cardiac catheterisation evidence of right heart failure was observed in any COPD affected horses. These observations were substantiated by the relative in-

frequency of right ventricular hypertrophy that was observed on post mortem examinations of horses affected with chronic pulmonary disease.

Chronic obstructive pulmonary disease (COPD): Effects of bronchodilator drugs on normal and affected horses

JILL R. MURPHY, E. A. McPHERSON and P. M. DIXON

University of Edinburgh Veterinary Field Station, Easter Bush, Roslin, Midlothian EH25 9RG

Summary

The effects of the bronchodilator drugs, atropine, isoprenaline and terbutaline, on normal horses and on horses affected with chronic obstructive pulmonary disease (COPD), were assessed by pulmonary function tests and clinical examination. Normal horses were not affected but COPD horses responded by a marked decrease in intrathoracic pressure, a decrease in respiratory rate, an initial decrease followed by an increase in arterial oxygen partial pressure and clinical improvement after treatment with all 3 drugs. These changes were temporary.

Introduction

CHRONIC obstructive pulmonary disease (COPD) in horses is associated with exposure to hay and straw containing moulds, including *Micropolyspora faeni* and *Aspergillus fumigatus* (McPherson *et al* 1979). The condition is usually reversed when affected horses are kept at grass or in a dust-free environment (Thurlbeck and Lowell 1964; Eyre 1972; Cook 1976; McPherson *et al* 1979). In the past, the disease was widely believed to be irreversible and was erroneously likened to alveolar emphysema in man, but recent studies have shown that, in fact, bronchiolitis is the major pathological feature of the disease, with emphysema being absent or confined to small areas of the lung (Nicholls 1978).

Additionally, a number of authors have postulated that bronchospasm also plays a role in the pathogenesis of COPD (Alegren and Carlström 1940; Obel and Schmitterlöw 1948; Cook and Rosedale 1963; Lowell 1964; Eyre 1972; Gerber 1973; McPherson and Lawson 1974; Cook 1976). Bronchodilator drugs, including the parasympatholytic drug, atropine, have been found to induce temporary relief of clinical dyspnoea (Obel and Schmitterlöw 1948; Lowell 1964; Muylle and Oyaert 1973; Sasse and Hajer 1977). Clinical improvement has also been reported in COPD affected horses with the use of sympathomimetic bronchodilators, including adrenaline, noradrenaline, scopolamine hydrobromide and NAB 365 (Boehringer Sohn, Ingleheim, BRD) (Obel and Schmitterlöw 1948; Schatzmann, Straub and Gerber 1972; Sasse and Hajer 1977; Corbella 1978). However, Corbella (1978) reported only slight and transitory clinical improvement following treatment of COPD affected animals with the sympathomimetic drugs, ephedrine, orciprenaline and salbutamol.

Many of these studies have been carried out on animals diagnosed as suffering from COPD on clinical grounds only and the response to treatment assessed by clinical obser-

vation alone. This paper describes some studies on the effect of 3 bronchodilator drugs, namely atropine (a parasympatholytic drug), isoprenaline and terbutaline (both sympathomimetics) on some pulmonary functions and clinical parameters in normal and COPD affected horses.

Materials and methods

Animals

COPD affected horses often coughed, were dyspnoeic, manifested a double expiratory effect and had harsh chest sounds, including wheezing and crepitant sounds in some cases (McPherson *et al* 1978). Additionally, all horses showed maximum intrathoracic pressure changes (max Δ Ppl) of ≥ 6 mm Hg and resting arterial oxygen partial pressures (PaO₂) of ≤ 82 mm Hg. Most of the 37 COPD affected horses were adult hunters and Thoroughbreds; a few were ponies and draught horses. The controls consisted of 20 horses of similar breeds which had no history of chronic respiratory illness and were normal on clinical and pulmonary function examinations.

Monitoring techniques

During all measurements the animals were standing, untranquillised and were handled quietly to prevent any excitement-induced respiratory or cardiac changes. Intrathoracic pressures including maximum expiratory, minimum inspiratory and maximum intrathoracic pressure changes (max Δ Ppl) were measured using an intra-oesophageal balloon (McPherson *et al* 1978). These values were estimated from the mean of 10 consecutive and representative respiratory tracings. Intrathoracic pressures were monitored at rest for 10 min to establish baseline values, for 30 min after drug administration and, thereafter at hourly intervals until the effects of the drug disappeared. Respiratory rates were measured from the intrathoracic pressure tracing.

Carotid arterial blood samples, for PaO₂ and carbon dioxide partial pressure (PaCO₂) respectively estimations, were collected using a previously described technique (McPherson *et al* 1978). Samples were obtained at rest and at 10, 20, 30, 60, 120 and 240 min after treatment and stored in iced water until analysed, within 1 hour after sampling. Blood gas estimations were carried out using a Corning pH/blood gas 161 analyser (Corning Medical, Halstead, Essex). The equipment was standardised according to manufacturer's specifications and the error for both PaO₂ and PaCO₂ estimations using this technique is reported to be less than 2 per cent.



Fig 1. Method of drug inhalation in the horse using a face mask and Wright's nebuliser.

Drugs and administration

Atropine sulphate (Macfarlane Smith Ltd, Edinburgh), isoprenaline sulphate (Thornton & Ross Ltd, Huddersfield) and terbutaline (Bricanyl respira tor solution, Astra Chemicals Ltd, Watford) were administered by inhalation. Dosage levels which would provide the maximum therapeutic effect without severe side effects were established by previous pilot experiments. For all drugs, a dose of 0.02 mg/kg was found suitable. For inhalation administration, drugs were dissolved with saline to a total volume of 4 ml, to standardise the time taken for drug nebulisation. The drugs were nebulised using 2 Wright's nebuliser pumps and were administered via plastic tubing to a face mask (Fig 1). Nebulisation took 7 min. Atropine sulphate (Bimeda UK Ltd, Liverpool) was also administered by intravenous injection over a 2 min period.

Statistical analysis of results

In normal and COPD affected horses, resting parameters were compared with parameters obtained at various intervals after drug administration by Student's *t* test as applied to paired observations. Max Δ Ppl and PaO₂ values of normal and COPD affected animals were compared at rest and at the time of peak response in COPD affected horses by Student's *t* test.

Results

Atropine by inhalation and intravenous injection was found to cause identical changes in all parameters except heart rate.

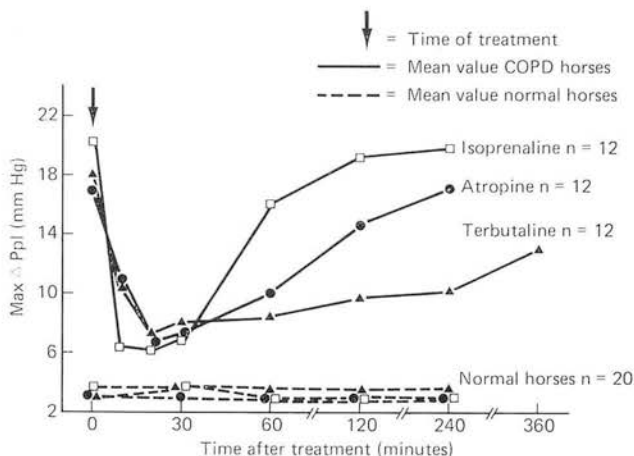


Fig 2. Effect of bronchodilator drugs on intrathoracic pressure in COPD affected and normal horses.

Intrathoracic pressure changes

Max Δ Ppl decreased significantly ($P < 0.001$) in COPD affected horses after administration of all 3 drugs. Max Δ Ppl was reduced by a mean of 72 per cent, 63 per cent and 68 per cent at the time of peak response to isoprenaline, terbutaline and atropine respectively, (Fig 2). The intrathoracic pressure decreases remained significant for 30 min following isoprenaline, 1 to 2 hours following atropine, and for 4 hours following terbutaline administration. Despite the large decreases in max Δ Ppl after all drugs, max Δ Ppl of COPD affected horses at times of maximum response still remained significantly different ($P < 0.01$) from that of the resting control horses. No significant max Δ Ppl changes were recorded in the normal animals.

Respiratory rate

Horses affected with COPD showed a decrease in respiratory rate after drug administration with the maximum response occurring 20 to 30 min after treatment (Fig 3). No significant changes in respiratory rate occurred in control animals.

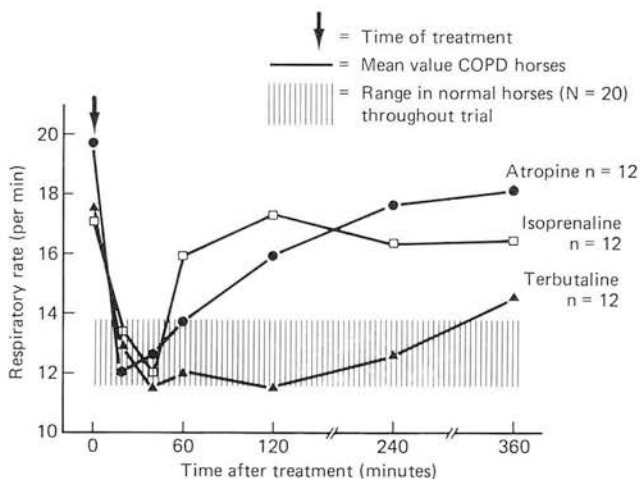


Fig 3. Effect of bronchodilator drugs on respiratory rate in COPD affected and normal horses.

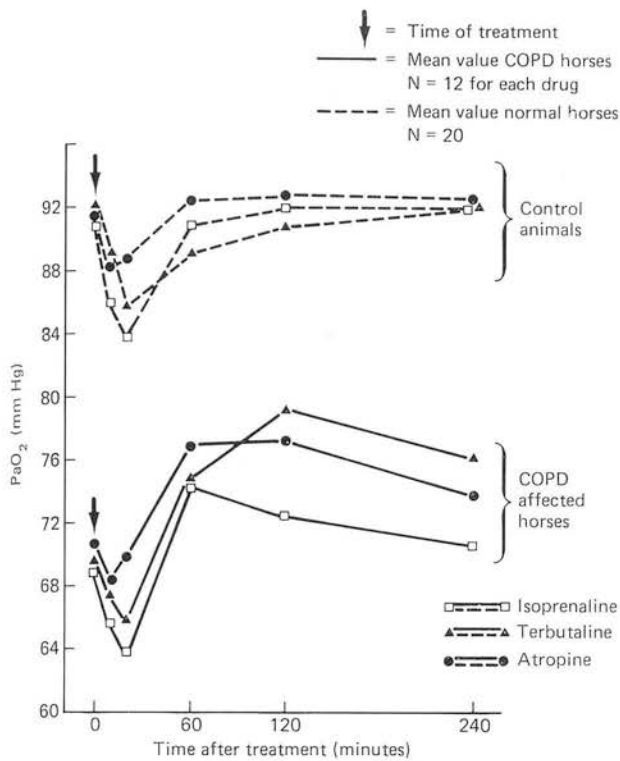


Fig 4. Effect of bronchodilator drugs on PaO_2 in normal and COPD affected horses.

Blood gases

The effects of the bronchodilator drugs on PaO_2 levels in COPD affected and normal horses are shown in Fig 4. All drugs caused a temporary drop in PaO_2 levels in both COPD affected and normal horses which was followed by a significant increase in PaO_2 to above resting levels in COPD affected horses (5, 9.5 and 6.5 mm Hg after isoprenaline, terbutaline, atropine by inhalation and atropine by intravenous injection, respectively). However, PaO_2 levels of COPD affected horses remained significantly lower than those of resting control horses, at all times after drug administration ($P < 0.01$). COPD affected horses showed a slight decrease (2-3 mm Hg) in $PaCO_2$ levels from 1 to 4 hours after treatment, corresponding to the times at which increased PaO_2 levels occurred. No significant $PaCO_2$ changes were recorded in the control animals.

Heart rate

Within 10 to 20 minutes of intravenous atropine and isoprenaline inhalation, the heart rate increased twofold in both COPD and normal horses. A slight transient increase in heart rate (\bar{x} 48/min) was recorded at 10 to 20 min after inhalation of atropine and terbutaline.

Clinical observations

After drug administration, COPD affected horses showed a temporary reduction in their expiratory effort, dyspnoea and flaring of the nostrils. All drugs alleviated wheezing chest sounds in affected animals. Clinical improvement persisted for 1 to 2 hours following isoprenaline and atropine and for 2 to 6 hours following terbutaline treatment. Atropine

administration caused mydriasis in all animals which persisted for 12 to 24 hours. The control animals showed no other clinical changes.

Discussion

Isoprenaline is a sympathomimetic drug which stimulates both β_1 (or cardiac receptors) and β_2 (or smooth muscle receptors) causing cardiac stimulation and bronchial muscle relaxation. Terbutaline is a sympathomimetic drug which exhibits selectivity for β_2 receptors, thereby causing bronchodilation with little or no cardiac stimulation. Isoprenaline has been used for many years for the treatment of human bronchial asthma, but recently its use has been superseded by the more selective β_2 stimulating agents (Formgren 1977).

The effects of isoprenaline were rapid in onset and marked but of short duration (ie, 1-2 hours) (Fig 2), because isoprenaline is rapidly metabolised by catechol-o-methyltransferase (COMT) (Hertting 1964). Terbutaline, in which the catechol nucleus of isoprenaline has been replaced by a resorcinol nucleus, is not a substrate for COMT and consequently, is longer acting. Although the decrease in max Δ Ppl with terbutaline was not as great as with isoprenaline, terbutaline's action was more prolonged with max Δ Ppl significantly decreased for 6 hours.

Atropine administration by either route produced a similar degree and duration of response but the peak response to inhalation occurred approximately 7 min later than after intravenous injection.

Intrathoracic pressure measurement was one of the main parameters used to assess pulmonary function in these experiments. Max Δ Ppl measurements have been shown to be increased in COPD affected horses (Gillespie, Tyler and Eberly 1966; Sasse 1971; Muylle and Oyaert 1973; McPherson *et al* 1978) indicating the presence of airway obstruction in this disease.

In these experiments max Δ Ppl was found to decrease greatly after all drugs. While this max Δ Ppl decrease could most obviously be attributed to a direct bronchodilating action, some other factors have also to be considered (ie, a marked decrease in respiratory rate was also recorded) which could have contributed to this max Δ Ppl decrease; it is also possible that a decrease in tidal volume could have caused a decrease in max Δ Ppl. However, Muylle and Oyaert (1973) failed to demonstrate any significant changes in tidal volume in COPD affected horses after intravenous atropine administration. Additionally, atropine could have decreased airway resistance by decreasing excess airway secretions. Because the secretions are not removed, but merely become more viscous in nature and because of the similar max Δ Ppl response in COPD affected animals to isoprenaline and terbutaline which do not have a drying effect on secretions, it is unlikely that this phenomenon contributed significantly to the decrease in max Δ Ppl after atropine administration.

After treatment with all drugs, the COPD affected horses showed a significant increase in PaO_2 levels and marked clinical improvement, corresponding with the time that the decrease in max Δ Ppl and respiratory rate occurred. It appears probable that this rapid improvement in pulmonary efficiency which was associated with a decrease in the work of respiration was, in fact, due to a functional decrease in airway resistance (ie, bronchodilation) induced by the drug administration. In contrast, no such response was observed in control horses and so these results suggest that bronchospasm is involved in the pathogenesis of equine COPD.

Furthermore, an increase in max Δ Ppl has been recorded in asymptomatic COPD affected animals within 2 hours of inhalation antigen challenge (Murphy and McPherson, personal observation) which suggests the involvement of a type I hypersensitivity reaction (bronchospasm) in this disease.

Despite the marked decrease in max Δ Ppl in COPD horses after bronchodilator administration, their max Δ Ppl and PaO₂ values still remained significantly different from those of the control horses indicating that their airway obstruction was not fully alleviated. This residual airway obstruction is undoubtedly due to the previously noted anatomical changes of COPD (ie, exudative bronchiolitis) (Nicholls 1978).

The effects of bronchodilator therapy on arterial blood gas tensions in horses affected with COPD have not been previously reported. The initial drop in PaO₂ levels in both normal and COPD animals occurred, without a simultaneous increase in PaCO₂ levels. De Moor (1968) also noted a transitory hypoxaemia in normal horses after intravenous atropine and hypoxaemia had been found to increase in human bronchial asthma patients after treatment with isoprenaline or atropine (Knudson and Constantine 1967; Field 1967; Ingram, Krumpke, Duffell and Maniscalco 1970; Chick, Nicholson and Johnson 1973). This fall in PaO₂ is generally thought to be caused by an intensification of the pre-existing ventilation-perfusion inequality, induced by these drugs (Field 1967; West 1976).

The dual action of isoprenaline on both β_1 and β_2 receptors is disadvantageous in that bronchodilation is usually accompanied by tachycardia as was observed by us in both normal and COPD affected horses. Although terbutaline exhibits a useful degree of selectivity for β_2 adrenoreceptors, the increased heart rates noted in our horses indicates that terbutaline even in clinical doses exerts significant β_1 receptor activity in the horse. During pilot experiments using terbutaline at doses of 0.04 mg/kg and above, increases in heart rate to 60/min occurred, accompanied by sweating and muscular tremor in many cases. Similar results were observed following administration of isoprenaline at higher dosage rates. The short duration of action of isoprenaline and its undesirable cardiac effects preclude its use as a therapeutic agent in the horse. Atropine, also, produces a tachycardia and causes increased viscosity of bronchial secretions, reduced bowel motility and mydriasis and so it is unsuitable as a long-term therapeutic agent. Terbutaline and other β_2 sympathomimetic drugs with their prolonged selective activity may prove to be of value in the symptomatic treatment of COPD in the horse, particularly if oral preparations prove effective.

Acknowledgements

This work was made possible by a grant from the Horse-race Betting Levy Board. Thanks are also due to the technical staff of the Department of Veterinary Medicine for their assistance.

References

- Alegren, A. and Carlström, B. (1940) Broken wind in horses. *Skand. Vet. Tidskr.* **30**, 10-25.
- Chick, T. W., Nicholson, D. P. and Johnson, R. L. (1973) Effects of isoproterenol on distribution of ventilation and perfusion in asthma. *Amer. Rev. Resp. Dis.* **107**, 869-873.
- Cook, W. R. (1976) Chronic bronchitis and alveolar emphysema in the horse. *Vet. Rec.* **99**, 448-451.
- Cook, W. R. and Rosedale, P. D. (1963) The syndrome of "broken wind" in the horse. *Proc. R. Soc. Med.* **56**, 972-977.
- Corbella, E. (1978) Clinico-therapeutic aspects of the use of beta-2-mimetic drugs in respiratory syndromes of the horse. *La Clinica Veterinaria* **101**, 343-366.
- Eyre, P. (1972) Equine pulmonary emphysema: A broncho-pulmonary mould allergy. *Vet. Rec.* **91**, 134-140.
- Field, G. B. (1967) The effects of posture, oxygen, isoproterenol and atropine on ventilation—perfusion relationships in the lung in asthma. *Clin. Sci.* **32**, 279-288.
- Formgren, H. (1977) Broncho- and cardioselective β -receptor active drugs in the treatment of asthmatic patients. *Scand. J. Resp. Dis. Supplementum No 97*. Copenhagen, Munksgaard.
- Gerber, H. (1973) Chronic pulmonary disease in the horse. *Equine vet. J.* **5**, 37-44.
- Gillespie, J. R., Tyler, W. S. and Eberly, V. E. (1966) Pulmonary ventilation and resistance in emphysematous and control horses. *J. Appl. Physiology* **21**, 416-422.
- Hertting, G. (1964) The fate of ³H-iso-proterenol in the rat. *Biochem. Pharmacol.* **13**, 1119-1128.
- Ingram, R. H., Krumpke, P. E., Duffell, D. M. and Maniscalco, B. (1970) Ventilation—perfusion changes after aerosolized isoproterenol in asthma. *Amer. Rev. Resp. Dis.* **101**, 364-370.
- Knudson, R. J. and Constantine, H. P. (1967) An effect of isoproterenol on ventilation—perfusion in asthmatic versus normal subjects. *J. Appl. Physiol.* **22**, 402-406.
- Lowell, F. C. (1964) Observations on "heaves". An asthma-like syndrome in the horse. *J. Allergy* **35**, 322-330.
- Moor, A. De (1968) Influence of atropine on arterial oxygen tension, acid-base status and tissue hypoxia in the conscious horse. *Zbl. VetMed. R.A.* **15**, 494-498.
- McPherson, E. A. and Lawson, G. H. K. (1974) Some aspects of chronic pulmonary diseases of horses and methods used in their investigation. *Equine vet. J.* **6**, 1-6.
- McPherson, E. A., Lawson, G. H. K., Murphy, J. R., Nicholson, J. M., Fraser, J. A., Breeze, R. G. and Pirie, H. M. (1978) Chronic obstructive pulmonary disease (COPD): Identification of affected horses. *Equine vet. J.* **10**, 47-53.
- McPherson, E. A., Lawson, G. H. K., Murphy, J. R., Nicholson, J. M., Breeze, R. G. and Pirie, H. M. (1979) Chronic obstructive pulmonary disease (COPD) in horses: Aetiological studies: Responses to intradermal and inhalation antigen challenge. *Equine vet. J.* **11**, 159-166.
- Muyllé, E. and Oyaert, W. (1973) Lung function tests in obstructive pulmonary disease in horses. *Equine vet. J.* **5**, 37-44.
- Nicholls, J. M. (1978) A pathological study of chronic pulmonary disease in the horse. PhD Thesis, University of Glasgow.
- Obel, N. J. and Schmitterlöw, C. G. (1948) The action of histamine and other drugs on the bronchial tone in horses suffering from alveolar emphysema (heaves). *Acta. Pharmacol.* **4**, 71-80.
- Sasse, H. H. L. (1971) Some pulmonary function tests in horses. PhD Thesis, University of Utrecht.
- Sasse, H. H. L. and Hajer, R. (1977) Veterinary and clinical experience of the use of a beta-2-receptor-stimulating sympathomimetic agent (NAB 365[®]) in horses with respiratory diseases. *Tijdschr. Diergeneesk.* **102**, 1237-1238.
- Schatzmann, U., Straub, R. and Gerber, H. (1972) Aspiration of bronchial secretions in the horse. *Schweiz. Arch. Tierheilk.* **114**, 395-403.
- Thurlbeck, W. M. and Lowell, F. C. (1964) Heaves in horses. *Amer. Rev. Resp. Dis.* **89**, 82-88.
- West, J. B. (1976) Ventilation—perfusion and diffusion disturbances in asthma. In *Bronchial Asthma: Mechanisms and Therapeutics*, pp 295-316. E. B. Weiss and M. S. Segal (Edts) Little Brown & Co, Boston.

Résumé

Les effets d'agents bronchodilatateurs tels que l'atropine, l'isoprenaline et la terbutaline sur des chevaux normaux et sur des chevaux atteints de maladie pulmonaire chronique obstructive, ont été appréciés par des examens cliniques et par des tests. Les chevaux normaux n'en parurent point affectés, mais les malades témoignèrent d'une pression intrathoracique diminuée, d'un ralentissement du rythme respiratoire et d'une variation en moins, d'abord, ensuite augmentée de la pression artérielle d'oxygène. Il en résulta pour une amélioration clinique suivant l'emploi des trois médicaments.

Zusammenfassung

Die Wirkung verschiedener Bronchodilatoren (Atropin, Isoprenalin und Terbutalin) auf normale Pferde und auf Tiere mit chronisch-obstruktiven Lungenleiden (COPD) wurde mit Lungenfunktionsprüfungen und durch klinische Untersuchungen beurteilt. Gesunde Pferde zeigten keine Veränderungen, aber COPD-Tiere beantworteten die Medikation mit einer Herabsetzung des intrathorakalen Drucks, der Atemfrequenz und des arteriellen Sauerstoffpartialdrucks, wobei der letztgenannte Wert nachher anstieg. Klinisch zeigten die Tiere eine Besserung. Alle beobachteten Wirkungen waren temporärer Natur.

Accepted for publication 14.8.79

ABSTRACTS**Metabolism and nutrition**

Utilisation of a corn oil supplemented diet by the pony

KANE, E., BAKER, J. P. and BULL, L. S. (1979). *J. Anim. Sci.* **48**, 1379-1384.

The utilisation by 6 mature Shetland pony geldings of a maize oil supplemented diet was studied to determine the apparent digestibility of fat and the digestible energy (DE), metabolisable energy (ME) and net energy (NE) for weight gain using an open circuit calorimeter. The maize oil provided 0.15 and 30 per cent of the digestible energy of a maintenance diet of oats, vitamins and limestone. The oil did not depress dry matter or acid detergent fibre digestibility and ether extract of the corn oil had a digestibility coefficient of 0.93. The DE, ME and NE for gain of the corn oil were 36.8, 35.1 and 29.7 kJ/g.

The DE requirement for energy maintenance expressed as kJ/kg^{0.75} daily was calculated to be 397 for the ponies receiving the oat-maize oil diet. Similarly the ME for energy maintenance was 345 kJ/kg^{0.57} daily. The ME of the oil was used for body fat synthesis with an efficiency of 0.85.

D. L. FRAPE

Energy and protein under-nutrition in the weanling filly foal

ELLIS, R. N. W. and LAWRENCE, T. L. J. (1979). *Br. vet. J.* **135**, 331-337.

Six Welsh weanling filly foals were allocated to each of 4

treatments for a winter period of 112 days. Three iso-energetic diets were individually fed. In 2 treatments the diet contained 14.8 per cent crude protein and 0.70 per cent total lysine and was fed either to maintain constant weight (LP) or to induce a weight gain of 0.45 kg/day (HP). In 2 other treatments the diets contained 6.0 per cent crude protein and either 0.28 per cent (LPP) or 0.70 per cent (LPPL) total lysine, achieved by including L-lysine HCl in the latter diet. These 2 diets were fed to maintain constant bodyweight. After this all animals grazed together for 126 days (May to September).

During winter and (summer) periods the daily weight changes were +0.30 (+0.41), +0.03 (+0.57), -0.12 (+0.53) and -0.09 (+0.50) kg for the HP, LP, LPP and LPPL treatments respectively.

In spite of being maintained at or near constant weight the ponies on the 3 low-plane treatments made small gains in height and length of skeletal parts while soft tissue was lost. Compensatory growth in the latter during the summer was the greater as measured by width of chest, heart girth, body girth, hocks to pins, width of hocks and circumference of legs. The decreases in liveweight during winter amongst the LPP and LPPL groups largely reflected the method of adjusting food intake to liveweight at weekly intervals.

D. L. FRAPE

SOME SEROLOGICAL STUDIES IN THE HORSE IN RELATION
TO THE PATHOGENESIS OF CHRONIC OBSTRUCTIVE PULMONARY
DISEASE.

ANDREW G. MATTHEWS

B.V.M.&S. (UNIVERSITY OF EDINBURGH)

M.R.C.V.S.

Degree of Doctor of Philosophy
Department of Veterinary Medicine
Royal (Dick) School of Veterinary Studies
University of Edinburgh.

June 1981

SUMMARY

The study described in this thesis was designed to examine some serological factors which may be involved in the pathogenesis of chronic obstructive pulmonary disease (C.O.P.D.) of horses.

In the first section, zone electrophoresis of normal horse serum on agarose gels (pH 8.6) was studied and the serum electrophoretic profiles of normal and C.O.P.D. affected horses and ponies were compared. No differences between the serum electrophoretic profiles of healthy and C.O.P.D. affected horses and ponies were observed which could be attributed to the presence of the disease.

In the second section, the nature of the two major, electrophoretically distinct antiproteases in horse serum was investigated prior to examining the possible association of antiprotease deficiency with the onset of C.O.P.D. in the horse, analogous to the association of the inherited dysproteinaemia of alpha-1 antitrypsin deficiency and chronic lung disease in man. The electrophoretically faster antiprotease, a functional homologue of human alpha-1 antitrypsin, was shown to appear in the prealbumin region of horse serum after acidic starch gel electrophoresis (pH 4.3). This polymorphic antiprotease corresponded to the allele products of the Pr locus of horse serum described by Braend (1970). The genetically determined polymorphism of the Pr antiprotease was examined by acid starch

gel electrophoresis, isoelectric focusing and immunofixation electrophoresis. The occurrence of a second antiprotease in the acidic prealbumin region of horse serum was postulated, although its nature remains to be established.

The electrophoretically slower antiprotease of horse serum was identified as alpha-2 macroglobulin, and was shown to contribute 48 percent of the total serum antiproteolytic activity. As in man, horse alpha-2 macroglobulin is able to inhibit the proteolytic activity of trypsin, but has only limited inhibitory activity on its esterolytic activity. Native alpha-2 macroglobulin was shown to possess esterase activity and the possible association of the macroglobulin and plasma pseudocholinesterase is discussed. No inherited polymorphism of horse alpha-2 macroglobulin was observed.

The Pr antiprotease allele frequencies in healthy and C.O.P.D. affected Thoroughbred horses were compared and no significant differences were observed. There was however an apparently increased frequency of the PrW allele amongst C.O.P.D. affected horses and ponies of mixed breeding, although the significance of this observation could not be established. Significantly increased levels of immunochemically measured circulating Pr protein were observed in a C.O.P.D. affected population, although no corresponding increase in biochemically measured serum trypsin inhibitory capacity (STIC) was observed in this same population.

It was concluded that serum antiprotease deficiency and consequent predisposition to the development of C.O.P.D. was unlikely to occur in the horse, although a possible deficiency of local bronchiolar anti-proteases, resulting in an increased chance of hypersensitization to the protease antigens of the fungi commonly incriminated in C.O.P.D., could not be excluded.

In the third section the occurrence of a serum homocytotropic antibody in the horse, homologous to human IgE, was investigated. A passively transferable homocytotropic antibody against Culicoides pullicaris was demonstrated in the serum of horses and ponies affected with recurrent seasonal dermatitis. Like human IgE, this antibody is heat-labile, susceptible to thiol reducing agents and persists for long periods in homologous skin. The elution characteristics of the horse antibody on DEAE-anion exchange chromatography are similar to those of human IgE. Anti-human IgE was shown to induce reversed anaphylaxis-like reactions in horse skin and immunfluorescent studies provided preliminary evidence of the binding of anti-human IgE to horse mast cells. These observations on the equine homocytotropic antibody satisfy Vaerman's (1970) criteria of interspecies protein homology suggesting that the antibody is homologue of human IgE.

Chronic obstructive pulmonary disease anatomical cardiac studies

P. M. DIXON, J. R. NICHOLLS*, E. A. McPHERSON, G. H. K. LAWSON and J. R. THOMSON
University of Edinburgh, Veterinary Field Station, Easter Bush, Roslin, Midlothian EH25 9RG

H. W. PIRIE and R. G. BREEZE

**University of Glasgow Veterinary School, Bearsden Road, Bearsden, Glasgow G61 1QH*

Summary

An abattoir survey on horses diagnosed as suffering from chronic obstructive pulmonary disease (COPD) on clinical grounds showed that the right ventricular wall thickness was not significantly different from that of normal horses. However, the weight ratio between the left and right ventricles was found to be significantly ($P < 0.001$) different in COPD affected, compared with control, horses in a study of 17 physiologically and pathologically confirmed COPD cases, using ventricular weight measurements. No clinical evidence of right heart failure nor post mortem evidence of right heart dilatation was observed in any COPD affected animals. It is suggested that the low incidence and degree of cor pulmonale in equine COPD may be related to the reversibility of the pulmonary hypertension associated with this disease.

Introduction

COR pulmonale, ie, right ventricular (RV) hypertrophy secondary to chronic pulmonary disease (World Health Organisation 1963), is caused by long-standing pulmonary hypertension. It is a common finding in human chronic obstructive pulmonary disease (COPD), with some post mortem reports indicating an incidence of RV hypertrophy in up to 40 per cent of cases (Otto, Zeilhofer and Reissinger 1969; Haselton 1973). Because pulmonary hypertension is an invariable finding in horses suffering from COPD (Beltran 1974; Bergsten 1974; Dixon 1978), it is surprising that RV hypertrophy has not been well documented in this species. There appear to be only 2 descriptions of its occurrence (Salutini 1959; Sporri and Schlatter 1959). Salutini (1959) noted that deaths could occur in horses with chronic pulmonary disease due to cor pulmonale. Sporri and Schlatter (1959) found marked pulmonary hypertension in 2 horses with chronic pulmonary disease and, on post mortem examination, they found right ventricular hypertrophy in both but gave no details of how they assessed this hypertrophy. It is unclear from the literature whether the low recorded incidence of cor pulmonale in the horse reflects a true low incidence or it is because of poor observation.

This study was undertaken to assess the incidence of RV hypertrophy in equine COPD. It consists of 2 parts: a preliminary abattoir study on COPD cases diagnosed on clinical grounds, followed by a study on clinicopathologically confirmed COPD cases.

Preliminary study

Materials and methods

The survey was carried out in a horse abattoir (North Kilkenny Meat Exporters, Freshford, Eire). Horses were kept indoors in pens for a few days before slaughter and, at this stage, were examined clinically with particular emphasis placed on the respiratory and cardiovascular systems. They were then divided into 2 groups, normal and COPD affected, using the clinical criteria of McPherson *et al* (1978), in so far as was possible because the histories were often incomplete. Animals in either group showing evidence of any other disease were excluded from the survey.

Slaughter was by stunning followed by exsanguination. The hearts were examined 20 mins after death, at which time both ventricles are fully contracted in systole (Rooney 1970). Routine post mortem examinations were carried out with particular emphasis on the cardiac and respiratory systems and animals showing evidence of diseases other than COPD were also excluded from the survey.

Hearts were examined for the presence of RV hypertrophy by comparing relative ventricular wall thickness. RV wall thickness was measured midway on a line joining the subauricular papillary muscle origin and the tricuspid valve. Left ventricular (LV) wall thickness was measured midway between the origins of the subauricular and subatrial papillary muscles and the LV:RV wall thickness ratio was determined. The significance of differences between the means (\pm sd) of both groups were compared using Students' *t* test.

Results

Three hundred and sixteen normal and 29 COPD affected horses were examined. No gross evidence of RV dilatation or RV hypertrophy was observed in any animal in either group. The normal horses had a mean LV:RV wall thickness ratio of 2.37 ± 0.58 whereas the COPD affected group had thicker RV walls and a LV:RV thickness ratio of 2.20 ± 0.71 a difference which was not statistically significant ($P < 0.05$).

Study on confirmed COPD cases

Materials and methods

Seventeen adult horses consisting mainly of hunters and ponies with histories and clinical signs of COPD were used. The

mean period since recognition of COPD was 3 years and 3 months, although it is likely that, in many of the cases the disease was present, but unrecognised, for longer. Eleven cases were confirmed by pulmonary function tests using the techniques of McPherson *et al* (1978) and all were confirmed by gross and histological post mortem examinations to have typical COPD changes according to the following criteria of Nicholls (1978): Macroscopically—over-inflation of the lungs with limited emphysema in small areas of the cranial lobe and in the periphery of the caudal lobe; Histologically—widespread bronchiolitis involving all airways of less than 2 mm diameter characterised by epithelial hyperplasia, goblet cell metaplasia, peribronchiolar cellular accumulations and intraluminal exudation of cells and mucus.

Thirty-eight clinically normal horses, of similar breeds and age, were examined similarly as control. Animals were killed by stunning and exsanguination and were subjected to post mortem examination. The external circumferences of the aorta and pulmonary artery were measured at their origins and the aorta:pulmonary artery (AO:PA) circumference ratio calculated.

To compare the weight of the RV with that of the LV and interventricular septum (LV + S), the technique of Fulton, Hutchinson and Morgan-Jones (1952) was used. The RV was dissected free from its border with the right atrium and interventricular septum. The atria were then dissected off both ventricles. The RV and LV + S were individually weighed and LV + S:RV weight ratio was calculated. The significance of differences between the means (\pm sd) of AO:PA circumference and LV + S:RV weight ratios of both groups were compared using Students' *t* test.

Results

No gross evidence of cardiac dilatation was seen but 2 of the cases showed some macroscopic RV hypertrophy. The mean (\pm sd) AO:PA circumference ratio for controls was 1.04 ± 0.10 which was significantly ($P < 0.001$) wider than that for COPD affected horses (0.89 ± 0.11), indicating a relatively wider pulmonary artery. The controls had a mean LV + S:RV weight ratio of 3.42 ± 0.39 , whereas in the COPD affected group the mean ratio was 2.81 ± 0.65 , indicating significantly ($P < 0.001$) relatively heavier right ventricles.

Discussion

It is generally accepted that the most accurate method of assessing RV hypertrophy is to compare the weight of the RV with that of the LV along with that of the anatomically confluent interventricular septum (Fulton *et al* 1952; Medical Research Council 1975). It has been noted that RV hypertrophy could also cause slight interventricular septum hypertrophy, but this is not believed to be a major drawback to this technique (Bove and Scott 1966).

In man, with a relatively limited range of adult body weights (bwt), absolute increase in RV weight above the normal limits has also been used to diagnose RV hypertrophy (Fulton *et al* 1952) but, because of the major interbreed differences in bwt and in relative heart weight, such a technique would not be applicable to the horse. The LV + S:RV weight ratio technique, therefore, would have been the technique of choice for both studies. However, this was not possible because the preliminary study was performed in an abattoir used for intercommunity EEC trade, whose regulations ban the sale of non-intact viscera. The main objections to assessing RV hypertrophy by ventricular thickness measurement is that ventricular wall thickness can vary greatly from site to site and also vary due to cardiac dilatation or post mortem contraction. By using fixed ventricular measurement points and by taking measurements during

bilateral systole it was hoped to overcome these drawbacks. Despite using the fixed measuring point it was found that some wall irregularity occurred at these sites even in normal horses, as can be seen from the considerable standard deviation obtained in their LV:RV wall thickness ratio (2.37 ± 0.58). Because of the limitations both in the confirmation of COPD and of the RV hypertrophy assessment technique used in the preliminary study, less reliance must be placed upon the results of this study.

The incidence and degree of RV hypertrophy in COPD affected horses was low in both studies. However, the confirmed cases had significantly relatively heavier RVs and this group included 2 horses with LV + S:RV weight ratios of 1.44 and 1.43. No evidence of cardiac dilatation was seen in either survey. No clinical evidence of right heart failure was observed in any of the COPD cases nor has any clinical evidence of cor pulmonale been seen in the hundreds of confirmed COPD cases, many severe and long-standing, studied by the Edinburgh group over the past decade.

Evidence of relatively wider pulmonary artery circumferences was seen in the confirmed COPD affected group and this could possibly be attributed to pulmonary hypertension. With persistent or severe pulmonary hypertension in man, secondary muscular hypertrophy and arteriosclerotic changes commonly occur in the pulmonary arterioles (Silber and Katz 1975). This causes an additional and permanent increase in pulmonary arterial vascular resistance, added to the functional increased vascular resistance initiated by the hypoxic vascular reflex. These anatomical vascular changes are not a feature of equine COPD, even in longstanding cases (Nicholls 1978).

The reversibility of pulmonary hypertension in the horse during remission stages of COPD has been demonstrated by Dixon (1978). Long term studies have shown that the pulmonary arterial pressures of COPD affected horses constantly fluctuate in inverse relation to their arterial oxygen partial pressures which, in turn, depend upon exposure of the horses to the aetiological agents of COPD. Even after many years of intermittent and often severe pulmonary hypertension (eg, maximum 80mmHg), their pulmonary arterial pressures quickly revert to normal limits during remission phases of the disease (Dixon 1979).

It would appear that this great reactivity of the equine pulmonary vascular bed and the absence of anatomical pulmonary arteriolar changes allow respites from constant pulmonary hypertension in COPD. This absence of persistent pulmonary hypertension and, consequently, of constant RV extra workload may be the factor which limits the incidence and degree of RV hypertrophy and the subsequent development of clinical signs of cor pulmonale in equine COPD. This is not to imply that in equine COPD the presence of pulmonary hypertension, albeit temporarily, is insignificant. It is likely that in symptomatic animals the extra RV workload it causes is an additional factor to the coexistent systemic hypoxaemia in detracting from performance, particularly in competitive animals. The present study was primarily concerned with COPD but if horses were affected by non-intermittent chronic pulmonary diseases, other than COPD, it appears likely that they would suffer a more constant pulmonary hypertension and consequently be more likely to develop RV hypertrophy.

Acknowledgements

Our thanks to Professor J. T. Baxter of the department of veterinary medicine, University of Edinburgh and to Professor W. F. H. Jarrett of the department of veterinary pathology, University of Glasgow in whose departments most of this work was carried out, to Dr R. W. Else for constructive criticism of the manuscript and to the Horserace Betting Levy Board for financial support for this study.

References

- Beltran, L. E. (1974) A critical evaluation of right heart catheterisation in the horse. PhD thesis, University of London
- Bergsten, G. (1974) Blood pressure, cardiac output and blood gas tension in the horse at rest and during exercise. *Acta. Vet. Scand. suppl.* **48**.
- Bove, K. E. and Scott, R. C. (1966) The anatomy of chronic cor pulmonale secondary to intrinsic lung disease. *Prog. cardiovasc. Dis.* **9**, 227-238.
- Dixon, P. M. (1978) Pulmonary artery pressure in normal horses and in horses affected with chronic obstructive pulmonary disease. *Equine vet. J.* **10**, 195-198.
- Dixon, P. M. (1979) Some blood pressure studies in normal horses and in horses affected with chronic obstructive pulmonary disease. PhD thesis, University of Edinburgh
- Fulton, R. M., Hutchinson, R. C. and Morgan-Jones, A. (1952) Ventricular weight in cardiac hypertrophy. *Br. Heart J.* **14**, 413-420.
- Haselton, P. (1973). Right ventricular hypertrophy in emphysema. *J. Path.* **110**, 27-36.
- McPherson, E. A., Lawson, G. H. K., Murphy, J. R., Breeze, R. G. and Pirie, H. M. (1978) Chronic obstructive pulmonary disease (COPD). Identification of affected horses. *Equine vet. J.* **10**, 47-53.
- Medical Research Council (1975) Report of a committee on research into chronic bronchitis. *Thorax* **30**, 241.
- Nicholls, Julia M. (1978) A pathological study of chronic pulmonary disease in the horse. PhD thesis, University of Glasgow
- Otto, H., Zeilhofer, R. and Reissinger, O. (1969) Vergleichende unter Suchungen zur Klinik unsymptomatik Morphologisch gesicherter Emphysemfalle. *Prax. Pneumol.* **23**, 771-776, 776-785.
- Rooney, J. R. (1970) *Autopsy of the horse* Williams & Wilkins Co, Baltimore
- Salutini, E. (1959) Cuore polmonare cronico e bolsaggine, *Nvova Vet* **35**, 101-104.
- Silber, E. W. and Katz, L. N. (1975) *Heart Disease*. 1st edn. Macmillan Publishing Co, New York
- Sporri, H. and Schlatter, C. (1959) Blutdruckerhöhungen in Lungenkreislauf. *Schweizer Arch. Fierheilk* **101**, 525-541.
- World Health Organisation (1963) Chronic cor pulmonale. Tech. Rep. Series No 213, WHO, Geneva.

Résumé

Un contrôle à l'abattoir effectué sur des chevaux identifiés cliniquement comme étant atteints de maladie pulmonaire chronique obstructive a montré que l'épaisseur de la paroi du ventricule droit n'était pas différente de celle des chevaux normaux d'une manière significative. Cependant le rapport des poids entre les deux ventricules fut trouvé différent de manière significative chez les chevaux atteints de maladie pulmonaire obstructive chronique ($P < 0.001$) par référence aux animaux de contrôles. Ces mesures portèrent sur 17 chevaux représentant autant de cas confirmés pour la maladie concernée. On ne trouva aucune preuve d'insuffisance cardiaque droite ni de dilatation cardiaque droite à l'examen post mortem, chez les chevaux atteints de la maladie. On suggère que la rareté et la faible intensité du syndrome "coeur pulmonaire" chez le cheval atteint d'emphysème sont dus à la réversibilité de l'hypertension pulmonaire associée à la maladie.

Zusammenfassung

Eine Untersuchung an Schlachtpferden, die klinisch an chronisch-obstruktiven Lungenkrankheiten (COPD) gelitten haben sollen, wies nach, dass die Dicke der rechten Kammerwand nicht signifikant von derjenigen normaler Pferde verschieden war. Immerhin erwies sich die Gewichtsbeziehung von linker zu rechter Kammer als signifikant ($P < 0.001$) von derjenigen der Kontrollpferde abweichend. Diese Studie bezog sich auf 17 physiologisch und pathologisch bestätigte COPD-Fälle und bediente sich der Wägung der Kammern. Bei keinem der COPD-Patienten konnten klinisch oder autoptisch Anzeichen eines Versagens beziehungsweise einer Dilation des rechten Herzens beobachtet werden. Es wird vermutet, dass die Seltenheit unter der leichten Grad von cor pulmonale bei COPD des Pferdes mit der Reversibilität des Lungenhochdrucks bei dieser Krankheit zusammenhänge.

Accepted for publication 11.6.81

ABSTRACT

Surgery

Esophageal healing in the pony: comparison of sutured vs non-sutured esophagotomy

STICK, J. A., KREHBIEL, J. D., KURZE, D. J. and WORTMAN, J. A. (1980) *Am. J. vet. Res.* **42**, 1506-1513

A 5 cm longitudinal cervical oesophagotomy was created in 10 clinically normal ponies under general anaesthesia. In 5 the oesophageal and skin wounds were sutured and in the others, left unsutured. In the first group the oesophageal mucosa and submucosa were apposed with a continuous suture of 3-0 polypropylene material and the musculature with simple interrupted 2-0 medium chronic gut. In both groups a cervical oesophagotomy tube was inserted distal to the initial wound and ponies were fed identical diets of pelleted food via the tube until mucosal healing occurred. Wounds were treated with warm water and soap but no antibiotics were used. Oesophageal

wounds were examined endoscopically and radiographically at regular intervals. Mean oesophageal healing time was assessed clinically in 9 of the ponies (one died) and they were euthanased 90 days postoperatively. The oesophageal wounds were examined histologically. Mean oesophageal healing time in the first group was 7.5 days and in the unsutured group 25.6 days. Skin wounds also healed on average 23.4 days more quickly in the first group of ponies. Radiographic evidence of a sinus tract was present 30 days postoperatively in one pony in Group I and 3 in Group II, and traction diverticuli in all ponies in Group II, but only 1 in the first group. However, other complications were more common in the first group (eg, subcutaneous abscessation). Histological appearance of the oesophagus at the surgical site was identical in both groups of animals.

T. R. C. GREET

STUDIES ON THE THERAPY OF EQUINE CHRONIC OBSTRUCTIVE
PULMONARY DISEASE.

JILL R. THOMSON

B.V.Sc. (UNIVERSITY OF PRETORIA)

M.R.C.V.S.

Degree of Doctor of Philosophy
Department of Veterinary Medicine
Royal (Dick) School of Veterinary Studies
University of Edinburgh.

February 1982.

Summary

The therapy of equine chronic obstructive pulmonary disease (COPD) by means of environmental control (bedding horses on shredded paper and feeding a complete cubed diet) and by the use of several therapeutic agents, was assessed by clinical examination and pulmonary function tests, i.e. maximum change in intrathoracic pressure, tidal volume, minute volume, inspiratory and expiratory flow rates and blood gas analyses.

Environmental control caused symptomatic COPD affected horses to become asymptomatic within 4 to 32 days (mean \pm S.D.: 9.0 ± 4.8 days). When asymptomatic, their pulmonary function values did not differ significantly from those of normal horses which indicates that the pathophysiological changes occurring in equine COPD are reversible.

Inhalation or intravenous administration of the following bronchodilator drugs; atropine, isoprenaline, terbutaline, clenbuterol and etamiphylline camsylate to symptomatic COPD affected horses brought about a temporary, marked improvement clinically and in the pulmonary function parameters examined. This indicates that airway spasm is involved in the pathogenesis of equine COPD and that parenteral or inhaled bronchodilator therapy can be of value in the treatment of acute attacks.

Orally administered bronchodilator drugs (clenbuterol and etamiphylline camsylate) were not found to be effective in the treatment of COPD. Neither drug significantly improved pulmonary function in symptomatic COPD affected horses maintained in the natural challenge environment (exposed to poor quality hay and straw bedding which was dusty and visibly contaminated with moulds). In addition, neither drug significantly hastened the remission of clinical signs which normally occurred when symptomatic COPD affected horses were housed in the controlled environment.

Prophylactic sodium cromoglycate inhalation prevented asymptomatic COPD affected horses from becoming symptomatic following artificial Micropolyspora faeni inhalation challenge or after exposure to the natural challenge environment. A linear response existed between the number of successive days sodium cromoglycate treatment and the duration of protection in the COPD horses exposed to the natural challenge environment. The protective period was 3.6 ± 1.1 (mean \pm S.D.) after a single sodium cromoglycate treatment and increased to 24.3 ± 13.4 days after 4 days treatment. Two successive days sodium cromoglycate treatment administered at weekly intervals over a 28 day period was effective in preventing the onset of COPD in 6 out of 8 affected horses housed in the natural challenge environment.

Serum protein electrophoresis in horses and ponies

A. G. MATTHEWS

Department of Veterinary Medicine, University of Edinburgh, Veterinary Field Station, Easter Bush, Roslin, Midlothian EH25 9RG

Summary

A method of electrophoresis of horse serum on agarose gels (pH 8.6) is described, together with a system for interpreting changes in the electrophoretic zones based upon the relative distribution of the major serum proteins. Differences in the protein composition of the individual electrophoretic zones of horses and ponies were recorded, although this variation probably reflects differences in management and the presence of subclinical disease.

Introduction

ELECTROPHORESIS of horse serum using a wide range of support media and techniques has been reported (Kristensen and Firth 1977). However, variation in the quality of resolution of the protein zones and bands achieved with each electrophoretic system has led to confusing and conflicting published data on the number, identity and nomenclature of the electrophoretic zones. Nevertheless, quantitative serum protein electrophoresis has been used as an aid to diagnosis in equine medicine for some time (Jeffcott 1971), although in human medicine Laurell (1973) argued that qualitative interpretation of the electrophoretogram accompanied by specific serum protein assays will provide the maximum diagnostically useful information. Laurell (1972) stated that quantitative variation in plasma proteins whose minimum concentration lies between 0.1 and 0.5 g/litre may significantly alter the electrophoretic profile. In the horse, the lower limits of the normal serum concentration of transferrin, alpha-2 macroglobulin and the immunoglobulins IgG, IgM, IgA and IgG(T) are in excess of this threshold level (Lavergne and Raynaud 1970; McGuire, Crawford and Henson 1972; Ek 1981). Thus, variation in the circulating levels of these proteins will be reflected in the electrophoretic profile. Other proteins which, by virtue of their normally high serum concentration, may alter the electrophoretic profile are albumin, haptoglobin (Allen and Archer 1971), lipoproteins (Straub, Gerber and Petitjean 1975) and, in the perinatal foal, alphafoetoprotein (Lock, Morgan and Mock 1976). In the present paper, interpretation of the horse serum electrophoretogram based upon the distribution of these individual proteins is discussed and variation in the electrophoretic profiles of adult horses and ponies is examined.

Materials and methods

Electrophoresis

A commercially available agarose electrophoresis system (Corning Medical, Halstead, Essex) employing an agarose-sucrose gel in 0.05M barbital — EDTA buffer (pH 8.6) and a continuous gel-electrode buffer system was used. Electrophoresis was carried out at 100V for 21 mins. Routine protein staining was carried out using amido black B following by destaining in glacial acetic acid.

The relative protein composition of the electrophoretic zones was determined as percentage optical absorbance at 520nm using a Phoroscope Densitometer (Millipore Corporation, Bedford, Massachusetts, USA) and the absolute concentration (g/litre) calculated from the total serum protein determined using the Biuret method.

Sera

Horse sera were obtained from clinically normal animals aged one year or more. These horses were working animals housed under stable conditions. Pony sera were obtained from 2 different sources. **Group A** comprised 47 sera from clinically normal animals maintained outdoors on marginal vegetation throughout the year with little supplementary management. **Group B** comprised 15 sera from animals managed under conditions similar to those of the horses used in this study. Ponies in **Groups A** and **B** were all aged one year or more.

Electrophoretic distribution of serum proteins

The distribution of alpha-2 macroglobulin and IgG, IgM, IgA and IgG(T) after agarose electrophoresis (pH 8.6) was derived from the results of exclusion and exchange chromatography of horse serum presented by Vaerman, Querinjean and Heremans (1971). The distribution of transferrin after agarose electrophoresis (pH 8.6) has been described by Ek (1981), and that of alphafoetoprotein by Lock *et al* (1976).

The distribution of horse haptoglobin and lipoprotein fractions after agarose electrophoresis was determined using O-dianisidine/hydrogen peroxide staining of serum-haemoglobin mixtures and fast red 7B staining of fresh serum respectively.

Results

The typical densitometric profile of horse serum after agarose electrophoresis (pH 8.6) is shown in Fig 1a. The distribution of

A. G. Matthews' present address is Ratley Lodge, Ratley, Banbury, Oxfordshire

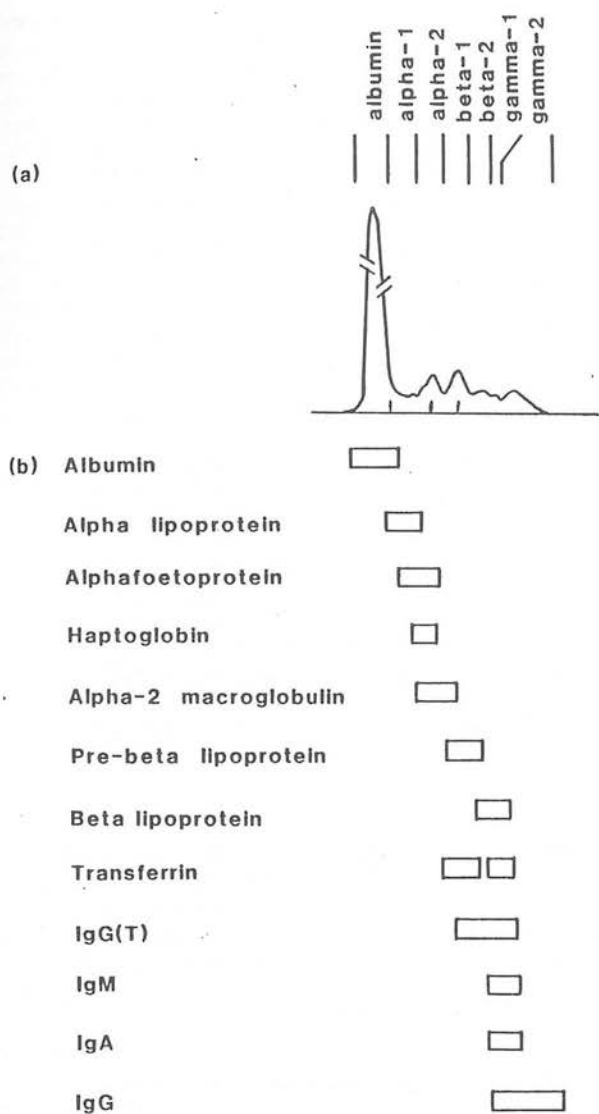


Fig 1. (a) Densitometric tracing of horse serum after agarose electrophoresis (pH 8.6) showing the division into the electrophoretic zones. (b) Distribution of the major serum proteins in relation to the densitometric scan

the major serum proteins and the electrophoretic zones derived from this distribution are shown in Figs 1b and 1a respectively. In this series the albumin zone excludes the cathodal shoulder of the albumin peak, which is included within the alpha-1 zone. The alpha-1 zone contains predominantly alpha lipoprotein. Haptoglobin migrates predominantly within the anodal alpha-2 zone. Braend and Efremov (1965) found no phenotypic differences in horse haptoglobin after starch gel electrophoresis and variation in its fast alpha-2 mobility after agarose electrophoresis is not expected. Alpha-2 macroglobulin was the second major component of the alpha-2 zone. In the perinatal foal alphafoetoprotein was the major component of the alpha-2 zone.

The beta-1 zone contains the electrophoretically faster phenotypic variants of transferrin and also pre-beta lipoprotein. The beta-2 zone contains predominantly the electrophoretically slower phenotypic variants of transferrin, beta lipoprotein and IgG(T). The gamma zone was artificially divided by the insert slit into gamma-1 and gamma-2 zones. The

TABLE 1: Total serum protein and electrophoretic zone concentrations of horses and ponies in Groups A and B (g/litre, mean \pm sd)

Electrophoretic zone	Horses (n=30)	Ponies: Group A (n=47)	Ponies: Group B (n=15)
Total protein	65.85 \pm 6.64	70.25 \pm 7.90	71.81 \pm 7.20
Albumin	29.89 \pm 3.39	25.05 \pm 3.67	27.88 \pm 5.50
Alpha-1	6.40 \pm 1.21	5.24 \pm 1.00	6.34 \pm 1.26
Alpha-2	6.72 \pm 1.84	9.88 \pm 3.32	9.22 \pm 2.35
Beta-1	9.01 \pm 3.30	12.65 \pm 2.77	9.41 \pm 2.57
Beta-2	5.39 \pm 1.60	7.85 \pm 2.00	8.14 \pm 1.84
Gamma-1	2.22 \pm 0.71	2.20 \pm 0.69	2.26 \pm 0.63
Gamma-2	6.33 \pm 1.54	7.65 \pm 2.26	8.49 \pm 2.14

gamma-1 zone contained predominantly IgM, IgA, IgG(T) and the IgGc subclass, while the gamma-2 zone contained predominantly the IgGAb subclass.

The mean total protein and electrophoretic zone protein concentration (g/litre) of the horses and Group A and B ponies are shown in Table 1.

Discussion

The delineation of the electrophoretic zones in this study was derived from the distribution of the major serum proteins. Using this system, changes within each zone may be related to changes in the constituent proteins, thus providing a basis for interpretation of variation in the electrophoretic profile as an aid in clinical diagnosis.

In addition to alpha-lipoprotein the alpha-1 zone contains a number of minor proteins (Juneja, Gahne and Sandberg 1978). However, the acidic protease inhibitor of horse serum, analogous to human alpha-1 antitrypsin, migrates within the albumin zone (Matthews 1979; Ek 1981).

Increased circulating levels of haptoglobin are observed in man and laboratory animals during acute inflammatory responses, and in the horse increased haptoglobin levels probably contribute to the marked increase in the more anodal alpha-2 region observed in acute inflammatory and infectious conditions. Horse alpha-2 macroglobulin is capable of binding both antigen-antibody complexes and proteolytic enzymes (Laverne and Raynaud 1970; Matthews 1979) and can neutralise the infectivity of human influenza-A virus (Pepper 1968). Erickson (1975) stated that horse alpha-2 macroglobulin is an acute phase reactant protein, although this is not the case in man (Laurell, Jeppsson and Tejler 1978).

The beta zones of horse serum present difficulty in interpretation arising from the electrophoretic heterogeneity of transferrin. The discrete protein bands observed within the beta zones contain predominantly transferrin (Ek 1981), although electrophoretically slower transferrin phenotypes, which migrate within the beta-2 zone, contain less protein than those faster phenotypes appearing within the beta-1 zone (Ek 1981). On visual inspection of the electrophoretogram, absence or reduced staining intensity of the beta-1 transferrin band in the presence of a distinct beta-2 band is likely to indicate a homozygous slow phenotype or a heterozygous slow and fast phenotype respectively. A distinct beta-1 band in the absence of a beta-2 band is likely to indicate a homozygous fast transferrin phenotype. Clinically, increased transferrin levels have been reported in the serum of anaemic horses (Thoren-Tolling 1977), while decreased transferrin levels are observed in horses with acute infectious conditions (Ek 1981) and cirrhosis (Thoren-Tolling 1977).

The beta-1 zone normally contains small amounts of the pre-beta lipoprotein. Large increases in this lipoprotein fraction in pony serum have been observed during starvation (Morris,

Zilversmit and Hintz 1972), suggesting that increased lipolysis may contribute to the hyperbetaglobulinaemia observed in cases of severe parasitism (Ooms *et al* 1976) and chronic liver disease (Merritt, Bolton and Crimprich 1975). Large increases in beta globulins have been observed in helminthiasis (Ooms *et al* 1976), arising primarily from increased IgG(T) levels (Groudswaand and Noordzij 1980).

Increased gammaglobulin levels caused by polyclonal immunoglobulin production have been reported in chronic infections (Jeffcott 1971) and lymphosarcoma (Ek 1981). Fluctuation in the gammaglobulin concentration is observed during the first weeks of life and electrophoresis provides a useful screening method for the detection of failure of colostral immunoglobulin transfer in young foals.

Considerable variation in mean electrophoretic zone concentration was observed between the 3 groups of animals in this study. Neither sex nor age has been shown to influence markedly the electrophoretic zone concentration of adult horses and ponies (Massip and Fumiere 1974; Kristensen and Firth 1977). However, the complex influences of plane of nutrition and subclinical parasitism on the electrophoretic profile suggests that the differences encountered between horses and ponies are not simply breed dependant. Such variation in serum globulin components among apparently healthy animals casts doubts upon the use of quantitative electrophoresis in the case of an individual clinical case. In such cases visual inspection of the stained electrophoretogram to determine patterns of change in the globulin zones is likely to provide more clinically useful information.

Acknowledgements

The author thanks the Horserace Betting Levy Board for their financial support and Dr P. Imlah and Mr E. A. McPherson for their guidance during the course of this work. Thanks are also due to Dr P. Dixon, Mr G. Keay and Mr R. H. C. Thursby-Pelham for their constructive criticism of the manuscript.

References

- Allen, B. V. and Archer, R. K. (1971) Haptoglobins in the horse. *Vet. Rec.* **89**, 106-109.
- Braend, M. and Efremov, G. (1965) Haemoglobins, haptoglobins and albumins of horses. *Proc. 9th European Conf. anim. blood Grps. Prague*, pp 253-259.
- Ek, N. (1981) Studies on the prealbumin (Pr) protein and transferrin phenotypes in the serum of healthy and sick horses and investigation of serum levels of the immunoglobulin IgG(T). Dissertation, Veterinary College of Norway, Oslo.
- Erickson, E. D. (1975) Alpha globulins of equine serum. *Proc. 1st Int. Symp. equine Haematology, Michigan*, pp 152-157.
- Groudswaand, J. and Noordzij, A. (1980) Immunology in Veterinary Laboratory Diagnosis: An introduction. *Proc. 2nd Int. Symp. vet. lab. Diagnosticians, Lucerne* **1**, 74.
- Jeffcott, L. B. (1971) In discussion of Doxey, D. L. (1971) Laboratory aids to clinical diagnosis in equine practice. *Equine vet. J.* **3**, 25-30.
- Juneja, K., Gahne, B. and Sandberg, K. (1978) Genetic polymorphism of the vitamin D binding protein and another post albumin protein in horse serum. *Anim. blood Grps. Biochem. Genets.* **9**, 29-36.
- Kristensen, F. and Firth, E. C. (1977) Analysis of serum proteins and cerebrospinal fluid in clinically normal horses using agarose electrophoresis. *Am. J. vet. Res.* **38**, 1089-1092.
- Laurell, C. B. (1972) Composition and variation of the gel fractions of plasma, cerebrospinal fluid and urine. *Scand. J. clin. Lab. Invest.* **29**, Suppl. **124**, pp 71-82.
- Laurell, C. B. (1973) Electrophoresis, specific protein assays, or both in the measurement of plasma proteins. *Clin. Chem.* **19**, 99-102.
- Laurell, C. B., Jeppsson, J. G. and Tejler, L. (1978) Plasma protein analysis. Worthington Diagnostics, Millipore Corporation, New Jersey.
- Lavergne, M. and Raynaud, M. (1970) Preparation at proprietes de l'alpha 2 macroglobulins de cheval. *Ann. Inst. Pasteur.* **119**, 27-49.
- Lock, T. F., Morgan, D. O. and Mock, R. E. (1976) Equine alpha-fetoprotein. Isolation and characterisation. *Proc. 4th Int. Conf. equine Infect. Dis.* Eds J. T. Bryans and H. Gerber, Veterinary Publishing Inc., New Jersey. pp 201-208.
- Massip, A. and Fumiere, I. (1974) Analyse electrophoretique des proteines du serum sanguin de chevaux adultes normaux ages de quatre a dix ans. *Ann. Med. Vet.* **118**, 129-229.
- Matthews, A. G. (1979). The identification and characterisation of the major protease inhibitors in horse serum and an investigation of their role in chronic obstructive pulmonary disease. *Equine vet. J.* **11**, 177-182.
- Merritt, A. M., Bolton, J. R. and Crimprich, R. (1975) Differential diagnosis of diarrhoea in horses over six months of age. *J.S. Afr. vet. Ass.* **46**, 73-76.
- Morris, M. D., Zilversmit, D. B. and Hintz, H. F. (1972) Hyperlipoproteinaemia in profiling ponies. *J. Lipid. Res.* **13**, 383-389.
- McGuire, T. C., Crawford, T. B. and Henson, J. B. (1972) The isolation, characterisation and functional properties of equine immunoglobulin classes and subclasses. *Proc. 3rd. Int. Conf. equine Infect. Dis.* Eds. J. T. Bryans and H. Gerber. S. Karger, Basel, pp 364-381.
- Ooms, L., Oyaert, W., Muylle, C., Van Den Hende, C. and Decraemere, H. (1976) Electrophoresis as an aid to the diagnosis of helminthiasis and especially of verminous aneurysm in the horse. *Vlaams diergeneesk. Tijdschr.* **45**, 290-299.
- Pepper, D. S. (1968) Isolation and properties of γ inhibitor. An equine alpha-2 macroglobulin subfraction. *Biochim. biophys. Acta.* **156**, 327-339.
- Straub, R., Gerber, H. and Petitjean, J. (1975) Serum lipids in horses, ponies, donkeys and mules. *Proc. 1st Int. Symp. equine Haematology, Michigan*. pp 228-236.
- Thoren-Tolling, K. (1977) Serumproteinvariationer vid patologiska tillstand hos hast och hund. *Sw. Vet. Tidn.* **29**, 917-927.
- Vaerman, J. P., Querinjean, P. and Heremans, J. F. (1971) Studies on the IgA system of the horse. *Immunology* **21**, 443-454.

Résumé

On décrit une méthode d'électrophorèse du sérum de cheval sur gels d'agarose (pH 8.6). On décrit également un système d'interprétation des changements observés dans les zones électrophorétiques fondé sur la répartition relative des principales protéines du sérum. Des différences ont été constatées dans la composition protéinique des zones électrophorétiques individuelles de chevaux et de poneys; on pense que ces variations explicitaient des différences dans le management et la présence de maladies subcliniques.

Zusammenfassung

Eine Methode der elektrophoretischen Untersuchung von Pferdeserum auf Agarosegel (pH 8.6) wird beschrieben, zusammen mit einem Interpretationssystem für Veränderungen der elektrophoretischen Zonen. Das System stützt sich auf die relative Verteilung der wichtigen Serumproteine. Unterschiede in der Proteinzusammensetzung individueller elektrophoretischer Zonen bei Pferden und Ponies wurden beobachtet. Die Variationen rühren wahrscheinlich von Unterschieden in der Haltung und vom Vorhandensein in subklinischer Krankheitszustände her.

Research articles

A REAGIN-LIKE ANTIBODY IN HORSE SERUM: 1. OCCURRENCE AND SOME BIOLOGICAL PROPERTIES

A.G. MATTHEWS¹, P. IMLAH² and E.A. McPHERSON¹

¹Department of Veterinary Medicine and ²Department of Animal Health, University of Edinburgh Veterinary Field Station, Easter Bush, Roslin, Midlothian, EH25 9RG (Great Britain)

Dr. Matthews' present address: Ratley Lodge, Ratley, Banbury, Oxfordshire (Great Britain)

(Accepted 28 May 1982)

ABSTRACT

Matthews, A.G., Imlah, P. and McPherson, E.A., 1983. A reagin-like antibody in horse serum: 1. Occurrence and some biological properties. *Vet. Res. Commun.*, 6: 13-23.

The demonstration of a reagin-like antibody against Culicoides pulicaris extract in the serum of horses and ponies affected with recurrent seasonal dermatitis (sweet itch) is reported. This antibody can confer Prauznitz-Küstner (P-K) sensitivity on homologous skin for up to 5 days and, like human IgE, is thermolabile and susceptible to 2-mercaptoethanol reduction. It is eluted on diethylaminoethyl dextran-52 anion exchange chromatography independently of IgG, IgG(T) and IgM, and its elution characteristics indicate similarity in net molecular charge to human IgE.

The P-K response observed in horse skin is biphasic, and is morphologically similar to the late cutaneous anaphylactic response in man. Both phases of the P-K response are dependent upon the reagin-like antibody, although other serum factors appear involved in the delayed phase of the response.

INTRODUCTION

Human reagin (immunoglobulin E) is a passively transferable gamma globulin capable of sensitising circulating basophils and tissue mast cells and of mediating the release of intracellular vasoactive compounds upon subsequent antigen or antiglobulin challenge (Bennich et al., 1976). An IgE-like immunoglobulin has been described in a number of mammalian species (Nielsen, 1977). These immunoglobulins share a number of properties with human IgE, and in most instances satisfy the three orders of criteria proposed by Vaerman (1970) for the identification of interspecies protein homology. These are: immunological cross reactivity and amino acid homology, the association of a protein with a particular function, and a range of biological and physiological homologies.

Passive transfer of systemic and cutaneous anaphylactic hypersensitivity has been described in the horse (Ritzenthaler, 1924; Eyre, 1972; Schatzman et al., 1973) and, in the United Kingdom, recurrent seasonal dermatitis of horses (RSD) is believed to be a cutaneous hypersensitivity to the biting midge Culicoides pulicaris (McCaig and Mellor, 1974).

This paper reports on investigations into some biological and physicochemical properties of a reagin-like antibody found in the serum of RSD-affected horses and ponies. Evidence of antigenic cross reactivity between human IgE and an equine reagin-like antibody is the subject of a second paper.

MATERIALS AND METHODS

Serum

Serum (active stage serum) was obtained from 12 horses and ponies showing typical signs of RSD and from 7 horses and ponies unaffected with the disease but with a history of the disease during previous summers (inactive stage serum). Serum was stored at -20°C until used.

Antigen

20 mg of whole C. pulicaris was macerated in 5 ml sterile 0.01M phosphate buffered saline (pH 7.4) (PBS). After centrifugation, the supernatant was diluted 1:5 in sterile PBS and passed through a 0.22 μm membrane filter, giving a final protein concentration of 200 $\mu\text{g}/\text{ml}$. Aliquots of antigen were stored at -20°C until used.

Prauznitz-Küstner (P-K) test

0.1 ml of test serum was injected intradermally (i/d) over the clipped lateral cervical region of recipient horses having no known history of RSD. Sensitised sites were challenged 24 hours later by i/d injection of 0.1 ml of antigen, placed so that the centre of the resulting dermal bleb lay as close as possible to an ink spot marking the centre of the sensitisation bleb. Sites which were injected subcutaneously were excluded from further consideration. The horizontal diameters (mm) of the resulting reactions were measured at 5, 30, 60, 120, 360 minutes and 24 hours.

Foal serum and physiological saline were included as negative sensitisation controls. Antigen and PBS were included as negative challenge controls. To avoid error arising from variation in recipient each P-K procedure was carried out on a single animal.

Persistence of the reagin-like antibodies in homologous skin

Antigen challenge of sites that had been sensitised with pooled P-K positive serum was carried out 2, 4, 24, 48 hours and 5 and 9 days after sensitisation.

Heat and thiol sensitivity of the reagin-like antibodies

Each of two 0.5 ml aliquots of pooled P-K positive serum was either heated to 56°C for 2 hours or dialysed against 250 ml of 0.1M 2-mercaptoethanol (2-ME) for 3 hours at room temperature. The latter was subsequently dialysed against 500 ml 0.02M iodoacetamide for 4 hours at room temperature and then against PBS for 24 hours at 4°C. Control serum was dialysed against iodoacetamide and PBS only.

The heat and 2-ME treated sera and controls were examined for P-K activity.

Elution of reagin-like activity from horse serum using ion-exchange chromatography

After dialysis against equilibrating buffer, 5 ml of pooled P-K positive serum was applied to a 1.5 cm x 25 cm diethylaminoethyl dextran-52 (DEAE-52) cellulose anion exchange column equilibrated with 0.04M Tris-HCl (pH 8.0). A Tris-HCl gradient of 0.04M (pH 8.0) to 0.5M (pH 8.0) was applied to the column over 16 hours giving a flow rate of 40 ml per hour. Individual protein peaks were collected, concentrated by evaporation to approximately 0.5 ml and dialysed against PBS at 4°C for 48 hours. After passage through a 0.22 µm membrane filter, the peaks were examined for P-K activity.

The distribution of IgG, IgM and IgG(T) in the column eluate was determined by slide double immunodiffusion employing 1% agar in 0.1M Tris-HCl (pH 8.0). IgG_{ab} and IgG_c were differentiated by their mobility after immunoelectrophoresis against anti-whole horse serum (Allen and Dalton, 1975). Immunoelectrophoresis was carried out as described by Hirschfeld (1960).

RESULTS

Fraunzitz-Küstner activity of sera from cases of equine recurrent seasonal dermatitis

Following antigen challenge, weal-like reactions were observed at 8 of the 12 sites sensitised with active stage sera. These reactions were apparent within 30-60 minutes and continued to enlarge for up to 6 hours after challenge. In all cases they had disappeared by 24 hours. Only transient dermal blebs were observed at the four remaining sites sensitised with active stage sera, the sites sensitised with inactive stage sera and the control sites. The mean and standard

deviation (SD) of the horizontal diameters of the dermal reactions observed at the 8 responding active stage sites (Group A), the 4 non-responding active stage sites (Group B) and the 7 inactive stage sites (Group C) are shown in Table 1 along with the horizontal diameters of the reactions at the control sites.

McPherson et al. (1979) deemed positive those responses following direct i/d challenge of horses where the diameter exceeded the mean plus 2 SD of the control diameters. In the present study, the reactions at sites sensitised with Group A sera (Table 1) were in excess of the mean plus 2 SD of the reactions at the sites sensitised with inactive stage sera (Group C). The former were deemed positive P-K responses, indicative of reagin-like antibodies in the sensitising sera. The responses at sites sensitised with Group B sera (Table 1) did not exceed the mean plus 2 SD of the Group C sera sensitised sites and were deemed negative responses. Reactions at Group B and Group C sera sensitised sites did not differ from those at the negative control sites.

Persistence of the reagin-like antibodies in homologous skin

Typical positive P-K responses were elicited up to 5 days after sensitisation. A negative response was elicited 9 days after sensitisation.

Heat and thiol sensitivity of the reagin-like antibodies

The effects of heat and 2-ME reduction-alkylation on the P-K activity of pooled P-K positive serum are shown in Table 2. Both treatments resulted in the elimination of the P-K response. Alkylation alone had no effect on the P-K activity.

Elution of reagin-like activity from horse serum using ion-exchange chromatography

The distribution of positive P-K and immunoglobulin activity in the DEAE fractions of horse serum is shown in Figure 1. P-K activity was eluted in fractions 2-6, over a molar range of 0.05M - 0.1M Tris-HCl (pH 8.0). This activity was independent of the major immunoglobulin classes. However, the P-K responses at sites sensitised with DEAE fractions 2-6 reached a maximum 30-60 minutes after antigen challenge and did not progress from that time. At sites sensitised by fractions 2, 3 and 5 the reactions had dispersed by 360 minutes.

DISCUSSION

The occurrence of positive P-K responses at skin sites sensitised with active stage RSD sera after challenge with midge (C. pulicaris) extract indicates the

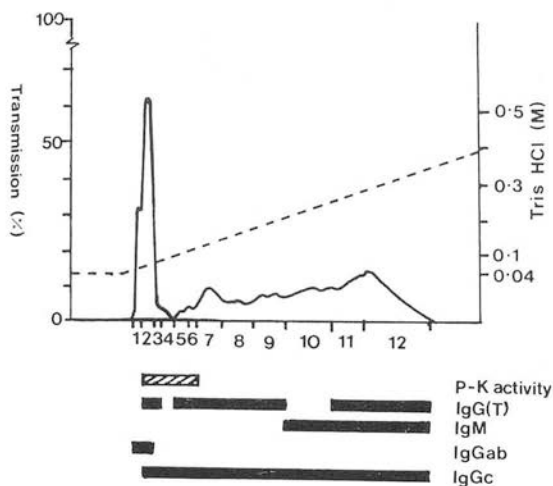


Fig. 1. Elution of Prauznitz-Kustner (P-K) activity and immunoglobulin classes Gab, Gc, M and G(T) from pooled P-K positive horse serum on DEAE-52 chromatography using a linear Tris-HCl (pH 8.0) gradient.

presence of reagin-like antibodies to the midge antigen in the sera of these affected animals. This is in accordance with the earlier observations of Baker (1978) on a single case of RSD and of Riek (1954) on a number of horses naturally hypersensitised to the bite of the midge *C. robertsi*.

The magnitude of the positive P-K responses are similar to those resulting from both direct i/d challenge of sensitised horses with fungal antigens (Halliwell et al., 1979; McPherson et al., 1979) and from P-K testing of sensitised horse serum and *Parascaris equorum* antigen (Mansman and Mansman, 1975). The persistence of swellings at negative test or negative control sites for up to 6 hours has been reported during both P-K and direct i/d testing of horses (Schatzman et al., 1973; McPherson et al., 1979) and probably reflects the extreme sensitivity of horse skin to relatively innocuous challenge noted by De Weck (1972).

The reaginic antibody in horse serum shares a number of physiological and biological properties with human IgE in accordance with Vaerman's third criterion of interspecies protein homology. The horse reagin remained detectable in

TABLE I

Mean and standard deviation of the horizontal diameters (mm) of dermal responses at Praunnitz-Küstner test sites and the horizontal diameters (mm) at control sites

Time (min)	Test Sites			Control Sites			
	Active stage serum Responding sites (Group A: n = 8)	Active stage serum Non-responding sites (Group B: n = 4)	Inactive stage serum (Group C: n = 7)	Sensitisation		Challenge	
				Saline	Foal serum	Antigen	PBS
5	11.6 ± 1.07	12.0 ± 1.63	11.9 ± 1.35	11	11	10	11
30	16.7 ± 1.58	12.0 ± 2.16	12.9 ± 1.07	12	12	9	10
60	16.5 ± 1.31	12.0 ± 1.41	11.7 ± 0.76	13	13	9	9
120	21.1 ± 2.47	13.0 ± 1.15	13.3 ± 0.95	D	14	D	D
360	22.9 ± 1.96	D	D	D	D	D	D
24 hr	D	D	D	D	D	D	D

D: reactions dispersing

PBS: phosphate buffered saline

TABLE II

Heat and 2-ME reduction-alkylation of pooled P-K positive serum. Horizontal diameters (mm) of dermal responses following antigen challenge of sites sensitised with treated and untreated serum; reactions at control sites are also shown.

Time (min)	Test Sites				Control Sites			
	Untreated P-K positive serum	56°C for 2 hours	2-ME/iodoacetamide	Iodoacetamide alone	Sensitisation		Challenge	
					Saline	Foal serum	Antigen	FBS
5	10	11	12	11	10	10	9	10
30	16	11	14	15	12	11	9	10
60	16	12	14	18	D	10	10	11
120	20	14	15	22	D	12	D	D
360	21	14	D	22	D	12	D	D
24 hr	D	D	D	D	D	D	D	D

D: reactions dispersing FBS: phosphate buffered saline

homologous skin for between 5 and 9 days. Although this is less than the latent period reported for the reaginic antibody in other species (Stanworth, 1973), the exponential decay of both reaginic antibody activity and IgE in homologous skin (Augustin, 1967; Ishizaka and Ishizaka, 1971) indicates that the duration of detectable levels will be dependent upon the initial antibody titre. Thus interspecies variation in latency is to be expected.

Like human IgE (Dorrington and Bennich, 1973), horse reagin is thermolabile at 56°C and is sensitive to thiol reduction. In contrast, IgG antibodies are typically thermostable at 56°C (Bryant et al., 1975) and retain their biological activity when dialysed against thiol reducing agents at neutral pH (Stanworth and Turner, 1973).

P-K activity in the DEAE-cellulose eluate was detected overlapping in part the major IgG peak and extending into trailing IgG fraction, but not overlapping the IgM fraction. After DEAE-Sephadex chromatography of whole reaginic human serum or isolated immunoglobulin fractions using Tris-HCl (pH 8.0) buffer, the P-K activity is similarly distributed about the IgG and IgM peaks and is eluted in the molar range 0.1M - 0.3M (Ishizaka and Ishizaka, 1967; Johansson et al., 1968; Perelmutter and Liakopoulou, 1971). Although the Sephadex chromatography medium used by these authors differs from the cellulose medium used in the present study, the DEAE anion exchange group is the same and the similarity in elution characteristics of the homocytotropic antibody in horse and human serum, particularly in relation to the major immunoglobulin classes, indicates some similarity in net molecular charge.

The distribution of detectable P-K activity after DEAE chromatography of horse serum does not parallel that of the immunoglobulin classes Gab, Gc, G(T) and M, suggesting that this activity resides outside these classes. However, the possibility remains that the equine homocytotropic activity may be associated with the remaining immunoglobulin classes, namely IgA, AI (aggregating immunoglobulin) and 10S_{γ1} immunoglobulin. The respective biological roles of AI and the 10S_{γ1} immunoglobulin have not been elucidated. However, the latter immunoglobulin, occurring in anti-β lactoside sera, is sensitive to 2-mercaptoethanol reduction (Raynaud and Iskaki, 1970) and probably merits further investigation of its relationship with the reagin-like antibody.

Unlike the classical weal and flare response in human skin, the P-K response in horse skin elicited using whole serum appears biphasic. The initial weal-like response observed within 30-60 minutes is followed by a period during which the dermal response increases in size for up to 6 hours. Similar reactions have been observed in horse skin following both i/d challenge of sensitised individuals with parasitic antigens and i/d challenge with artificial mast cell degranulating compounds (Mansman and Mansman, 1975). Whether the prolonged phase of the P-K responses observed in the present study is the result of an Arthus-type response

mediated by non-homocytotropic antibodies is not known. However, the elimination of both phases of the P-K response following heating and thiol reduction of the sensitising serum suggests the entire response is dependent upon reaginic-type antibodies. Furthermore, the persistence of a biphasic response up to 5 days after sensitisation suggests that non-cytotropic IgG antibodies play no role in the genesis of the response, the estimated half-life of these antibodies in skin being 8-12 hours (Kuhns, 1961). The occurrence of isolated 30-60 minute P-K responses at sites sensitised by DEAE-52 peaks 2-6 indicates, however, that one or more additional serum factors may be involved in the genesis of the 2-6 hour phase of the reagin dependent response. Alternatively, should the delayed phase of the P-K response be dependent on high titres of sensitising antibody, then dilution and loss of the antibody during DEAE fractionation may have resulted in insufficient amounts of the antibody in the final fractions to initiate the delayed response.

The biphasic P-K response in horse skin is morphologically similar to the IgE dependent late cutaneous anaphylactic response (LCAR) in man, typically elicited by direct i/d challenge of allergic individuals with high doses of allergen (Umemoto et al., 1976). Less intense LCARs have been observed following P-K testing of human serum in homologous skin (Dolovich et al., 1973).

The absence of positive P-K responses at 4 sites sensitised with active stage RSD sera may have arisen due to the absence of detectable levels of reagin-like antibodies in the sensitising serum, or from the presence of antigen specific blocking antibodies arising from challenge during the previous summer. Antigen specific IgG antibodies have been induced in man following prolonged antigen exposure (Devey et al., 1976), although their biological role in the inhibition of reagin dependent hypersensitivity responses is not fully understood (Ishizaka and Ishizaka, 1973). Fisher and Connel (1962) have shown that antigen specific blocking antibodies in human sera can confer anaphylactic sensitivity on heterologous skin. In the horse, Cordal and Margni (1974) have demonstrated the occurrence of IgG antibodies in hyperimmune serum which are capable of conferring short-term anaphylactic sensitivity on heterologous skin, suggesting that horses may produce an antibody functionally similar to a human IgG blocking antibody.

The results of this study have shown the occurrence of a reagin-like antibody in horse serum functionally similar to human IgE, and sharing a number of biological properties in common with human IgE. In these respects this antibody fulfils Vaerman's second and third criteria of interspecies protein homology, suggesting the antibody may correspond to human IgE.

ACKNOWLEDGEMENTS

The Horserace Betting Levy Board is thanked for providing financial support

for Dr. Matthews during the course of this work.

REFERENCES

- Allen, P.Z. and Dalton, E.J., 1975. Studies on equine immunoglobulins. IV. Immunoglobulins of the donkey. *Immunology*, 28: 187-197.
- Augustin, R., 1967. Demonstration of reagins in the sera of allergic subjects. In: D.M. Weir (Editor), *Handbook of Experimental Immunology*. Blackwell Scientific Pub., Oxford and Edinburgh, pp. 1056-1151.
- Baker, K.P., 1978. The rational approach to the management of sweet itch. In: C.S. Grunsell and F.W.G. Hill (Editors), *Veterinary Annual*, 18th edition. Scientifica, Bristol, pp. 163-168.
- Bennich, H., Johansson, S.G.O. and Bahr-Lindstrom, H., 1976. Immunoglobulin E. In: E.B. Weiss and M.S. Segal (Editors), *Bronchial Asthma: Mechanisms and Therapeutics*. Little Braun and Company Inc., Boston, pp. 171-179.
- Bryant, J.H., Burns, M.W. and Lazarus, L., 1975. Identification of IgG antibody as a carrier of reaginic activity in asthmatic patients. *J. Allergy clin. Immunol.*, 56: 417-428.
- Cordal, M.E. and Margni, R.A., 1974. Isolation, purification and biological properties of horse precipitating and non-precipitating antibodies. *Immunochemistry*, 11: 765-770.
- Devey, M.E., Wilson, D.V. and Wheeler, A.W., 1976. The IgG subclasses of antibodies to grass pollen allergens produced in hay fever patients during hypsensitization. *Clin. Allergy*, 6: 227-236.
- De Weck, A., 1972. In discussion of McGuire, T.C., Crawford, T.B. and Henson, J.B., 1972. The isolation, characterisation and functional properties of equine immunoglobulin classes and subclasses. *Proc. 3rd Int. Conf. Equine Inf. Dis.* Karger, Basel, pp. 364-381.
- Dolovich, J., Hargreaves, F.E., Chalmers, R., Shier, K.J., Gaudie, J. and Bienenstock, J., 1973. Late cutaneous allergic responses in isolated IgE dependent reactions. *J. Allergy clin. Immunol.*, 52: 38-45.
- Dorrington, K.H. and Bennich, H., 1973. Thermally induced structural changes in immunoglobulin E. *J. biol. Chem.*, 248: 8378-8384.
- Eyre, P., 1972. Anaphylactic (skin sensitising) antibodies in the horse. *Vet. Rec.*, 90: 36-37.
- Fisher, J.P. and Connel, J.T., 1962. Passive cutaneous anaphylaxis in the guinea pig with serum of allergic patients treated with ragweed emulsions. *J. Allergy*, 33: 59-61.
- Halliwell, R.E.W., Fleischman, J.B., Mackay-Smith, M., Beech, J. and Gunson, D.E., 1979. The role of allergy in chronic pulmonary disease of horses. *J. Am. vet. med. Assoc.*, 174: 277-281.
- Hirschfeld, J., 1960. Immunolectrophoresis: procedure and application to the study of group specific variations in sera. *Science Tools*, 7: 18-25.
- Ishizaka, K. and Ishizaka, T., 1967. Identification of YE-antibodies as a carrier of reaginic activity. *J. Immunol.*, 99: 1187-1198.
- Ishizaka, K. and Ishizaka, T., 1971. IgE immunoglobulins of human and monkey. In: E.L. Becker and K.F. Austen (Editors), *Biochemistry of the Acute Allergic Reactions*. Blackwell Scientific Pub., Oxford, pp. 13-30.
- Ishizaka, K. and Ishizaka, T., 1973. The role of IgE and IgG antibodies in reaginic hypersensitivity in the respiratory tract. In: K.F. Austen and L.M. Lichtenstein (Editors), *Asthma*. Academic Press, New York and London, pp. 55-68.
- Johansson, S.G.O., Bennich, H. and Wide, L., 1968. A new class of immunoglobulin in human serum. *Immunology*, 14: 265-272.
- Kuhns, W.J., 1961. Disappearance of diphtheria antitoxin from human passive transfer skin sites. *Proc. Soc. exp. Biol. Med.*, 108: 63-67.

- Mansman, R.A. and Mansman, R.A.D., 1975. Equine immunology in health and disease. Am. Assoc. Equine Pract. Newsletter, 2: 44-72.
- McCaig, J. and Mellor, P.S., 1974. The probable cause of "sweet itch" in England. Vet. Rec., 95: 411-415.
- McPherson, E.A., Lawson, G.H.K., Murphy, J.R., Nicholson, Janet M., Breeze, R.G. and Pirie, H.M., 1979. Chronic obstructive pulmonary disease (COPD): Aetiological studies: responses to intradermal and inhalation antigen challenge. Equine vet. J., 11: 159-166.
- Neilsen, K.H., 1977. Bovine reaginic antibody. III. Cross reaction of anti-human IgE and antibovine reagin immunoglobulin with sera from several species of mammals. Can. J. comp. Med., 41: 345-348.
- Perelmutter, L. and Liakopoulou, A., 1971. Detection of IgE mediated immediate hypersensitivity reactions in the sera of ragweed sensitive individuals using rat mast cells. Int. Arch. Allergy, 40: 481-494.
- Raynaud, M. and Iskaki, S., 1970. Classification of equine immunoglobulins. Proceedings of the International Symposium on Antilymphocyte Serum, Versailles. Karger, Basel, pp. 101-124.
- Riek, R.F., 1954. Studies on allergic dermatitis (Queensland itch) of the horse: the aetiology of the disease. Aust. J. agric. Res., 5: 109-129.
- Ritzenthaler, M., 1924. L'anaphylaxie du cheval. Arch. int. Physiol., 24: 54-71.
- Schatzman, U., De Weck, A.L., Gerber, H., Lazary, S., Straub, R. and Pauli, B., 1973. Active and passive cutaneous anaphylaxis in the horse following immunisation with benzoyl-penicilloyl-bovine gammaglobulin (BPO₂₀-BGG). Res. vet. Sci., 15: 347-352.
- Stanworth, D.R., 1973. Immediate hypersensitivity. North Holland Research Monographs, vol. 28. North Holland Publishing Company, Amsterdam.
- Stanworth, D.R. and Turner, M.W., 1973. Immunochemical analysis of immunoglobulins and their subunits. In: D.M. Weir (Editor), Handbook of Experimental Immunology, 2nd edition. Blackwell Scientific Pub., Oxford and Edinburgh, chapter 10.
- Umamoto, L., Poothullil, J., Dolovich, J. and Hargreave, F.E., 1976. Factors which influence late cutaneous allergic responses. J. Allergy clin. Immunol., 58: 60-68.
- Vaerman, J.P., 1970. Cited by Halliwell, R.E.W., Schwartzman, R.M. and Rockey, J.H., 1972. Antigenic relationship between human IgE and canine IgE. Clin. exp. Immunol., 10: 399-407.

A REAGIN-LIKE ANTIBODY IN HORSE SERUM

II. ANTI-HUMAN IgE INDUCED REVERSED CUTANEOUS ANAPHYLAXIS-LIKE RESPONSES IN HORSE SKIN

A.G. MATTHEWS¹, P. IMLAH² and E.A. McPHERSON¹

¹Department of Veterinary Medicine and ²Department of Animal Health, University of Edinburgh, Veterinary Field Station, Easter Bush, Roslin, Midlothian, EH25 9RG (Great Britain)
Dr. Matthews' present address: Ratley Lodge, Ratley, Banbury, Oxford, (Great Britain)

(Accepted 1 July 1982)

ABSTRACT

Matthews, A.G., Imlah, P. and McPherson, E.A., 1983. A reagin-like antibody in horse serum. II. Anti-human IgE induced reversed cutaneous anaphylaxis-like responses in horse skin. *Vet. Res. Commun.*, 6: 111-122.

Fc specific anti-human IgE serum induced prolonged reversed cutaneous anaphylaxis (RCA)-like reactions in horse skin. Morphologically and histologically, these reactions resembled passively induced late cutaneous anaphylaxis responses in human skin, but differed from reversed passive Arthus responses induced in horse skin using anti-horse IgG serum. The induction of RCA-like responses in horse skin by anti-human IgE indicates shared Fc antigenic determinants on human IgE and a horse homocytotropic or reagin-like antibody.

INTRODUCTION

Reagin-like antibodies, homologues of human IgE, have been identified in the serum of a number of mammalian species (Nielsen, 1977).

Vaerman (1970) proposed three orders of criteria to be applied in the identification of interspecies protein homology. The second and third orders of criteria, those of functional and biological homology, have been satisfied in the case of human IgE and an equine reagin-like antibody (Matthews, 1981; Matthews et al., 1983). The first order criterion establishing homology of these antibodies, that of immunological cross reactivity and amino acid sequence homology, is examined in the present paper.

Ishizaka and Ishizaka (1968) described the induction of typical weal and flare responses following intradermal (i/d) injection of anti-human IgE into human volunteers. This reaction was described as reversed cutaneous anaphylaxis (RCA)

and i/d injection of anti-human IgG, IgM, IgA and IgD serum failed to induce similar responses. Subsequently, Ishizaka and Ishizaka (1969) demonstrated that the apparent bridging of adjacent IgE molecules on the surface membranes of basophils by the bivalent F(ab¹)₂ fragment of an antibody directed specifically against the Fc antigenic determinants on the IgE molecules resulted in the release of intracellular vasoactive mediators, and it was postulated that a similar mechanism probably resulted in the anti-IgE mediated RCA responses via IgE coated dermal mast cells.

Thus, if common heavy chain antigenic determinants exist on the Fc fragments of heterologous reaginic antibodies, inoculation of Fc specific anti-human IgE sera should elicit RCA-type responses in heterologous skin. Yet, it is only in the dog and a marsupial, the quokka (Setonix brachyurus), that this relatively simple indicator of shared antigenic determinants has been described (Halliwell et al., 1972; Lynch and Turner, 1974). In the present series of experiments the induction of RCA-like responses in horse skin by anti-human IgE is described and compared to the responses elicited by inoculation of antisera against horse immunoglobulins and immunoglobulin fragments.

MATERIALS AND METHODS

Horses

Eight horses from the experimental stock maintained by the Department of Veterinary Medicine of the University of Edinburgh were used.

Intradermal procedures

Lateral cervical test sites were used throughout.

Anti-human IgE

0.1 ml of Fc specific sheep anti-human IgE serum (Sh Ah IgE) (Nordic Immunological Laboratories, Maidenhead, England) and 0.1 ml of fresh sheep serum were injected i/d at separate sites into horses 1 to 6. The response to challenge with globulin and control serum was recorded as the horizontal diameter of the reaction (mm) at 5, 30, 60, 120 and 360 minutes, and at 24 hours.

Anti-horse immunoglobulins

0.1 ml of the anti-immunoglobulin serum and 0.1 ml of the respective control serum were injected i/d into separate sites on the experimental horses as follows: Fc specific rabbit anti-horse IgG serum (Rb Aeq IgG) into horses 6, 7 and 8, rabbit anti-whole horse serum (Rb Aeq WS) into horses 1 and 2, Fc specific goat

anti-horse IgG (T) serum (Gt Aeq IgG (T)) into horse 1, and pig anti-horse light chain (kappa and lamda determinants) serum (Sw Aeq Lc) into horse 3. These sera were purchased from Miles Laboratories Ltd., Slough, England.

The antibody activity of the test antisera was checked by immunoelectrophoresis prior to i/d use.

The dermal responses were recorded as above.

Dermal biopsy

Sh Ah IgE and control sheep serum were each inoculated at three sites in horses 4 and 5. Rb Aeq IgG and control rabbit serum were each inoculated at three sites in horses 7 and 8. On each animal a separate pair of sites, consisting of one test site and one control site, were biopsied at 30 minutes, 4 hours and 24 hours post inoculation. Full thickness dermal biopsies were removed from the centre of the lesion using an 8 mm biopsy punch under local anaesthesia. Biopsy specimens were transferred into Bouin's fixative and, after processing to wax, 6 μ m sections were prepared and stained with haematoxylin and eosin, Giemsa and carbol chromotrope. For reference, biopsies were taken from untreated sites in two horses.

RESULTS

Intradermal procedures

The horizontal diameters of the reactions at increasing intervals after i/d inoculation of Sh Ah IgE and the various anti-homologous whole sera and immunoglobulin sera are presented in Table I.

Anti-human IgE

Following i/d inoculation of Sh Ah IgE, a discrete weal-like reaction was apparent within 30 minutes in all six horses, the diameter of which was 5 or more mm greater than that of the initial i/d bleb. These reactions continued to increase in size for up to 360 minutes and, with the exception of horse 6, had dispersed by 24 hours. In horse 6, a diffuse raised reaction was evident at both the challenge and control sites at 24 hours. Comparable responses did not occur after sheep serum challenge of horses 1 to 5, although the initial injection bleb remained apparent for up to 2 hours. In horse 6, a reaction was apparent at the control site at 360 minutes, although this response was less marked than at the Sh Ah IgE site.

TABLE I

The response of normal horse skin to intradermal challenge with anti-human IgE, anti-whole horse serum, and anti-horse IgG, IgG (T) and immunoglobulin light chain, expressed as horizontal diameter (mm) of the reaction

Challenge	Horse number	Minutes				Hours		Note
		5	30	60	120	24	360	
Sheep anti-human IgE	1	12	18	20	22	28	D	
	2	10	19	20	22	27	D	
	3	10	16	21	23	24	D	
	4	10	17	17	22	22	D	Biopsied
	5	10	16	20	22	24	D	Biopsied
	6	10	16	17	19	26	Diffuse raised area	
Sheep serum	1	10	11	D	D	D	D	
	2	10	10	D	D	D	D	
	3	10	10	12	12	D	D	
	4	10	10	10	D	D	D	Biopsied
	5	10	12	13	13	D	D	Biopsied
	6	12	12	12	13	17	Diffuse raised area	
Rabbit anti-horse IgG	6	10	10	13	17	29	Diffuse raised area	
	7	6	6	10	15	21	Diffuse raised area	Biopsied
	8	10	8	10	13	20	D	Biopsied
	6	13	14	14	14	13	Diffuse raised area	
Rabbit serum	7	6	6	8	7	D	D	Biopsied
	8	10	8	10	8	12	D	Biopsied
	1	10	14	14	22	50	55 R	
Rabbit anti-whole horse serum	2	10	13	14	25	50	D	
	1	10	11	11	10	D	D	
Rabbit serum	2	10	13	13	D	D	D	
	3	10	14	14	25	25	D	
Pig anti-horse light chain	3	10	14	13	6	D	D	
	1	10	12	13	15	20	D	
Goat anti-horse IgG (T)	1	10	11	14	16	20	D	
	1	10	11	14	16	20	D	

D = dispersed

R = reaction present at 48 hours as a discrete raised plaque

Anti-
Foll
tion bl
by 360
at 360
oedema.
apparen
serum f
although
of horse
I/d c
like res
I/d c
at 120 m
and was
challeng
rabbit s

Dermal b

The h
with Sh
Table II.

Anti-
Biopsi
reaction
Accompany
vessel wa
and there
neutrophi
biopsies
cellular
Four h
dominantl
4 hours an
of the va
there was
showed ess
morphonuc
in the dec

Anti-horse immunoglobulins

Following i/d inoculation of Rb Aeq IgG, no increase in diameter of the injection bleb was apparent for 1 to 2 hours, although a marked response was apparent by 360 minutes. The reactions induced by Sh Ah IgE and Rb Aeq IgG were identical at 360 minutes, each showing heat, sensitivity and, in some cases, dependent oedema. In two of the three Rb Aeq IgG recipients, the reactions were still apparent at 24 hours as diffuse plaque-like dermal swellings. Normal rabbit serum failed to elicit a comparable response in the three recipient horses, although a diffuse swelling was observed 24 hours after rabbit serum challenge of horse 6.

I/d challenge with Gt Aeq IgG (T) and control goat serum resulted in weal-like responses which increased in diameter for up to 360 minutes in both cases.

I/d challenge with Sw Aeq Lc and Rb Aeq WS resulted in a weal-like response at 120 minutes, which in the latter case increased markedly up to 360 minutes and was still present in horse 1 as a discrete weal-like lesion 48 hours after challenge. In these recipients no reactions resulted from challenge with pig or rabbit serum controls.

Dermal biopsies

The histopathological findings of the dermal biopsies taken after challenge with Sh Ah IgE and Rb Aeq IgG and their respective controls are summarised in Table II.

Anti-human IgE

Biopsies taken 30 minutes after i/d inoculation of Sh Ah IgE showed a vigorous reaction involving the venules and precapillaries of the middle and deep dermis. Accompanying the vasodilation and oedema, the endothelial cells in some of the vessel walls appeared to be swollen, with pale or occasionally pyknotic nuclei and there was a marked intravascular accumulation and transmural migration of neutrophil leucocytes. Eosinophil leucocytes were not observed in the 30-minute biopsies from either horse. In the control biopsies, comparable vascular and cellular reactions were not present.

Four hours after challenge with Sh Ah IgE, the cellular reaction was predominantly perivascular. Transmural neutrophil migration was still occurring at 4 hours and there was evidence of cytoplasmic swelling, vacuolation and pyknosis of the vascular endothelial cells. Oedema was still apparent and in horse 5 there was perivascular lymphatic distension. The control biopsies at this time showed essentially normal dermis, although a limited perivascular, mainly polymorphonuclear infiltrate was observed associated with some of the blood vessels in the deep dermis of horse 5.

TABLE II

Summary of histopathological findings of skin biopsies taken 0.5, 4 and 24 hours after intradermal challenge of 4 horses with sheep anti-human IgE (Sh Ah IgE) or rabbit anti-equine IgG (Rb Aeq IgG) and their respective serum controls

Horse number	Challenge	Biopsy time (hr)	Dermal oedema	Vasculitis	Cellular infiltration			Cell types				
					Intra-vascular ^a	Trans-mural ^b	Peri-vascular ^c	Collagenous phils	Neutro-phils	Eosino-phils	Mono-nuclear	
4	Sh Ah IgE	0.5	2	1	3	3	1	-	1	-	-	
		4	2	1	1	2	3	3	3	-	2e	
		24	-	-	-	-	1	1	1	1	-	1f
	Sheep serum	0.5	1	-	-	-	-	-	-	-	-	
		4	-	-	-	-	-	-	-	-	-	
		24	1	-	-	-	1	1	1	1	-	2f
5	Sh Ah IgE	0.5	2	1	2	3	-	-	1	-	-	
		4	4	1	1	2	4	3	3	-	1e	
		24	2	1	-	-	3	2	1	1	-	3f
	Sheep serum	0.5	1	-	-	-	-	-	-	-	-	
		4	1	-	-	-	1	1	1	2	-	1e
		24	1	1	-	-	1	1	1	1	-	3g
7	Rb Aeq IgG	0.5	-	-	-	-	-	-	-	-	-	
		4	3	1	2	2	3	-	5	-	1f	
		24	3	2-3	-	2	5	5	3	-	1g	
	Rabbit serum	0.5	1	-	-	-	-	-	-	-	-	
		4	-	-	-	-	-	-	-	-	-	
		24	1	1	-	-	2	1	6	-	-	1f
8	Rb Aeq IgG	0.5	-	-	1	-	-	-	1	-	-	
		4	3	2-3	1	3	4	2	4	-	1g	
		24	4	2-3	-	3	5	5	3	-	1g	
	Rabbit serum	0.5	1	-	-	-	-	-	-	-	-	
		4	1	1	-	-	-	-	-	-	-	
		24	1	1	-	-	2	3	5	3	-	1f

Table II (continued)

Explanatory notes

The severity of dermal oedema and cellular infiltration in the biopsy sections is graded from absent (-) to maximum (5). The relative numbers of the cell types present are similarly graded.

Dermal oedema: disruption of collagen bundles in dermis with accumulation of proteinaceous fluid in the interstitial spaces. Occasional lymphatic distension.

Vasculitis: 1 endothelial swelling
2 degeneration of the vascular endothelium
3 frank perivascular necrosis.

Cellular infiltration: a clumping of cells within dermal blood vessels
b cells within the walls of dermal vessels
c cells grouped around dermal blood vessels
d cellular infiltration of dermal interstitium unassociated with blood vessels.

Cell type: e mononuclear cells, predominantly lymphocytes
f mononuclear cells, predominantly lymphocytes with occasional macrophages
g mixed mononuclear cells, predominantly macrophages.

The dermal biopsies 24 hours after Sh Ah IgE and normal sheep serum challenge in both horses showed a predominantly mononuclear perivascular and collagenous cellular reaction which was more marked in horse 5. Lymphocytes predominated with occasional macrophages and eosinophils. Both control biopsies showed a limited perivascular inflammatory response, possibly the result of foreign protein challenge.

Anti-horse IgG

Thirty minutes after i/d challenge with Rb Aeq IgG, the biopsy from horse 7 showed normal dermis while that from horse 8 showed limited intravascular accumulation of neutrophil leucocytes affecting only a few vessels. Both control biopsies showed only normal dermis.

Four hours after Rb Aeq IgG challenge, biopsies from both horses showed a vigorous cellular reaction in and around dilated and occasionally thrombosed precapillaries and venules in the middle and deep dermis. There was transmural migration and perivascular accumulation of predominantly neutrophil leucocytes, many of which appeared pyknotic and dying. The majority of the infiltrating mononuclear cells were macrophages. Dermal oedema, haemorrhage and accumulation of homogeneous eosinophilic material were present in both biopsies and, in the biopsy taken from horse 8, focal areas of necrosis around obliterated blood vessels were apparent in the deep dermis. Control biopsies showed no changes from normal, with the exception of interstitial oedema in horse 8.

Twenty-four hours after i/d Rb Aeq IgG challenge of both horses, there was marked cellular infiltration, extensive interstitial oedema, haemorrhage and accumulation of homogeneous eosinophilic material. Many of the blood vessels appeared to be obliterated by overt necrosis. A mixed population of cells was present in both test biopsies, the relative numbers of mononuclear cells having increased. Examination of both 24-hour control biopsies showed a diffuse, predominantly neutrophil response with evidence of limited vascular degeneration. Relatively fewer macrophages were present in the control in comparison to the test reactions.

DISCUSSION

These observations on i/d Sh Ah IgE inoculation in the horse indicate that the antiglobulin is capable of inducing an immediate and prolonged response similar to the response elicited in horse skin using a Prauznitz-Küstner (P-K) model (Matthews et al., 1983). Furthermore, the anti-IgE induced response appears to be morphologically similar to that reported following i/d inoculation of both histamine and Compound 48/80 (Mansman and Mansman, 1975), suggesting that the response is a reversed anaphylaxis associated with antiglobulin induced basophil

degranulation. By analogy with RCA responses in primate skin (Ishizaka and Ishizaka, 1969) the anti-human IgE induced response in horse skin will be dependent upon interaction of the $F(ab^1)_2$ fragment of the antiglobulin with the antigenic determinants on the Fc fragment of a horse homocytotropic or reagin-like antibody.

Although the RCA responses induced in primates (Ishizaka and Ishizaka, 1969) and dogs (Halliwell et al., 1972) by i/d inoculation of anti-human IgE are typically transient weal and flare responses, both Dolovich et al. (1973) and Umemoto et al. (1976) reported prolonged late cutaneous anaphylaxis-like responses (LCARs) after i/d inoculation of anti-human IgE in atopic and non-atopic human subjects. These reversed LCAR-like responses were morphologically similar to the anti-human IgE responses observed in the present study.

Both the 30-minute and 4-hour biopsies of sites challenged with Sh Ah IgE show many of the histological features of passive cutaneous anaphylaxis (PCA) in laboratory animals (Fisher and Cooke, 1957). However, the RCA-like reactions in the present study develop more slowly than the PCA responses in laboratory animals, as shown by the presence of significant transmural leucocyte migration associated with blood vessels in the 4-hour anti-IgE biopsies. By 4 hours, leucocytic infiltration of PCA biopsies is predominantly perivascular.

The extent of the oedema, cellular infiltration and vascular damage observed in the 4-hour anti-IgE biopsies in the present series is similar to that reported by Solley et al. (1976) in 4-hour biopsies of LCARs elicited in human skin during P-K testing of ragweed sensitive human serum. These authors reported approximately 1:1 ratios of polymorphonuclear and mononuclear cells in the infiltrate, in contrast to the predominantly polymorphonuclear cell infiltrate in the present series. However, Dolovich et al. (1973) earlier reported a predominantly polymorphonuclear leucocyte infiltration of LCAR biopsies in non-atopic subjects after challenge with anti-human IgE or anti- $F(ab^1)_2$ of human IgE.

Although the histopathological features of RCA-like responses in the present study indicate the participation of both leucotactic and vasoactive substances in the genesis of the response, a major difference between these responses and previous reports of dermal anaphylactic-like responses in the horse is the absence of eosinophil leucocytes (Riek, 1954; Baker and Quinn, 1978). Mansman and Mansman (1975) observed LCAR-like responses following i/d injection of Compound 48/80 in horses although, histologically, eosinophilia of the dermis was not apparent. Compound 48/80 is a non-cytolytic releaser of vasoactive substances from mast cells (Kazimierczak and Diamant, 1978). However, in contrast to comparable mast cell acting agents, such as Concanavalin A, Compound 48/80 is a poor releaser of histamine from both human and horse blood basophils (Siraganian and Siraganian, 1975; Kings and de Weck, 1980). Histamine is potentially eosinophilotactic (Archer, 1960), but it is only one of a number of

compounds released during systemic and local anaphylaxis in the horse, including 5-HT, kinins and prostaglandins (Hanna et al., 1982). These latter compounds have not been shown to be eosinophilotactic (Broder, 1979). Although the relative individual importance of these vasoactive mediators in the genesis of dermal anaphylaxis in the horse is not known, Burka et al. (1976) published preliminary data showing that in the horse the relative percentages of the available mediators in a tissue preparation which are released after immunological or non-immunological basophil disruption vary with the nature of the disruptive challenge. Furthermore, there is evidence to indicate that the histamine releasing activity of chemical mast cell "degranulating" agents varies between tissues and species (Kazimierczak and Diamant, 1978). Hypothetically, therefore, Compound 48/80 and anti-human IgE inoculated into horse skin may result in the release of a spectrum of vasoactive and cytotoxic agents different from that released by direct antigen challenge of sensitised skin, and may provoke a typical anaphylactic response without eosinophilia.

Unlike the Sh Ah IgE induced RCA response, an Rb Aeq IgG response was inapparent at 30 minutes, but by 4 hours had developed into an oedematous dermal weal which histologically showed a severe necrotising vasculitis. This contrasts with the infiltrative perivascular cellular response observed in the 4-hour anti-IgE biopsy. By 24 hours, the anti-IgG response in two of the three recipient horses was still apparent as a diffuse plaque-like lesion, which histologically showed evidence of widespread perivascular necrosis and interstitial cellular infiltration in contrast to the receding anti-IgE reactions at 24 hours. This Rb Aeq IgG response is a model of the reversed passive Arthus response (RPAR) (Anderson, 1976), classically elicited by i/d antibody inoculation followed by intravenous antigen challenge, the antigen in this case being endogenous circulating IgG. Morphologically, the RPARs in this series show the characteristic features of a Type III hypersensitivity reaction (Ranadive and Movat, 1979). Similar RPAR-like responses were also observed after i/d inoculation of anti-whole horse serum and anti-horse light chain serum. However, the failure of anti-IgG (T) to induce a response is unexpected, but may be the result of low circulating IgG (T) levels in this horse.

Since the RCA-like responses induced by Sh Ah IgE in horse skin, which have been described in the present study, appear to result from antigenic cross reactivity of the antiglobulin with basophil bound homocytotropic antibody, the morphological similarity of the RCA and RPAR-like responses at 4 hours might suggest that the later stages of the former response may be an Arthus reaction mediated by anti-IgE-homocytotropic antibody complexes. However, the histopathological differences between the two responses indicate that the reactions occur independently of one another and that the prolonged RCA-like responses are primarily dependent upon common Fc antigenic determinants on the horse homocyto-

tropic or reagin-like antibody and human IgE.

Both Vaerman et al. (1969) and Neoh et al. (1973) have stated that immunological cross reactivity is a practical alternative to sophisticated amino acid sequencing as a means of demonstrating homology in primary polypeptide chain structure. The results of the present study indicate some degree of immunological cross reactivity between human IgE and horse homocytotropic antibody, thus satisfying Vaerman's (1970) first order criterion of interspecies protein homology, namely that of immunological cross reactivity and amino acid sequence homology.

ACKNOWLEDGEMENTS

The Horserace Betting Levy Board is thanked for its financial support of Dr. Matthews during the course of this work. Thanks are also due to Mr. A.C. Rowland for his invaluable help in the histological interpretation.

REFERENCES

- Anderson, J.R., 1976. Immunopathology. In: J.R. Anderson (Editor), Muir's Textbook of Pathology, 10th edition. Arnold Ltd., London, pp. 128-143.
- Archer, R.K., 1960. Eosinophil leucocyte attracting effect of histamine in skin. *Nature*, 187: 155-156.
- Baker, K.P. and Quinn, P.J., 1978. A report on the clinical aspects and histopathology of "sweet itch". *Equine Veterinary Journal*, 10: 243-248.
- Broder, I., 1979. Anaphylaxis. In: H.Z. Movat (Editor), *Inflammation, Immunity and Hypersensitivity*, 2nd edition. Harpers Row Publishers, Hagerstown, pp. 319-374.
- Burka, J.F., Delina, T.R., Holroyde, M.C. and Eyre, P., 1976. Chemical mediators of anaphylaxis (histamine, 5-HT and SRS-A) released from horse lung and leucocytes in vitro. *Research Communications in Chemical Pathology and Pharmacology*, 13: 379-388.
- Dolovich, J., Hargreave, F.E., Chalmers, R., Shier, K.J., Gauldie, J. and Bienenstock, J., 1973. Late cutaneous allergic responses in isolated IgE-dependent reactions. *Journal of Allergy and Clinical Immunology*, 52: 38-45.
- Fisher, J.P. and Cooke, R.A., 1957. Passive cutaneous anaphylaxis (PCA) in the guinea pig. An immunologic and pathologic study. *Journal of Allergy*, 28: 150-169.
- Halliwell, R.E.W., Schwartzman, R.M. and Rockey, J.H., 1972. Antigenic relationship between human IgE and canine IgE. *Clinical and Experimental Immunology*, 10: 399-407.
- Hanna, C.J., Eyre, P., Wells, P.W. and McBeath, D.G., 1982. Equine immunology 2: Immunopharmacology - Biochemical basis of hypersensitivity. *Equine Veterinary Journal*, 14: 16-24.
- Ishizaka, K. and Ishizaka, T., 1968. Reversed type allergic skin reactions by anti-γE globulin antibodies in humans and monkeys. *Journal of Immunology*, 100: 554-562.
- Ishizaka, K. and Ishizaka, T., 1969. Immune mechanisms of reversed type reaginic hypersensitivity. *Journal of Immunology*, 103: 588-595.
- Kazimierczak, W. and Diamant, B., 1978. Mechanisms of histamine release in anaphylactic and anaphylactoid reactions. *Progress in Allergy*, 24: 295-337.

- Kings, M.A. and de Weck, A.L., 1980. Pharmacological and immunological aspects of histamine release from horse leucocytes. *International Archives of Allergy and Applied Immunology*, 62: 397-408.
- Lynch, N.R. and Turner, K.J., 1974. Antigenic homology between a marsupial homocytotropic antibody and human IgE. *Immunochemistry*, 11: 753-755.
- Mansman, R.A. and Mansman, R.A.D., 1975. Equine immunology in health and disease. *American Association of Equine Practitioners, Newsletter*, 2: 44-72.
- Matthews, A.G., 1981. Some serological studies in the horse in relation to the pathogenesis of chronic obstructive pulmonary disease. Ph.D. Thesis, University of Edinburgh.
- Matthews, A.G., Imlah, P. and McPherson, E.A., 1983. A reagin-like antibody in horse serum. 1. Occurrence and some biological properties. *Veterinary Research Communications*, 6: 13-23.
- Neilsen, K.H., 1977. Bovine reagenic antibody. III. Cross reaction of antihuman IgE and antibovine reagin immunoglobulin with sera from several species of mammals. *Canadian Journal of Comparative Medicine*, 41: 345-348.
- Neoh, S.H., Jahoda, D.M., Rowe, D.S. and Voller, A., 1973. Immunoglobulin classes in mammalian species identified by cross reactivity with antisera to human immunoglobulin. *Immunochemistry*, 10: 805-813.
- Ranadive, N.S. and Movat, H.Z., 1979. Tissue injury and inflammation induced by immune complexes. In: H.Z. Movat (Editor), *Inflammation, Immunity and Hypersensitivity*, 2nd edition. Harpers Row Publishers, Hagerstown, pp. 409-444.
- Riek, R.F., 1954. Studies on allergic dermatitis (Queensland itch) of the horse. The aetiology of the disease. *Australian Journal of Agricultural Research*, 5: 109-129.
- Siraganian, R.P. and Siraganian, P.A., 1975. Mechanisms of action of Concanavalin A on human basophils. *Journal of Immunology*, 114: 886-893.
- Solley, G.O., Gleich, G.J., Jordan, R.E. and Schroeter, A.L., 1976. The late phase of the immediate wheal and flare skin reaction. *Journal of Clinical Investigation*, 58: 408-421.
- Umemoto, L., Poothullil, J., Dolovich, J. and Hargreave, F.E., 1976. Factors which influence late cutaneous allergic responses. *Journal of Allergy and Clinical Immunology*, 58: 60-68.
- Vaerman, J.P., 1970. Cited by Halliwell, R.E.W., Schwartzman, R.M. and Rockey, J.H., 1972.
- Vaerman, J.P., Heremans, J.F. and von Kerchoven, G., 1969. Identification of IgA in several mammalian species. *Journal of Immunology*, 103: 1421-1423.

A STUDY ON THE POSSIBLE ROLE OF CHYMOTRYPSIN IN THE AETIOLOGY OF
EQUINE CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

JILL R. THOMSON, E.A. McPHERSON, G.H.K. LAWSON, PENNY WOODING and R. BROWN

University of Edinburgh, Royal (Dick) School of Veterinary Studies, Veterinary
Field Station, Easter Bush, Roslin, Midlothian EH25 9RG (Great Britain)

(Accepted 11 August 1982)

ABSTRACT

Thomson, J.R., McPherson, E.A., Lawson, G.H.K., Wooding, P. and Brown, R., 1983.
A study on the possible role of chymotrypsin in the aetiology of equine
chronic obstructive pulmonary disease (COPD). Vet. Immunol. Immunopathol.,
4: 387-395.

The chymotrypsin activity of seven batches of Micropolyspora faeni and of
five batches of Aspergillus fumigatus culture extracts, prepared for inhalation
challenge in horses, was assayed and was found to range between 0.29 and 1.45
units/mg protein and 0.02 and 0.20 units/mg protein respectively. Horses
affected with chronic obstructive pulmonary disease (COPD) were challenged with
two batches of each antigen which had different chymotrypsin activities and no
significant correlations were found between the degree of response to challenge
and the chymotrypsin activity of the antigens.

Inhalation of two doses of nebulised, purified chymotrypsin over 4 days did
not induce signs of respiratory disease in COPD-affected horses. However,
repeated chymotrypsin inhalations after an interval of 3 weeks caused an
exacerbation of signs of COPD in one horse. These studies suggest that,
although repeated inhalation of purified chymotrypsin may induce respiratory
hypersensitivity in horses, the chymotrypsin-like enzymes of M. faeni and
A. fumigatus do not play a major role in the precipitation of clinical signs
of equine COPD.

INTRODUCTION

A wide range of enzymes are known to be produced by bacteria and fungi as
metabolic by-products of their growth processes. Many of these enzymes are
released extracellularly on to the substrate and some may enter the atmosphere
on dust particles and may thus be inhaled (Matsubara and Feder, 1970). Such
enzymes, many of which are proteolytic, are widely used in the manufacturing
industry and repeated exposure to them has been found to cause respiratory
disease in man. This occurs, for example, in factory workers exposed to
Bacillus subtilis proteases in the manufacture of enzyme detergents (Flindt, 1969;

Pepys et al., 1969) and in factory workers exposed to papain in the manufacture of meat tenderiser (Flindt, 1978). Certain enzymes may also give rise to pulmonary emphysema (Goldring et al., 1972). It has also been postulated that enzymes released from Micropolyspora faeni and Aspergillus fumigatus may be involved in the aetiology of farmer's lung and pulmonary aspergillosis in man (Nicolet et al., 1975).

The process by which these inhaled enzymes give rise to respiratory disease is not fully understood, but the following hypotheses have been proposed:

- i) Enzymes may simply act as antigens and induce an immunological response without being pharmacologically or enzymatically active.
- ii) Enzymes may act pharmacologically on the respiratory mucosa giving rise to inflammation, airway constriction and mucosal secretion and, due to their enzymatic action, may digest lung tissue resulting in irreversible lung damage (Nicolet et al., 1975).

M. faeni, a thermophillic actinomycete frequently recovered from spoiled hay, is the major allergen involved in farmer's lung in man and animals (Pepys, 1969; Pirie et al., 1971). Walbaum et al. (1969) and Biguet et al. (1974) have shown that farmer's lung patients usually show precipitating antibodies against enzymes of M. faeni, some of which are chymotrypsin-like. The same phenomenon has been observed in cattle with farmer's lung (Nicolet et al., 1974, 1977). Additionally, human patients with pulmonary aspergillosis have been shown to have precipitins against the chymotrypsin-like enzymes of A. fumigatus (Biguet and Vernes, 1974). This demonstrates that these chymotrypsin-like enzymes provide strong antigenic stimuli and that they may be involved in the pathogenesis of farmer's lung and pulmonary aspergillosis in man.

The pharmacological effects of the enzymes is less well documented but experimental inhalation of trypsin, chymotrypsin and other proteases has resulted in bronchoconstriction in dogs and cats not previously exposed to these enzymes (Goldberg et al., 1960; Walbaum et al., 1969).

Equine chronic obstructive pulmonary disease (COPD) has long been associated with the exposure of horses to antigens in mouldy hay, straw and stable dust. In northern Britain, M. faeni and A. fumigatus have been identified as playing a major aetiological role (McPherson et al., 1979). COPD has been shown to involve a respiratory hypersensitivity to these micro-organisms and it is possible that the chymotrypsin-like enzymes released by them into the environment may play a part in the aetiology of the disease.

This study investigates the possible role of chymotrypsin inhalation in the pathogenesis of equine COPD by measuring the chymotrypsin activity of antigens used for inhalation challenge and by correlating the chymotrypsin activity of these antigens with the responses of the COPD-affected horses to challenge. In order to assess the pharmacological effects of inhaled chymotrypsin, COPD-

affected horses were challenged over a short period of time with purified chymotrypsin and the response recorded. The antigenicity of chymotrypsin in horses was also tested by repeating the chymotrypsin inhalations over a longer period.

MATERIALS AND METHODS

1. Assay of the chymotrypsin activity of *M. faeni* and *A. fumigatus* antigens

Seven batches of *M. faeni* and five batches of *A. fumigatus* culture extracts were prepared using the techniques of Lawson et al. (1979). The chymotrypsin activity in each batch of antigen was assayed spectrophotometrically by measuring the increase in absorbency at 256 nm resulting from the hydrolysis of benzoyl-L-tyrosine-ethyl-ester (BTEE). One unit of chymotrypsin hydrolyses one μmol of BTEE per minute at pH 7.8 at 25°C (Hummel, 1959).

2. The response of COPD-affected horses to *M. faeni* and *A. fumigatus* inhalation challenge

Fifteen COPD-affected horses were challenged with *M. faeni*, batch 1, and 19 COPD-affected horses were challenged with *M. faeni*, batch 4. Similarly, *A. fumigatus* batches 2 and 5 were used to challenge 10 and 8 COPD-affected horses respectively. The challenge dose of *M. faeni* and *A. fumigatus* antigens was 12 mg, suspended in 5 ml normal saline. The antigens were aerosolised using a Wright's nebuliser and administered via a face mask which covered the horse's mouth and nose (McPherson et al., 1979).

Prior to antigen challenge, the horses were examined clinically and some respiratory function parameters, i.e. maximum change in intrathoracic pressure (max. Δ Ppl) and partial pressure of arterial oxygen (PaO_2), were recorded as described by McPherson et al. (1978). Following antigen challenge, horses were examined clinically at hourly intervals and max. Δ Ppl and PaO_2 were re-measured after 5 hours. The criteria of McPherson et al. (1979) were used to identify a positive response to antigen challenge, i.e. an increase in max. Δ Ppl at 5 hours after challenge of 15% or more of the pre-exposure value was taken as indicative of a positive response.

The significance of the correlation between the chymotrypsin activity of each batch of antigen and the degree of response to challenge, i.e. the increase in max. Δ Ppl at 5 hours after challenge, was tested by linear regression (Snedecor and Cochran, 1967).

3. Response of COPD-affected horses to purified chymotrypsin inhalation challenge

Eight COPD-affected horses were housed in a controlled environment, i.e. exposure to the aetiological antigens was minimised by bedding horses on peat moss and feeding a complete cubed diet, for 14 days prior to and during the chymotrypsin inhalation studies. Alpha-chymotrypsin, type IV, of bovine pancreas origin (CHY 5S, Sigma London Chemical Company Ltd., Poole, Dorset), activity 37 units/mg protein, was used for inhalation challenge. The 8 COPD-affected horses were challenged with 17.5 units chymotrypsin by inhalation on day 1 and with 52.5 units chymotrypsin on day 4. Two of the 8 COPD-affected horses (A and B) were re-challenged with 17.5 units chymotrypsin on days 22 and 29, and with 52.5 units chymotrypsin on days 25 and 32. The techniques for drug administration, monitoring the horses' respiratory function and assessing the horses' responses to chymotrypsin inhalation were as described for antigen inhalation challenge.

RESULTS

1. Assay of the chymotrypsin activity of *M. faeni* and *A. fumigatus* antigens

The chymotrypsin activities recorded in the different batches of *M. faeni* and *A. fumigatus* antigens are shown in Table I. The chymotrypsin activity of *M. faeni* antigen varied considerably between the batches, i.e. from 0.84 to 17.4 units per challenge dose. The chymotrypsin activity in the batches of *M. faeni* antigen was, in general, far higher than that in the batches of *A. fumigatus* antigen, where the activity ranged from 0.24 to 2.40 units per challenge dose.

2. The response of COPD-affected horses to *M. faeni* and *A. fumigatus* inhalation challenge

Of the 15 COPD-affected horses challenged with *M. faeni*, batch 1, in which the chymotrypsin activity was 5.64 units per challenge dose, 13 horses responded positively and 2 failed to respond. Fifteen of the 19 COPD-affected horses challenged with *M. faeni*, batch 4 (chymotrypsin activity 17.4 units per challenge dose), responded positively, whilst 3 horses failed to respond (Table II). It appears, therefore, that the differing chymotrypsin activities of these batches of antigen did not influence the number of positive responses to *M. faeni* inhalation challenge.

The number of positive responses to *A. fumigatus* inhalation challenge in COPD-affected horses also did not appear to be related to the chymotrypsin

TABLE I

The chymotrypsin activity recorded in different batches of Micropolyspora faeni and Aspergillus fumigatus antigens

Antigen	Batch No.	Units of chymotrypsin per mg protein	Units of chymotrypsin per challenge dose
<u>M. faeni</u>	1	0.47	5.64
	2	1.40	16.80
	3	0.07	0.84
	4	1.45	17.40
	5	0.77	9.24
	6	0.29	3.48
	7	0.44	5.28
<u>A. fumigatus</u>	1	0.18	2.16
	2	0.20	2.40
	3	0.08	0.96
	4	0.04	0.48
	5	0.02	0.24

TABLE II

Results of Micropolyspora faeni inhalation challenge in COPD-affected horses

Antigen batch no.	No. of horses challenged	No. of positive responses	No. of negative responses
1	15	13	2
4	19	15	3

TABLE III

Results of Aspergillus fumigatus inhalation challenge in COPD-affected horses

Antigen batch no.	No. of horses challenged	No. of positive responses	No. of negative responses
2	10	4	6
5	8	5	3

activity in the different batches of antigen. Four out of 10 COPD-affected horses challenged with A. fumigatus, batch 2 (chymotrypsin activity 2.40 units per challenge dose), responded positively to challenge. Of the 8 affected horses challenged with A. fumigatus, batch 5 (chymotrypsin activity 0.24 units per challenge dose), five responded positively (Table III).

There were no significant correlations ($P > 0.05$) between the degree of response to challenge (i.e. the increase in max. Δ Ppl at 5 hours after challenge, compared to pre-challenge values) and the chymotrypsin activity of either batch of M. faeni or A. fumigatus antigens ($r(26) = -0.257$ and $r(7) = 0.130$ respectively).

3. Response to COPD-affected horses to purified chymotrypsin inhalation challenge

The COPD-affected horses were asymptomatic prior to chymotrypsin inhalation challenge and their max. Δ Ppl and PaO_2 values were within the normal ranges of McPherson et al. (1978), i.e. max. Δ Ppl < 6 mmHg and $\text{PaO}_2 > 85$ mmHg, PaO_2 values being measured at 37.7°C . Inhalation of 17.5 units chymotrypsin on day 1 and 52.5 units chymotrypsin on day 4 did not induce signs of COPD in any of the 8 COPD-affected horses and the post-challenge max. Δ Ppl and PaO_2 values remained within normal ranges in all of the horses.

Neither horse A nor horse B responded to inhalation of 17.5 units chymotrypsin on days 22 and 29. Horse A did, however, respond positively to 52.5 units chymotrypsin inhalation on days 25 and 32. The horse showed clinical signs of COPD 5 hours after challenge and the respective max. Δ Ppl increases above the pre-challenge values on those days were 6.0 mmHg and 8.5 mmHg. Clinical signs of COPD persisted in this horse for 24 to 48 hours after challenge. Horse B failed to respond to challenge with 52.5 units chymotrypsin on either day.

DISCUSSION

A considerable variation was recorded in the chymotrypsin activity of the different antigen batches, particularly between the M. faeni batches, where the chymotrypsin activity ranged from 0.84 to 17.4 units per challenge dose. The reason for this variation is not known. The seven batches of M. faeni antigen were prepared using the same technique, from the same strain and stock cultures of M. faeni. The incubation time did not appear to affect the chymotrypsin activity of the M. faeni batches. Batches 1, 2, 4, 6 and 7 of M. faeni were incubated for 3 weeks, batch 3 was incubated for 4 weeks and batch 5 for 6 weeks. Batches 2 and 4 had the greatest chymotrypsin activity, i.e. 16.8 and 17.4 units respectively per 12 mg challenge dose. The batches of A. fumigatus were cultured from the same strain of A. fumigatus and were prepared similarly. The chymotrypsin

activity in the batches ranged from 0.24 to 2.40 units per challenge dose.

The number of positive responses to M. faeni and A. fumigatus inhalation challenge in COPD-affected horses did not appear to be related to their chymotrypsin activity. Neither did the degree of response to challenge correlate significantly with the chymotrypsin activity of the antigens. This suggests that the chymotrypsin component of the M. faeni and A. fumigatus antigens did not play a major role in the observed responses of COPD-affected horses to artificial inhalation challenge with those antigens.

The pharmacological effects of enzyme inhalation in COPD-affected horses was tested by inhalation of purified chymotrypsin. The initial 17.5 unit dose was chosen as that was equal to the highest enzyme content of an M. faeni challenge dose. This initial challenge dose did not induce signs of respiratory disease in any of the 8 horses. Increasing the dose three-fold to 52.5 units chymotrypsin also failed to induce respiratory signs in these 8 horses. It was, therefore, concluded that the pharmacological effects of chymotrypsin contained in the antigen challenge doses was unlikely to have caused the observed positive response to antigen challenge in COPD-affected horses.

Repeated inhalation of high doses of purified chymotrypsin induced signs of COPD in one horse which had not previously responded to the initial chymotrypsin challenge doses. This suggests that this animal developed respiratory hypersensitivity to the purified chymotrypsin protein. McPherson, E.A. (unpublished data) has previously induced respiratory hypersensitivity to avian albumin in a COPD-affected horse through repeated inhalation challenge with this antigen. This demonstrates that it is possible for COPD-affected horses to develop respiratory hypersensitivity to foreign proteins through repeated inhalation exposure. In man, sensitisation to trypsin and chymotrypsin has been described in laboratory workers after repeated handling of these enzymes (Howe et al., 1961).

The role of the chymotrypsin-like enzymes of M. faeni in the pathogenesis of farmer's lung in man and cattle and in equine COPD is not known. As previously noted, precipitating antibodies against M. faeni and the chymotrypsin-like enzymes of M. faeni have been demonstrated in healthy humans and cattle previously exposed to mouldy hay, as well as in patients with farmer's lung (Nicolet et al., 1972; Nicolet et al., 1977). The presence of precipitins against the chymotrypsin-like enzymes of M. faeni is evidence of their antigenicity and of an individual's previous exposure to these enzymes, but they do not necessarily indicate their aetiological role in the pathogenesis of farmer's lung.

These studies suggest that, although repeated inhalation of purified chymotrypsin may induce respiratory hypersensitivity in horses, the chymotrypsin-like enzymes of M. faeni and A. fumigatus do not appear to play a major role in the pathogenesis of naturally occurring COPD in Great Britain.

ACKNOWLEDGEMENTS

Thanks are due to the Horserace Betting Levy Board for their financial support and to Mr. G. Keay and Mr. G.M. Firth for their technical assistance.

REFERENCES

- Biguet, J. and Vernes, A. (1974). Antigenic structure of Aspergillus. In: R. de Haller and F. Suter (Eds), Aspergillosis and Farmer's Lung in Man and Animal, pp. 53-57. Hans Huber Publishers, Berne.
- Biguet, J., Walbaum, S. and Vernes, A. (1974). Physico-chemical aspects and antigenic structure of Micropolyspora faeni. In: R. de Haller and F. Suter (Eds), Aspergillosis and Farmer's Lung in Man and Animal, pp. 169-175. Hans Huber Publishers, Berne.
- Flindt, M.H.L. (1969). Pulmonary disease due to inhalation of derivatives of Bacillus subtilis containing proteolytic enzyme. Lancet, 1: 1177-1181.
- Flindt, M.H.L. (1978). Respiratory hazards from papain. Lancet, 1: 430-432.
- Goldberg, L., Martin, L.E., Sheard, P. and Harrison, C. (1960). The pharmacology of chymotrypsin administered by inhalation. British Journal of Pharmacology, 15: 304-312.
- Goldring, I.P., Park, S.S., Greenberg, L. and Ratner, M.D. (1972). Sequential anatomic changes in lungs exposed to papain and other proteolytic enzymes. In: C. Mittman (Ed), Pulmonary Emphysema and Proteolysis, pp. 389-409. Academic Press Inc., New York.
- Howe, C., Erlanger, B.F., Beiser, S.M., Ellison, S.A. and Cohen, W. (1961). Hypersensitivity to purified trypsin and chymotrypsin. New England Journal of Medicine, 265: 332-334.
- Hummel, B.C.W. (1959). A modified spectrophotometric determination of chymotrypsin, trypsin and thrombin. Canadian Journal of Biochemistry and Physiology, 37: 1394-1399.
- Lawson, G.H.K., McPherson, E.A., Murphy, Jill R., Nicholson, Janet M., Wooding, P., Breeze, R.G. and Pirie, H.M. (1979). The presence of precipitating antibodies in the sera of horses affected with chronic obstructive pulmonary disease (COPD). Equine Veterinary Journal, 11: 172-176.
- McPherson, E.A., Lawson, G.H.K., Murphy, Jill R., Nicholson, Janet M., Fraser, J.A., Breeze, R.G. and Pirie, H.M. (1978). Chronic obstructive pulmonary disease (COPD): Identification of affected horses. Equine Veterinary Journal, 10: 47-53.
- McPherson, E.A., Lawson, G.H.K., Murphy, Jill R., Nicholson, Janet M., Breeze, R.G. and Pirie, H.M. (1979). Chronic obstructive pulmonary disease (COPD) in horses: Aetiological studies: responses to intradermal and inhalation antigenic challenge. Equine Veterinary Journal, 11: 159-166.
- Matsubara, H. and Feder, J. (1970). Other bacterial, mould and yeast proteases. In: P.D. Boyer (Ed), The Enzymes, 3rd ed., Vol. 3, pp. 721-795. Academic Press Inc., London.
- Nicolet, J., Bannerman, E.S.N. and Haller, R. de (1975). Mycotic proteolytic enzymes. In: A.F. Juho and R. de Haller (Eds), Lung Metabolism, pp. 57-65. Academic Press Inc., New York.
- Nicolet, J., Bannerman, E.S.N., Haller, R. de and Wanner, M. (1977). Farmer's lung: immunological response to a group of extracellular enzymes of Micropolyspora faeni, an experimental and field study. Clinical Experimental Immunology, 27: 401-406.
- Nicolet, J., Bannerman, E.S.N. and Krawinkler, M. (1974). Observations on precipitins against a peptidase with chymotrypsin-like activity of Micropolyspora faeni, in sera of cattle exposed to mouldy hay. Pathologia et Microbiologia, 40: 237-238.

- Nicolet, J., Haller, R. de and Herzog, J. (1972). Serological investigations of a bovine respiratory disease ("Urner Pneumonie") resembling farmer's lung. *Infection and Immunity*, 6: 38-42.
- Pepys, J. (1969). Hypersensitivity diseases of the lungs due to fungi and organic dusts. In: *Monographs in Allergy*, Vol. 4. S. Karger, Basel.
- Pepys, J., Longbottom, J.L., Hargreave, F.E. and Faux, J. (1969). Allergic reactions of the lungs to enzymes of Bacillus subtilis. *Lancet*, 1: 1181-1184.
- Pirie, H.M., Dawson, C.O., Breeze, R.G., Wiseman, A. and Hamilton, J. (1971). A bovine disease similar to farmer's lung: extrinsic allergic alveolitis. *Veterinary Record*, 88: 346-351.
- Snedecor, G.W. and Cochran, W.G. (1967). *Statistical Methods*, 6th ed., pp. 246-248. Iowa State College Press, Ames.
- Walbaum, S., Biguet, J. and Tran-Van Ky, P. (1969). Structure antigenique de Thermopolyspora polyspora. Repercussions pratiques sur le diagnostic du poumon du fermier. *Annales de l'Institut Pasteur*, 117: 673-693.

Chronic obstructive pulmonary disease in the horse 1: Nature of the disease

E. A. McPHERSON and JILL R. THOMSON

Department of Veterinary Medicine, University of Edinburgh, Royal (Dick) School of Veterinary Studies, Veterinary Field Station, Easter Bush, Roslin, Midlothian, EH25 9RG

Summary

The aetiology, pathophysiological changes, pathology and clinical signs of the disease as presently understood are discussed. The condition appears to be a hypersensitivity of the respiratory system in some horses to poor quality hay and straw. *Micropolyspora faeni* is the chief agent identified in the northern part of the United Kingdom. In other locations, the chief agent is probably different. The principal changes are spasm of the airways and bronchiolitis of the small airways. Onset may be acute or insidious. The chief clinical signs are well known but the disease process is reversible if the source of the antigen is removed. In early cases, determination of increased maximum intrathoracic pressure changes and co-existent subnormal arterial oxygen pressure are the best objective confirmation of diagnosis. The same measurements may be made on asymptomatic horses if they have been challenged 4 to 5 h earlier by inhalation of the appropriate antigen, or even exposed to poor quality hay or straw. Other methods available to the clinician are discussed.

Introduction

CHRONIC obstructive pulmonary disease (COPD), also known as heaves, broken wind and alveolar emphysema, is probably the most common cause of chronic coughing in horses in the United Kingdom and western Europe. The disease is thought to have been recognised as far back as 333 BC by Aristotle, who described the characteristic expiratory effort or 'heave' associated with this condition (Smith 1919). The importance of COPD is well recognised and Gerber (1973) believed it to be the most frequent cause of premature retirement in the Swiss horse population. Horses from two years old and over may be affected and the prevalence increases with age. There is no breed or sex predisposition for COPD.

Aetiology

Dusty atmospheres and mould in the hay have been associated with the occurrence of the condition by many authors (Cook and Rossdale 1963; Gillespie and Tyler 1969; Eyre 1972). More recent studies have shown equine COPD to be a pulmonary hypersensitivity to organic dust antigens in the environment (McPherson *et al* 1979). In northern Britain, *Micropolyspora faeni* (a thermophilic actinomycete) and *Aspergillus fumigatus* (a fungus) are the predominant

aetiological antigens. Grass pollen and on occasions other minor as yet unidentified agents are also involved (McPherson *et al* 1979). In other parts of the world, the principal antigens may well be different. Thermophilic actinomycetes and fungi (hereafter loosely termed 'moulds') occur in large numbers (5 million/g) even in very good quality hay and straw and at levels of up to 1 billion/g in visibly contaminated material (Lacey 1974).

Modern methods of fodder and bedding conservation have exacerbated the difficulties experienced in obtaining good quality hay and straw.

Pathology

At autopsy, the lungs of a symptomatic COPD-affected horse in which the disease is long standing fill the freshly opened chest. They often have rib indentations on the surface. The lungs retain their over-inflated appearance for some time, often have puffy looking peripheral areas to the lobes and are pale pink.

Nicholls (1978) autopsied 25 COPD-affected horses and summarised her findings as follows: 'the principal lesion in all 25 horses examined was chronic bronchiolitis consisting of a hyperplastic bronchiolar epithelium, goblet cell metaplasia, peribronchial cellular infiltration and exudation of mucus or pus into the lumen. This combination produced narrowing of the airway and resultant alveolar over-infiltration. Emphysema estimated by examining slices of inflated lung was infrequent, occurring only in the cranial lobe or the periphery of the caudal lobe and seemed to be a late development in the course of the disease.' This author stated also that all the small airways (less than 2 mm in diameter) were affected and the lesion was characterised as quoted above.

More than half the cases examined had a pulmonary eosinophilia. It was also noted that the number and type of goblet cells in the bronchial epithelium were similar in COPD-affected horses and in normal horses. In addition there was no hyperplasia of the bronchial submucous glands and the author therefore concluded that the disease in the horse bears no pathological resemblance to chronic bronchitis and emphysema of man. In asymptomatic COPD-affected horses or symptomatic horses in which the current attack has been of short duration (ie, less than seven to 14 days), the lungs usually appear macroscopically normal.

Pathophysiology

Spörri and Leeman (1964), Gillespie, Tyler and Eberly (1966), Sasse (1971) and Willoughby and McDonnell (1979)

Jill Thomson's present address is East of Scotland College of Agriculture, Veterinary Investigation Centre, Bush Estate, Penicuik, Midlothian.

were all concerned with establishing methods of measuring the pathophysiological changes occurring in COPD-affected horses. The work of Sasse (1971) showed that affected horses suffer an increase in the work of breathing and that this is mainly caused by an increased maximum change in intrathoracic pressure during each breath. McPherson *et al* (1978) confirmed both the importance of these findings and the hypoxaemia which exists in such cases. McPherson *et al* (1979) showed that inhalation of extracts of *M faeni* and *A fumigatus* cultures may exacerbate the clinical disease or provoke asymptomatic horses to become symptomatically affected. The clinical disturbances can occur within 1 to 2 h of challenge; however, the reaction usually culminates 4 to 10 h after challenge.

The reason for this apparent hypersensitivity of certain horses to particular dusts is not at all clear though a respiratory allergy seems most likely. The result of exposure to these agents is a functional spasm of the airway smooth muscle and a widespread exudative bronchiolitis.

The addition of forceful expiration to the normal expulsive effort tends to exaggerate the normal collapse of the non-cartilaginous airways and partially collapses even the cartilaginous ones as demonstrated endoscopically by Fischer (1980). This, in addition to airway spasm and the above mentioned pathological changes, causes a reduced change of air in the alveoli with each breath because the residual alveolar volume is higher than normal. This leads to a reduction in the alveolar oxygen content (PA_{O_2}) which in turn results in reduced oxygen uptake by the blood. The oxygen level in arterial blood (the partial arterial oxygen pressure Pa_{O_2}), is therefore subnormal. This hypoxaemia causes pulmonary hypertension and consequently an increased right ventricular workload. In extremely advanced cases cor pulmonale is said to occur (Salutini 1959) although Dixon *et al* (1982) found that this was uncommon even in animals with long standing COPD.

These changes appear to be reversible if the animal is moved into a controlled environment (Thomson and McPherson 1982). However, the animal remains hypersensitive so that even a short exposure to the offending antigen will render an asymptomatic COPD horse symptomatic (ie, clinically affected).

Manifestations and clinical signs

The disease may manifest itself suddenly or develop gradually. The work performance is reduced and there is a chronic cough, ie, present for more than three months. (Coughing for over a year is highly indicative of COPD.) Nasal discharge is usually scanty, watery or mucoid. Dyspnoea with flaring of the nostrils as the animal inhales is often seen and the increased and double expiratory effort long associated by veterinarians and owners with 'broken wind' is usually evident. On auscultation harsh breathing sounds, mostly inspiratory, may be heard over a wide area of the chest. Wheezing or crepitant sounds, if present, are characteristic. In early cases it is difficult or impossible to detect abnormalities on auscultation. The rate and depth of breathing is often increased. Many authors assert that the area of expansion of the lungs is increased but Sasse (1971) deduced from the literature and his own experience that such a clinical sign depended on the degree of development of COPD. With this we agree, and this test is therefore generally considered too subjective and unreliable to be of diagnostic value.

Diagnosis

In most instances, COPD can be diagnosed on clinical

grounds and by response to therapy. Pulmonary function techniques which can be used to confirm the diagnosis include the maximum change in intrathoracic pressure during breathing (ΔP_{pl}) and Pa_{O_2} measurement. In COPD-affected horses, ΔP_{pl} is 6 mmHg or more and Pa_{O_2} is 82 mmHg or less measured at 37°C (85 mmHg or less measured at 37.7°C) (McPherson *et al* 1978).

In clinically affected horses the administration of a bronchodilator drug (eg, atropine sulphate 0.02 mg/kg body weight [bwt], clenbuterol [Ventipulmin; Boehringer] 0.8 µg/kg bwt or etamiphylline camsylate [Milliphylline — V; Dales Pharmaceuticals] 3 mg/kg bwt) provides clinical relief to horses affected with COPD within 10 to 20 mins after treatment. A retrospective diagnosis may be made by assessing the horse's response to a controlled environment.

The following techniques have also been investigated for the diagnosis of COPD.

Intradermal testing for antigen sensitivity

A positive antigen intradermal reaction is evidence that a horse has had previous exposure to that antigen and developed skin hypersensitivity. It does not necessarily indicate respiratory hypersensitivity in the horse. McPherson *et al* (1979) reported that significantly more COPD-affected horses showed skin hypersensitivity to *M faeni* than did normal horses and that 90 per cent of COPD cases with skin hypersensitivity to *M faeni* also showed respiratory hypersensitivity to the organism. Using other antigens, positive skin reactions were as likely to be found in normal horses as in those affected with COPD. It seems reasonable to presume that other agents predominate in different parts of the world. Halliwell *et al* (1979) also found this type of test unreliable as a means of diagnosing COPD although more of their chronic pulmonary diseased horses reacted to mould antigens than did their controls.

Antigen inhalation challenge

Exposure to antigen by this route either by exposure to mouldy hay or straw or, more specifically, to nebulised culture extracts (McPherson *et al* 1979) may be used to advantage for diagnosis. This test is particularly useful in early cases with a history suggestive of COPD but not showing overt clinical signs at the time of examination, or in well established cases which are in remission (or asymptomatic) as a result of being maintained in a controlled environment.

A crude natural inhalation challenge can be carried out in practice by exposing horses to mould-contaminated hay or straw for 12 to 24 h. Should a horse be sensitive to that environment, a distinct worsening of clinical signs should be evident within that time. The owner should observe the horse hourly during the first 8 h of the challenge in case it precipitates marked respiratory distress. If this occurs, the horse should be immediately removed from the challenge environment. As previously mentioned, horses may be sensitive to a range of different antigens, including pollens, and this should be borne in mind in the event of a horse failing to respond to challenge with mouldy hay or straw.

Serum precipitating antibody

Lawson *et al* (1979) found that precipitins to *M faeni* and *A fumigatus* were present in the sera of both normal and COPD-affected horses though they were found more frequently in affected horses. Many COPD-affected horses without detectable precipitins respond clinically to inhalation challenge with those antigens. As with intradermal testing this is not a reliable diagnostic tool.

Radiographic examination of lungs

Lateral radiographs of the lung of the horse can be technically satisfactory. Trimax films (fast) have given the best results, the exposure factors being 110 Kv, 300 mA for 0.04 secs with an AFD of 120 cm. Distances from tube to subject and subject to plate are difficult to keep standard. The images produced by the lung nearest the plate are sharp but those by the lung nearest the tube are enlarged and rather ill defined.

In horses which have been symptomatic for some time, a general increase in density gives an overall grey image of the lungs. Areas of greater density are sometimes seen. These are snowflake-like and presumably represent areas of more intense bronchiolitis. In asymptomatic and many early cases, the lungs appear radiologically normal. For this reason radiology is not a reliable diagnostic aid and, in addition, the distorted image of the lung nearest the tube makes interpretation difficult.

Conclusions

Equine COPD is a respiratory hypersensitivity to mould or occasionally to pollen in the environment. Hay and straw are the principal sources of the allergens. The onset may be sudden or gradual. The reaction takes the form of an airway spasm and a bronchiolitis, the latter involving the small bronchioles only. The earliest response of an asymptomatic but affected horse to challenge, which manifests in about 2 h, is in keeping with a type I allergy. The more obvious heaving 4 to 10 h after exposure may be a delayed type I or a type III allergic response.

Intradermal testing and precipitating antibody determination are of no help to the practitioner. Radiography offers difficulties in technique and interpretation and is not a reliable diagnostic method. In practice, observation of the effect of deliberate exposure to poor quality hay or straw, or the introduction of environmental control measures offer the best diagnostic aid in horses which are not showing overt clinical signs.

The terminology of the disease is considered by many to be unsatisfactory. Eyre (1972) and Littlejohn (1978) prefer the term *equine asthma*. On the other hand, Nicholls (1978) writing as a pathologist, expressed a preference for *chronic bronchiolitis*. The term *reversible chronic obstructive pulmonary disease* or *equine respiratory allergy* might be more appropriate and useful to the clinician although, on balance, it would be preferable to postpone introducing yet another new term at this stage of the unfolding of the *broken-winded horse* syndrome.

References

- Cook, W. R. and Rosedale, P. D. (1963) The syndrome of 'broken wind' in the horse. *Proc. R. Soc. Med.* **56**, 972-977.
- Dixon, P. M., Nicholls, J. M., McPherson, E. A., Lawson, G. H. K., Thomson, J. R., Pirie, H. M. and Breeze, R. G. (1982) Chronic obstructive pulmonary disease, anatomical cardiac studies. *Equine vet. J.* **14**, 80-82.
- Eyre, P. (1972) Equine pulmonary emphysema: A broncho-pulmonary mould allergy. *Vet. Rec* **91**, 134-140.
- Fischer, J. (1980) Bronchoskopische untersuchungen als beitrage zur klinischen und atiologicalischen diagnostik bei pferden mit atemwegserkrankungen. Doctoral thesis, Hanover.
- Gerber, H. (1973) Chronic pulmonary disease in the horse. *Equine vet. J.* **5**, 26-33.
- Gillespie, J. R., Tyler, W. S. and Eberly, V. E. (1966) Pulmonary ventilation and resistance in emphysematous and control horses. *J. appl. Physiol.* **21**, 416-422.
- Gillespie, J. R. and Tyler, W. S. (1969) Chronic alveolar emphysema in the horse. *Adv. vet. Sci.* **13**, 59-99.
- Halliwel, R. E. W., Fleischman, J. B., Mackay-Smith, M., Beech, J. and Gunson, D. E. (1979) The role of allergy in chronic pulmonary disease of horses. *J. Am. vet. med. Ass.* **174**, 277-281.
- Lacey, J. (1974) The microbiology of hay and straw. In: *Aspergillosis and Farmer's Lung in man and in animal*. Eds R. De Haller and F. Suter. Hans Huber, Berne. pp16-23.
- Lawson, G. H. K., McPherson, E. A., Murphy, J. R., Nicholson, J. M., Wooding, P., Breeze, R. G. and Pirie, H. M. (1979) The presence of precipitating antibodies in the sera of horses with chronic obstructive pulmonary disease (COPD). *Equine vet. Jo.* **11**, 172-176.
- Littlejohn, A. (1978) Studies on the physiopathology of chronic obstructive pulmonary disease in the horse. Doctoral thesis, University of Pretoria.
- McPherson, E. A., Lawson, G. H. K., Murphy, J. R., Nicholson, J. M., Breeze, R. G. and Pirie, H. M. (1978) Chronic obstructive pulmonary disease (COPD): Identification of affected horses. *Equine vet. J.* **10**, 47-53.
- McPherson, E. A., Lawson, G. H. K., Murphy, J. R., Nicholson, J. M., Breeze, R. G. and Pirie, H. M. (1979) Chronic obstructive pulmonary disease (COPD) in horses: Aetiological studies: Responses to intradermal and inhalation antigenic challenge. *Equine vet. J.* **11**, 159-166.
- Nicholls, J. M. (1978) A pathological study of chronic pulmonary disease in the horse. PhD thesis, University of Glasgow.
- Salutini (1959) Cuore polmonare cronico e bolsaggine. *Nuova Vet.* **35**, 101-104.
- Sasse, H. H. L. (1971) Some pulmonary function tests in horses. An aid to the diagnosis of chronic obstructive pulmonary disease (heaves) in horses. PhD thesis, University of Utrecht, Holland.
- Smith, F. (1919) *History of Veterinary Medicine*. Balliere Tindall, London.
- Sporri, H. and Leeman, W. (1964) Zur untersuchung der lungenmechanik bei grosstieren. *Sch. Arch. Tier.* **106**, 699-714.
- Thomson, Jill R. and McPherson, E. A. (1982) Chronic obstructive pulmonary disease in the horse 2: Therapy. *Equine vet. J.* **15**, 207-210.
- Willoughby, R. A. and McDonell, W. N. (1979) Pulmonary function testing in horses *Vet. Clin. North Am., Large anim. Pract.* **1**, 171-196.

Résumé

On discute l'étiologie, les changements physiopathologiques, la pathologie et les signes cliniques de la maladie telle qu'on l'appréhende aujourd'hui. Cette maladie paraît être une hypersensibilité du système respiratoire de certains chevaux en présence de foin ou de pailles de mauvaise qualité. *Micropolyspora faeni* est l'agent principal identifié dans le nord du Royaume Uni. Les troubles majeures sont des spasmes des voies respiratoires et une bronchiolite au niveau des voies secondaires. L'apparition de la maladie peut être aigue ou bien insidieuse. Les signes cliniques essentiels sont bien connus, l'évolution de la maladie est réversible si la source d'antigène est supprimée. Dans les cas récents, la détermination d'un accroissement de la pression intrathoracique maximum avec une pression d'oxygène artériel sub normale simultanée ne fournit la meilleure confirmation diagnostique. Les mêmes mesures peuvent être faites sur des chevaux sans symptômes qu'on a soumis 4 ou 5 heures plus tôt à une inhalation de l'antigène adéquat ou que l'on a exposés à des fourrages de mauvaise qualité. D'autres méthodes à la portée du clinicien sont discutées.

Zusammenfassung

Die Aetiologie, die pathophysiologischen Veränderungen, die Pathologie und die klinischen Zeichen der Krankheit werden diskutiert. Es scheint sich um eine Ueberempfindlichkeit der Atemwege bestimmter Pferde gegen Heu und Stroh schlechter Qualität zu handeln. *Micropolyspora faeni* ist das hauptsächlich verantwortliche Agens in den nördlichen Teilen des Vereinigten Königreiches. In anderen Gebieten sind wahrscheinlich andere Agenten verantwortlich. Die vorherrschenden Störungen bestehen in Spasmen der Atemwege und in einer Bronchiolitis der 'kleinen Luftwege.' Der Anfang der Krankheit kann sich akut oder schleichend

einstellen. Die wichtigsten klinischen Zeichen sind gut bekannt, aber die Krankheitsprozesse sind reversibel, wenn die Antigenquelle eliminiert wird. In frühen Fällen stellt die Bestimmung eines erhöhten maximalen intrathorakalen Drucks und einer gleichzeitigen Senkung des arteriellen Sauerstoffpartialdrucks die beste objektive Bestätigung der Diagnose dar. Diese Messungen können auch an asymptomatischen Pferden durchgeführt werden, die 4 bis 5 Stunden vorher einer Inhalation des betreffenden Antigens oder auch nur einer Exposition mit Heu/Stroh schlechter Qualität ausgesetzt worden sind. Auch andere, dem Kliniker zugängliche Methoden werden besprochen.

Accepted for publication 1.3.82.

ABSTRACTS

Nervous system and diseases

Migration of a spiruroid nematode through the brain of a horse

MAYHEW, I. G., LICHTENFELS, J. R., GREINER, E. C., MACKAY, R. J. and ENLOE, S. C. (1982) *J. Am. vet. med. Ass.* **180**, 1306-1311.

A PREGNANT 10-year-old mare was examined because of an acute neurological disturbance. Central nervous system signs consistent with asymmetric brain stem disease were noted on physical examination, including head tilt, turning of the head and neck, asymmetric eye drop and, on blindfolding, tight circling to the left side.

Equine protozoal myeloencephalitis, head trauma, helminth migration, cerebrospinal myiasis, basilar epidural empyaema with possible extension from a left otitis media-interna, tumour, and viral encephalomyelitides, including rabies, were considered, in that order, as the possible aetiologies.

Haematology, serum biochemistry and lumbosacral cerebrospinal fluid (CSF) analysis were within normal limits. Atlanto-occipital CSF collection was unsuccessful. Antiprotozoal therapy with trimethoprim-sulfadiazine (30 mg/kg, *per os* twice daily) was initiated and folic acid (75 mg intramuscularly) was given once weekly. Treatment continued for five weeks and the mare improved throughout this period. However, neurological signs recurred and despite increasing the trimethoprim-sulfadiazine to 60 mg/kg twice daily and the folic acid to 75 mg twice weekly, the mare continued to deteriorate and was destroyed.

At necropsy, large malacic tracts were found extending through the brain stem and cerebral cortex. Cross sections of a nematode were seen on microscopic examination and were subsequently identified as belonging to a single gravid female *Draschia* (*Habronema*) *megastoma*.

The authors stress the value of repeated efforts at harvesting CSF from the collection site nearest the lesion and emphasise that CSF eosinophilia has been of value in identifying cases of equine cerebrospinal parasite migration. They provide a useful table of the major neurological syndromes seen with nematode and fly parasite migrations through the CNS, which contains a suggested parasiticide therapy for each syndrome.

Abstractor's comment — This excellent paper is of value to the clinician because the details and methods of neurological examination are described in a clear and readily understood manner and the rationale followed in this case would be valuable to anyone investigating a similar case.

D. P. LEADON

Neoplasia

Endoscopic diagnosis of squamous cell carcinoma of the equine stomach

KEIRN, D. P., WHITE, K. K., KING, J. M. and TENNANT, B. C. (1982) *J. Am. vet. med. Ass.* **180**, 940-942.

AN 11-year-old gelding of mixed breeding was examined for weight loss over a three month period. There had been progressive reduction in food intake with, latterly, a refusal to drink water. At admission, the rectal temperature, heart rate and respiratory rate were normal. No abnormal masses were identified by rectal examination. Haematology and blood biochemistry figures were all within normal limits. Abdominal paracentesis led to the collection of fluid containing 3.3 g of protein/dl and a white cell count of 4.2×10^9 /litre with 90 per cent neutrophils and 10 per cent mononuclear cells. It was not possible to examine the stomach via the nares because of the inadequate length of the fibrescope.

An exploratory laparotomy was performed under general anaesthetic. Palpation of the stomach revealed a large firm mass within the wall of the stomach and associated adhesions. A midcervical oesophagostomy was performed and the same fibrescope was passed down the oesophagus towards the stomach. Resistance was met at the cardia but was overcome and the luminal surface of the stomach visualised. Within the oesophageal region of the stomach a rough nodular (cauliflower-like) mass with areas of ulceration and necrosis was observed. The mass was poorly demarcated. A biopsy specimen was obtained and the animal killed.

At necropsy, the mass was confined to the oesophageal region of the stomach and showed histological features characteristic of squamous cell carcinoma.

Abstractor's comment — This paper draws attention to the difficulties experienced in diagnosing squamous cell carcinoma of the stomach. Because of these problems, they are often at an advanced stage before diagnosis is made and non-invasive procedures, which allow early recognition, are required. Unfortunately, as this paper emphasises, fibrescopes longer than 180 cm are not yet available. Thus any visualisation of the stomach requires an oesophagostomy.

G. A. MUNROE

Chronic obstructive pulmonary disease in the horse 2: Therapy

JILL R. THOMSON and E. A. McPHERSON

Department of Veterinary Medicine, University of Edinburgh, Royal (Dick) School of Veterinary Studies, Veterinary Field Station, Easter Bush, Roslin, Midlothian, EH25 9RG

Summary

The therapy of equine chronic obstructive pulmonary disease (COPD) essentially entails minimising the horse's exposure to the aetiological antigens which are predominantly thermophilic actinomycetes and moulds occurring in hay and straw. This can be achieved, for example, by keeping affected horses permanently out of doors, or when stabled, using shredded paper, wood shavings or peat moss as bedding and feeding a complete cubed diet. There should be no supplementary hay feeding apart from dust-free vacuum-packed hay. Applying such measures generally allows horses to become asymptomatic in seven to 14 days.

Bronchodilators and corticosteroids bring about a marked, but temporary, improvement and can be of value in the treatment of acute attacks. The use of oral bronchodilators in combination with environmental control measures may hasten the remission of clinical signs in affected horses. Inhaled sodium cromoglycate can be used prophylactically in asymptomatic horses to prevent the onset of COPD when unavoidable antigen exposure is anticipated.

Introduction

THE therapy of chronic obstructive pulmonary disease (COPD) involves the institution of environmental control measures which may be used in conjunction with chemotherapy. The currently available methods of treating COPD-affected horses are presented here.

Environmental control

The most favourable long term method of controlling COPD is to remove affected horses from contact with the aetiological antigens occurring in hay and straw. This can most readily be achieved by substituting for straw any of the following forms of stable bedding — shredded paper, hardwood shavings or peat — and feeding a complete cubed diet. Hay should not be fed. An alternative suitable feeding regime consists of vacuum-packed hay (Horsehage; Westway, Torbay) in combination with a type of cube appropriate to the horse's energy needs. The stable should, preferably, be at least 46 m (50 yards) to the windward side of the hay store, according to the direction of the prevailing wind. However, it is futile to take such precautions with the individual's environment if the dust generated in the

maintenance of other horses on the premises gains admission to the airspace in which the COPD-affected horse is being kept. All animals sharing an airspace with the affected horse should, therefore, be subject to the same environmental control measures.

The alternative method of environmental control is to keep affected horses at grass, provided the animals are not hypersensitive to pollen and the grasses are not in flower. In this connection, one should note that multiple hypersensitivity is not uncommon in COPD-affected horses. No supplementary hay should be fed and grazing areas should not be adjacent to or downwind of the hay or straw stores. Any supplementary feeding should be in the form of a cubed diet.

Even 'good quality' hay contains large amounts of fungal spores and, for this reason, attempts at controlling COPD by feeding 'only the best hay' will invariably fail. The feeding of hay which has been soaked for hours in water is not universally successful. This may be because dust generated in handling the hay (eg, by filling hay nets) blows into the vicinity of the horse, or it may be that wetting the hay does not prevent the effects of exposure to antigen. The effects of feeding wet hay contaminated with *Micropolyspora faeni* and *Aspergillus fumigatus* have not been studied.

It is never possible to eliminate fungal spores completely from the environment, because these spores can be airborne for many hundreds of miles. However, using environmental control measures it is possible to reduce the levels of the antigens to below a threshold required to cause clinical disease. The application of these measures allows COPD-affected horses to become asymptomatic in one to two weeks, in most cases. However, severely affected horses, in which the illness has been of long duration, may take six weeks or longer to become asymptomatic.

Chemotherapy

Two forms of chemotherapy can be applied: (1) treatment of symptomatic horses and (2) prophylactic treatment of asymptomatic horses. (The chemotherapeutic agents described cannot be used in competing horses in Great Britain and Ireland under the current FEI and Jockey Club regulations.)

TREATMENT OF SYMPTOMATIC HORSES

When COPD-affected horses are in severe respiratory distress, treatment with corticosteroids or bronchodilator drugs helps to alleviate their condition. The improvement, however, lasts only for the duration of treatment; some permanent environmental control measures should also be indicated.

Jill Thomson's present address is East of Scotland College of Agriculture, Veterinary Investigation Centre, Bush Estate, Penicuik, Midlothian

Corticosteroids

Parenteral corticosteroids have been used for the treatment of COPD (Cook and Rosedale 1963; Gerber 1973; Beech 1979). They act by suppressing the allergic response to antigen challenge and reducing airway inflammation. Corticosteroids (eg, betamethasone or prednisolone) are sometimes used to treat acute COPD cases but their action is not as immediate as bronchodilators. The temporary and partial improvement after corticosteroid therapy lasts only for the duration of treatment. Long term usage of these drugs carries the very considerable risks of side-effects, particularly that of laminitis.

Bronchodilator drugs

Bronchodilator drugs which have been used for the therapy of equine COPD include the parasympatholytic drug atropine, sympathomimetic agents (eg, isoprenaline and clenbuterol) and the xanthine derivatives (eg, etamiphylline camsylate). The main value of bronchodilator therapy in COPD is to treat cases of acute respiratory distress where the almost immediate effects of these drugs, when given intravenously or by inhalation, are beneficial. However, as previously noted, a major feature of COPD is a widespread exudative bronchiolitis which occurs in addition to airway spasm. Whereas bronchodilator drugs can correct the latter airway obstruction, they cannot greatly influence the former. Therefore, even if bronchodilators are totally effective in relieving the airway spasm in COPD, they can only cause a partial improvement in the animal's overall pulmonary airflow.

Parasympatholytic drugs. Parasympatholytic drugs such as atropine cause bronchodilation by decreasing parasympathetic tone of the airway smooth muscle. Atropine has been found to bring about clinical improvement in COPD-affected horses (Alexander 1959; Cook and Rosedale 1963; Muylle and Oyaert 1973). However, its short duration of action (1 to 2 h) (Murphy, McPherson and Dixon 1980) and its widespread systemic side-effects, including tachycardia, mydriasis, reduced bowel motility and increased viscosity of respiratory secretions, preclude its widespread use.

Sympathomimetic bronchodilators. The sympathomimetic amines bring about bronchodilation by stimulating the beta adrenergic receptors in the respiratory system. The beta-2 sympathomimetic bronchodilators show preferential selectivity for the beta-2 receptors, thereby causing relaxation of the airway smooth muscle with fewer cardiac stimulating effects. Two such beta-2 sympathomimetic agents, clenbuterol and terbutaline, have been shown to bring about a marked clinical improvement in COPD-affected horses which lasts for 4 to 8 h (Sasse and Hajer 1977; Murphy *et al* 1980). Clenbuterol is marketed commercially for equine use (Ventipulmin; Boehringer) and intravenous (iv) administration of this drug is undoubtedly of value in the treatment of severely affected horses (Denac and Pfister 1981). However, oral administration of clenbuterol for 14 days to COPD-affected horses maintained in a hay and straw environment has not been found to improve significantly their clinical signs or lung function measurements (Thomson 1982). Clenbuterol administration, in combination with environmental control measures, may speed the remission of clinical signs in affected horses. Side-effects, including sweating, muscle tremor and tachycardia, may occur after treatment with sympathomimetic bronchodilators and may persist for up to 3 h after treatment.

Xanthine derivatives. Xanthine derivatives, including theophylline and aminophylline, are potent bronchodilators (Pain 1973). Etamiphylline camsylate (Millophylline-V; Dales Pharmaceuticals) is a theophylline derivative and has been used in the treatment of equine COPD. The effects following iv injection are similar in magnitude to those of clenbuterol and terbutaline, but are only of 2 to 4 h duration. Tachycardia may also be observed following iv etamiphylline camsylate administration. As with clenbuterol, oral administration of etamiphylline camsylate failed to bring about any significant improvement in COPD-affected horses housed in a hay and straw environment (Thomson 1982). However, oral administration of a theophylline compound has been found to improve significantly the pulmonary function of symptomatic COPD-affected horses (Petermann 1981). This compound is not currently commercially available in the United Kingdom.

It can be concluded that bronchodilators cause very rapid clinical improvement when administered iv or by inhalation, using a nebuliser. Consequently, these drugs are useful in the initial management of acute clinical cases, at which stage permanent environmental control measures should also be implemented. However, these drugs do not bring about complete recovery and their effects are temporary (lasting only as long as adequate blood levels are maintained). Bronchodilator drugs, therefore, have no place in the long term treatment of equine COPD as a substitute for environmental control.

Mucolytic and expectorant therapy

Because hypersecretion of viscous mucus in the airways is an important pathological change in COPD, the use of mucolytic agents and/or expectorant therapy has been recommended in addition to the previously mentioned methods of treating symptomatic COPD-affected horses (Deegan, Lieske and Fischer 1980). Mucolytic agents, such as bromhexine hydrochloride (Bisolvon; Boehringer), reduce sputum viscosity and thereby aid expectoration. However, the use of bromhexine hydrochloride in symptomatic COPD-affected horses without any additional measures was not found to have any lasting beneficial effects (Schatzmann, Bürgi and Straub 1973; Cook 1976).

Expectorant therapy by means of iv infusion of large volumes of fluid in COPD-affected horses has been investigated (Deegan *et al* 1980). This involved the administration of 20 to 40 litres of 0.9 per cent isotonic saline at a rate of 10 litres/h, on three to six successive days. Immediately after treatment, moist rales were heard on auscultation, the horses coughed and showed a nasal discharge. Affected horses were found to have markedly improved after the course of treatment. However, side-effects including excitement, tachycardia, dyspnoea and colic were observed in some cases during and after fluid infusion.

PROPHYLACTIC TREATMENT OF ASYMPTOMATIC HORSES

Sodium cromoglycate

Inhaled sodium cromoglycate is widely used for the prophylactic treatment of allergic respiratory disease in man. It has no direct bronchodilatory or anti-inflammatory effects. The mechanism of action is not completely understood, but it is believed to stabilise sensitised membranes of pulmonary mast cells. This prevents degranulation of the mast cells after challenge with the aetiological antigens, thus inhibiting the release of pharmacologically active substances, eg, histamine, slow-reacting substance of anaphylaxis and 5-hydroxytrypt-

tamine (Cox 1976). This drug should be administered prophylactically and is not intended for therapy once the patient is showing clinical signs of disease.

Inhaled sodium cromoglycate (Cromovet; Fisons) has been shown to be effective for the treatment of equine COPD when administered prophylactically to asymptomatic horses. The duration of the protective period increases linearly with the number of successive days of treatment with the drug, eg, the protective period varies from about three days after a single sodium cromoglycate treatment to as long as 24 days after four successive days' treatment (Thomson and McPherson 1981). Twice-weekly sodium cromoglycate inhalations have been found to be effective in preventing the onset of COPD when affected horses are housed in a challenge environment (ie, on hay and straw) for longer periods. In this respect, a four day treatment schedule may be preferred because this would allow longer intervals, eg, 21 days, between treatments.

This therapy, although of proven efficacy, should not, if possible, be substituted for the management of cases by means of environmental control. However, there are some cases where exposure to the aetiological antigens may be unavoidable, eg, during transport or when horses are temporarily housed away from their home environment. In such instances, prophylactic sodium cromoglycate treatment may be useful. Alternatively, long term intermittent sodium cromoglycate treatment could facilitate the management of a horse kept at livery or in large stables where the provision of special environmental control measures may prove difficult to institute.

Conclusions

In most COPD-affected horses, the disease can be well controlled by minimising exposure to the aetiological antigens. The resulting improvement in a horse's clinical state may be such that even a skilled examiner would not be able to detect its affliction. This must pose a problem for a veterinarian asked in the summer grazing season to advise a purchaser, or to carry out a health examination for any other purpose. At any time of year, it is wise to note whether any special management procedures are being followed.

When horses are severely affected, rapid relief may be obtained by the use of parenteral or inhaled bronchodilators. Corticosteroids may be used in conjunction with bronchodilators in such cases. These agents only bring about a temporary improvement and environmental control measures should also, therefore, be applied. A course of oral bronchodilators and/or expectorant therapy may speed the horse's recovery but these forms of therapy have not been found essential to the complete remission of COPD.

Sodium cromoglycate may be used prophylactically in asymptomatic horses when unavoidable antigen exposure is anticipated. The duration of protection varies according to the number of inhalations on successive days. Consequently, this form of therapy may be used as a temporary measure where brief exposure is anticipated or as a long term measure by repeating inhalations at regular intervals.

References

- Alexander, A. F. (1959) Chronic alveolar emphysema in the horse. *Am. Rev. resp. Dis.* **80**, 141-146.
- Beech, J. (1979) Principles of therapy. *Vet. Clin. North Am. Large Anim. Pract.* **1**, 73-88.
- Cook, W. R. (1976) Chronic bronchitis and alveolar emphysema in the horse. *Vet. Rec.* **99**, 448-451.
- Cook, W. R. and Rosedale, P. D. (1963) The syndrome of 'broken wind' in the horse. *Proc. R. Soc. Med.* **56**, 972-977.
- Cox, J. S. G. (1976) Disodium cromoglycate (cromolyn sodium) in bronchial asthma. Eds E. B. Weiss and M. S. Segal. In: *Bronchial Asthma: Mechanisms and Therapeutics*. Little, Brown & Company, Boston. pp 805-836.
- Deegan, E., Lieske, R. and Fischer, J. (1980) Eine neue Methode der sekretolytischen Therapie bei Pferden mit chronisch obstruktiver Bronchitis. *Deutsche Vet. Ges.* 1-3 October 1980, pp 63-73.
- Denac, M. and Pfister, R. (1981) Der Einfluss des Beta-2-Rezeptoren-stimulierenden Sympathomimeticums Ventipulmin auf die Atmungsmechanik des Pferdes. *Tierarztl. Ums.* **36**, 188-194.
- Gerber, H. (1973) Chronic pulmonary disease in the horse. *Equine vet. J.* **5**, 26-33.
- Murphy, Jill R., McPherson, E. A. and Dixon, P. M. (1980) Chronic obstructive pulmonary disease (COPD): Effects of bronchodilator drugs on normal and affected horses. *Equine vet. J.* **12**, 10-14.
- Muylle, E. and Oyaert, W. (1973) Lung function tests in obstructive pulmonary disease in horses. *Equine vet. J.* **5**, 37-44.
- Pain, M. C. F. (1973) The treatment of asthma. *Drugs* **6**, 118-126.
- Petermann, U. (1981) Untersuchungen zur Wirksamkeit von Theophyllin bei Pferden mit chronisch obstruktiver Bronchitis. Thesis, Hanover.
- Sasse, H. H. L. and Hajer, R. (1977) Enkele veterinaire klinische ervaringen met het gebruik van een β_2 -receptoren stimulerend sympaticomymeticum NAB 365R bij paarden met longaandoeningen. *Tijd. Dier.* **102**, 1233-1238.
- Schatzmann, U., Bürgi, H. and Straub, R. (1973) Der Einfluss von Bisolvon auf das Tracheobronchial-secret des chronisch lungenkranken Pferdes. *Deutsche Tier. Woch.* **80**, 97-100.
- Thomson, Jill R. (1982) Studies on the therapy of equine chronic obstructive pulmonary disease. PhD thesis, University of Edinburgh.
- Thomson, Jill R. and McPherson, E. A. (1981) Prophylactic effects of sodium cromoglycate on chronic obstructive pulmonary disease in the horse. *Equine vet. J.* **13**, 243-246.

Résumé

Le traitement de la maladie pulmonaire chronique obstructive exige notamment de soustraire l'animal aux antigènes générateurs de la maladie. Ceux-ci sont surtout des actinomycètes thermophiles et des moisissures de foin et de la paille. Par exemple, on peut maintenir les chevaux malades au dehors en permanence; ou bien utiliser une litière de papier, de copeaux ou de tourbe et une alimentation en granulés complets. Il ne devrait pas y avoir d'alimentation à base de foin sauf emploi d'un foin totalement dépoussiéré et conditionné sous vide. L'emploi de ces mesures entraîne la disparition des symptômes dans un délai de 7 à 14 jours.

Les bronchodilatateurs et les corticostéroïdes apportent une amélioration temporaire et sont utiles pour le contrôle des crises aiguës. L'emploi des bronchodilatateurs en combinaison avec l'assainissement de l'environnement, peut hâter la régression des signes cliniques. Le cromoglycate de sodium en inhalation peut être employé à titre préventif contre l'installation de la maladie pulmonaire chronique obstructive, lorsqu'on prévoit une mise au contact des antigènes.

Zusammenfassung

Die Therapie der chronisch obstruktiven Lungenkrankheit

(COPD) des Pferdes umfasst im wesentlichen eine möglichst komplette Ausschaltung der aetiologischen Antigene, die vor allem aus thermophilen Actinomyceten und Schimmelpilzen im Heu und Stroh bestehen. Das kann zum Beispiel erreicht werden durch die permanente Weidehaltung oder — im Stall — durch Streue aus Papierschnitzeln, Hobelspänen oder Torf und ein gewürfeltes Alleinfutter. Es darf kein Heu gefüttert werden, ausser staubfreiem, vakuum-verpacktem Heu. Durch derartige Massnahmen klingen die Symptome innert sieben bis 14 Tagen im allgemeinen ab.

Accepted for publication 9.9.82.

ABSTRACTS

Surgery

Oesophageal diverticulectomy in a horse

FRAUENFELDER, H. C. and ADAMS, S. B. (1982) *J. Am. vet. med. Ass.* **180**, 771-772.

A THREE-year old Quarterhorse gelding was examined after a bout of "choke". Four months earlier the horse had been kicked on the side of the neck. Endoscopy after the first bout of choke revealed only a small area of hyperaemia in the oesophageal mucosa. Later, a large diffuse swelling appeared at the caudoventral third of the neck. There was some resistance to palpation of the swelling and endoscopy revealed a large mucosal defect filled with food on the ventral aspect of the distal third of the cervical oesophageal mucosa. Contrast radiography confirmed the diagnosis of an oesophageal diverticulum.

The animal was anaesthetised, placed in dorsal recumbency and a nasogastric tube passed into the oesophagus. Routine dissection between the neck muscles revealed a large fibrin covered diverticulum ventral to the trachea. Large amounts of clear yellow fluid oozed from the site. The diverticulum was resected and the remaining 10 cm longitudinal defect in the oesophageal mucosa closed with simple interrupted sutures, using 2/0 polypropylene with knots placed within the lumen. The edges of the tunica muscularis were debrided and closed, using simple interrupted sutures of 2/0 Dexon. The area was thoroughly flushed with a large quantity of Ringer's containing crystalline sodium penicillin.

A fenestrated drain was placed alongside the oesophagus and the neck muscles reopposed. The nasogastric tube was left in the oesophagus, sutured to the left nostril and plugged. A liquid mixture of alfalfa meal, horse cubes, bran, dextrose and electrolytes was administered via the nasogastric tube for four days after surgery. At this stage the drain was removed. Antibiotic therapy was continued for seven days after surgery and the animal discharged 18 days postoperatively. A liquid diet was continued for four weeks.

The authors considered this particular oesophageal diverticulum to be of the pulsion type which is thought to occur because of a mucosal herniation through acquired defects in the muscularis of the oesophagus. It is considered that this defect may have been caused by external trauma to the neck.

Abstractor's comment — This paper is of interest because it describes an alternative technique for surgical treatment of oesophageal diverticulum. Previous papers have suggested the repulsion of the redundant evaginated mucosa into the lumen and apposition of the edges of the muscularis defect. However in this case, because of the size of the diverticulum and the

Bronchodilatoren und Corticosteroide erzielen eine deutliche aber nur temporäre Besserung und können bei der Behandlung akuter Episoden von Nutzen sein. Die Verwendung oraler Bronchodilatoren in Verbindung mit Haltungsverbesserungen kann die Remission klinischer Anzeichen beschleunigen. Inhaliertes Na-Chromogluconat kann prophylaktisch bei symptomfreien Pferden eingesetzt werden, um das Auftreten von COPD bei unvermeidlicher und voraussetzbarer Antigenexposition zu verhindern.

possible degeneration of the integrity of the mucosa, the diverticulum was excised. The authors consider the correct selection of non-reactive suture material, placement of the mucosal suture knots within the lumen and the resting of the oesophagus for several days after surgery to be extremely important.

G. A. MUNROE

Haematology

Haemophilia A (factor VIII deficiency) in a colt

FELDMAN, B. F. and GIACOPUZZI, R. L. (1982) *Equine Pract.* **4**, 24-30.

THE clinical picture, laboratory investigation and diagnosis of a case of severe haemophilia A (factor VIII deficiency) in a three-day-old Quarterhorse cross colt are presented. On initial examination, the colt had marked joint swellings but was otherwise bright, active and nursing well. Joint taps of the knees revealed blood. There had been no previous history of foaling problems. A coagulogram showed a prolonged activated partial thromboplastin time (APTT) caused by less than 5 per cent factor VIII. All other coagulation assays, including one for the presence of an inhibitor to factor VIII, were normal. The packed cell volume was 0.22 litres/litre and total plasma proteins 46 g/litre. Treatment consisted of the administration of 2 litres of fresh equine blood. Over the next four days, the size of the joint swellings diminished, but on the sixth day the colt died. Post mortem examination revealed haemorrhages in several organs and all joints contained blood. The authors considered the case to be the first of classical haemophilia A in a horse with a Quarterhorse background.

Abstractor's comments — Haemophilia A is a rare inherited coagulation defect which has been reported previously in male Thoroughbreds, Arabs and Standardbreds. The disease seems to be extremely severe in the horse and, as a result, few affected animals survive beyond six months of age. This case appears to be the first recorded in a crossbred animal. As the authors point out, a normal prothrombin time with prolonged APTT in a young male horse with a history of spontaneous haemorrhage around joints, suggests an intrinsic coagulation defect such as haemophilia. Further laboratory tests, such as those carried out in this paper, are then required before a definite diagnosis of factor VIII deficiency can be made.

B. V. ALLEN

Effects of environmental control on pulmonary function of horses affected with chronic obstructive pulmonary disease

JILL R. THOMSON and E. A. McPHERSON

Department of Veterinary Medicine, Royal (Dick) School of Veterinary Studies, Veterinary Field Station, near Roslin, Midlothian

Summary

The effects of environmental control on horses affected with chronic obstructive pulmonary disease was assessed by clinical examination and pulmonary function tests, ie, maximum change in intrathoracic pressure, tidal volume, minute volume, non-elastic work of breathing, dynamic compliance, inspiratory and expiratory flow rates and arterial blood gas analysis. A controlled environment (ie, bedding horses on shredded paper and feeding a complete cubed diet) caused symptomatic COPD affected horses to become asymptomatic within four to 24 days (mean \pm sd 8.4 ± 4.8 days). When asymptomatic, their pulmonary function values did not differ significantly from those of normal horses, which indicates that the pathophysiological changes occurring in equine COPD are reversible. The time taken for horses to become asymptomatic correlated significantly with age, duration of illness and severity of disease as adjudged by the non-elastic work of breathing.

Introduction

SEVERAL authors have noted clinical improvement in horses affected with chronic obstructive pulmonary disease (COPD) as a result of environmental control (Thurlbeck and Lowell 1964; Eyre 1972; Schatzmann *et al* 1974; Cook 1976). Such measures aim to prevent exposure of susceptible horses to the aetiological agents of COPD which, in Great Britain, are usually thermophilic actinomycetes and moulds occurring in hay and straw. Minimal dust regimes include keeping horses permanently outdoors with no supplementary hay feeding, stabling horses on peat moss, wood shavings or shredded paper and feeding a complete cubed diet, along with stabling horses away from hay and straw.

Although clinical improvement has been noted by several authors as a result of environmental control there have been few studies into the effects of such measures on the pulmonary function of COPD-affected horses. Schatzmann *et al* (1974) recorded significant decreases in respiratory rate in COPD-affected horses following removal of hay and straw from their environment. Pulmonary function tests performed on two of these horses demonstrated decreases in maximum change in intrathoracic pressure (max Δ Ppl) and non-elastic work of breathing and an increase in dynamic compliance as a result of the above-named measures. Similarly, Meister, Gerber and

Tschudi (1976) and Dixon (1979) recorded significant increases in partial pressure of arterial oxygen (PaO_2) in COPD-affected horses which were prevented from access to hay and straw.

This study aimed to provide more information on the pulmonary function changes occurring in COPD-affected horses resulting from environmental control. A range of pulmonary function measurements were obtained from affected horses in the symptomatic and asymptomatic phases of COPD along with values from normal horses and these results compared. The time taken for horses to become asymptomatic in a controlled environment (ie, the remission time) and some factors playing a role therein were also investigated.

Materials and methods

Horses

Twenty horses were identified as being COPD-affected according to the criteria of McPherson *et al* (1978). These horses which had been exposed to dusty and mouldy hay and straw (**Group A1**) were examined clinically and the following pulmonary function parameters were measured: max Δ Ppl, tidal volume, minute volume, non-elastic work of breathing, dynamic compliance, maximum inspiratory and expiratory flow rates, inspiratory time (It), expiratory time (Et), Et:It ratio, PaO_2 , partial pressure of arterial carbon dioxide (PaCO_2) and arterial pH.

These horses were then housed in a controlled environment, ie, exposure to the aetiological agents was minimised by bedding with shredded paper (Shredabed Ltd, Exeter) and by feeding a complete cubed diet (Spillers Agriculture Ltd, London). Care was taken to ensure that hay and straw were not stored or handled in the vicinity of these horses. Stables were washed regularly to minimise dust levels. The horses were given daily clinical examinations and when clinically asymptomatic, the above-named parameters were remeasured. These horses were then classed as **Group A2**.

The same parameters were recorded in 20 clinically normal horses which had no recent history of respiratory illness (**Group B**). These horses were hay-fed and bedded on straw. Case details of the COPD-affected and normal horses are shown in Table 1. Both groups were of similar type, age and body weight (bwt).

Pulmonary function recording techniques

Max Δ Ppl was measured as described by McPherson *et al*

TABLE 1: Case details of COPD-affected and normal horses

COPD-affected horses							Normal horses				
Group and horse number	Breed/type	Sex	Age (years)	Weight (kg)	Duration of illness (months)	Remission time (days)	Group and horse number	Breed/type	Sex	Age (years)	Weight (kg)
A1	Thoroughbred	Mn	10	480.0	12	5	B1	Hunter	Mn	9	481.4
A2	Hunter	Mn	8	452.3	8	7	B2	Heavy hunter	Mn	13	584.5
A3	Hunter	F	15	511.8	48	7	B3	Hunter	F	9	434.5
A4	Pony	Mn	6	383.6	5	6	B4	Hunter	Mn	4	495.9
A5	Hunter	Mn	10	584.1	9	8	B5	Thoroughbred	F	5	539.1
A6	Hunter	Mn	12	509.1	10	5	B6	Hunter	Mn	8	553.6
A7	Hunter	Mn	12	519.1	30	10	B7	Thoroughbred	Mn	4	485.5
A8	Hunter	F	23	458.2	52	24	B8	Hunter	Mn	9	470.9
A9	Heavy hunter	Mn	13	636.7	36	4	B9	Hunter	Mn	20	614.6
A10	Heavy hunter	Mn	10	659.1	12	7	B10	Hunter	Mn	9	543.6
A11	Hunter	Mn	16	409.1	48	14	B11	Thoroughbred	Mn	7	586.4
A12	Hunter	Mn	10	521.8	60	16	B12	Hunter	F	10	493.2
A13	Thoroughbred	M	11	525.5	26	6	B13	Hunter	Mn	3	445.5
A14	Thoroughbred	Mn	7	469.3	5	8	B14	Thoroughbred	Mn	2	365.5
A15	Hunter	F	5	492.6	6	4	B15	Hunter	F	10	528.2
A16	Thoroughbred	Mn	14	515.7	14	9	B16	Heavy hunter	Mn	8	631.8
A17	Thoroughbred	Mn	12	529.0	12	10	B17	Norwegian Fjord	M	10	407.3
A18	Thoroughbred	F	8	488.2	16	5	B18	Hunter	F	12	489.1
A19	Hunter	F	7	526.4	6	6	B19	Thoroughbred	Mn	6	540.0
A20	Hunter	Mn	10	559.7	9	6	B20	Thoroughbred	Mn	10	459.0
Mean (± sd)			10.95 ± 4.08	511.57 ± 66.03	21.20 ± 17.96	8.35 ± 4.82				8.40 ± 4.03	507.93 ± 68.69

Mn Male neuter
F Female

(1978) using narrower polyethylene intraoesophageal tubing (3 mm bore, 2 mm wall × 190 cm long). The intrathoracic pressure changes were recorded in cm H₂O.

Tidal volume, minute volume, inspiratory and expiratory flow rates were measured using a face mask, pneumotachograph (F 3000L Flowhead; Mercury Electronics [Scotland] Ltd, Glasgow) and electronic spirometer (CS9 Electrospirometer; Mercury Electronics [Scotland] Ltd, Glasgow). The face mask, which covered the horse's mouth and nose, was constructed from a rigid polythene container (length 20 cm, top diameter 20 cm, bottom diameter 12 cm) with a 15 cm wide flexible plastic sleeve attached to its proximal end. A 5 cm diameter hole was cut in the base of the mask into which a rigid plastic cuff was sealed with perspex cement. This cuff protruded 3 cm from the base of the mask. The tapering entrance to the flowhead was fitted securely into this cuff and the connection sealed by adhesive bandage.

The intraoesophageal tube passed through an additional 8 mm diameter opening in the base of the mask and this connection was similarly sealed. The mask was held in position on the horse's head by a nylon strap over the poll. The proximal end of the mask was sealed air tight with a double rubber strap secured with self adhesive nylon strip. The pneumotachograph was connected by twin polyethylene tubing 2.5 m long, inner diameter 3 mm, to the spirometer.

The spirometer was calibrated using a Rotameter float gauge (GEC-Elliott, Process Instruments Ltd, Croydon). A vacuum pump provided a positive airflow through the pneumotachograph and the float gauge. A valve between the pump and the pneumotachograph allowed the flow level to be adjusted manually. The tidal volume was calibrated by passing 3 litres of air through the pneumotachograph from a 3 litre syringe.

Intrathoracic pressure, tidal volume, inspiratory and expiratory flow rates were recorded simultaneously and values for these parameters, inspiratory and expiratory time were

calculated from a mean of 10 consecutive and representative respiratory cycles. The minute volume was measured by electronically cumulating the tidal volume over 1 min.

To determine the viscous work of breathing, pressure-volume loops were constructed as described by Sasse (1971) and their area measured by planimetry. Dynamic compliance was calculated from the ratio of tidal volume to the difference in intrathoracic pressure between the points of zero airflow which corresponded to the beginning of inspiration and expiration. For these measurements, care was taken to select a representative respiratory cycle from an area of the tracing where the respirations were constant regarding frequency and amplitude.

Carotid arterial blood samples for PaO₂, PaCO₂ and pH estimations were collected as described by McPherson *et al* (1978), stored in iced water and analysed at 37.7°C with a Corning 168 pH/blood gas analyser (Corning Medical, Melfield, Massachusetts, USA).

Statistical analysis

The results from the three groups were compared by analysis of variance (Downie and Heath 1974) and Duncan's multiple-range test (Bliss 1967, 1970). Student's *t* test was used to compare results between Groups A1 and B and between Groups A2 and B. Student's paired *t* test was used to compare results of Groups A1 and A2. The significance of the correlation between the remission time in COPD-affected horses and their age, bwt, duration of illness, symptomatic max Δ Ppl, tidal volume, non-elastic work of breathing, dynamic compliance and PaO₂ was tested by linear regression (Snedecor and Cochran 1967).

Results

The mean (± sd) pulmonary function values for the three

TABLE 2: Pulmonary function values (mean \pm sd) for symptomatic COPD-affected horses (Group A1), asymptomatic COPD-affected horses (Group A2) and normal horses (Group B), with statistical differences between groups

Parameter	Group A1	Group A2	Group B	F value	Subsets
Respiratory rate (per min)	14.65 \pm 5.21	11.90 \pm 1.97	11.80 \pm 2.55	5.78**	A1, A2, A2, B
Max Δ Ppl (cm H ₂ O)	19.65 \pm 8.68	5.15 \pm 1.00	4.94 \pm 0.93	55.26**	A1, A2, B
Tidal volume (litres)	6.66 \pm 1.61	6.78 \pm 1.52	6.32 \pm 1.53	0.50 ^{NS}	—
Minute volume (litres/min)	93.14 \pm 33.56	78.68 \pm 13.81	72.25 \pm 15.93	4.37*	A1, A2, A2, B
Non-elastic work per breath (joules)	10.46 \pm 5.32	2.27 \pm 0.70	2.21 \pm 0.82	45.89**	A1, A2, B
Non-elastic work per litre tidal volume (joules/litre)	1.59 \pm 0.69	0.34 \pm 0.08	0.35 \pm 0.09	75.07**	A1, A2, B
Dynamic compliance (litres/cm H ₂ O)	0.82 \pm 0.45	2.52 \pm 0.89	2.33 \pm 0.93	27.99**	A1, A2, B
Max inspiratory flow rate (litres/min)	261.34 \pm 58.76	219.86 \pm 36.87	224.25 \pm 39.00	4.92*	A1, A2, B
Max expiratory flow rate (litres/min)	201.90 \pm 31.78	198.02 \pm 27.24	207.92 \pm 41.40	0.43 ^{NS}	—
Inspiratory time (It) (sec)	1.89 \pm 0.64	2.40 \pm 0.51	2.47 \pm 0.56	6.11**	A1, A2, B
Expiratory time (Et) (sec)	2.76 \pm 1.02	2.88 \pm 0.53	2.88 \pm 0.76	0.15 ^{NS}	—
Ratio Et:It	1.50 \pm 0.35	1.24 \pm 0.29	1.20 \pm 0.25	5.89**	A1, A2, B
PaO ₂ (mm Hg)	76.67 \pm 5.11	91.95 \pm 3.18	92.55 \pm 3.12	105.75**	A1, A2, B
PaCO ₂ (mm Hg)	40.39 \pm 3.02	40.22 \pm 2.71	40.36 \pm 3.29	0.02 ^{NS}	—
Arterial pH	7.438 \pm 0.015	7.435 \pm 0.011	7.437 \pm 0.019	0 ^{NS}	—

* P<0.05

** P<0.01

^{NS} Not significant

groups and the statistical differences between groups are presented in Table 2. Compared to normal horses, symptomatic COPD-affected horses, while in the challenge environment, had significantly increased respiratory rate, max Δ Ppl, minute volume, non-elastic work of breathing, maximum inspiratory flow rate and Et:It ratio and significantly decreased dynamic compliance, inspiratory time and PaO₂. There were no significant differences in tidal volume, maximum expiratory flow rate, expiratory time, PaCO₂ or arterial pH between these two groups.

When housed in the controlled environment, the affected horses become asymptomatic in four to 24 days (mean \pm sd 8.4 \pm 4.8 days). When this occurred, there were significant decreases in respiratory rate, max Δ Ppl, minute volume, non-elastic work of breathing, maximum inspiratory flow rate and Et:It ratio and significant increases in inspiratory time and PaO₂. There were no significant changes in the remaining parameters. Pressure volume loops from an affected horse in the symptomatic and asymptomatic phases of COPD are shown in Fig 1.

On testing the results by analysis of variance, the three groups fell into two subsets, **A1** and **A2, B** for all parameters

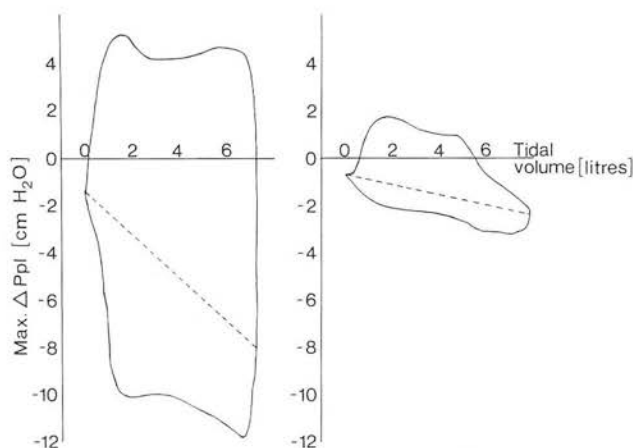


Fig 1. Respiratory pressure-volume loops from a COPD-affected horse (A19) when symptomatic (left) and asymptomatic (right). — — — Dynamic compliance line. Values for non-elastic work per breath: 10.25 joules (symptomatic); 2.18 joules (asymptomatic)

in which significant differences existed between groups, except respiratory rate and minute volume (**A1, A2** and **A2, B**) (Table 2). There were no significant differences in tidal volume, maximum expiratory flow rate, expiratory time, PaCO₂ or arterial pH between groups.

The individual remission times in COPD horses are presented in Table 1. Significant positive correlations existed between this time and age, duration of illness, symptomatic max Δ Ppl, non-elastic work of breathing and a significant negative correlation with dynamic compliance. No significant correlations occurred between the remission time and body-weight, symptomatic tidal volume or PaO₂ (Table 3).

Discussion

Lung volumes were not measured in this study; because the groups of horses were of similar body mass the results were considered comparable.

Symptomatic COPD-affected horses showed significant changes in many pulmonary function parameters, most notably max Δ Ppl, non-elastic work of breathing and PaO₂. This is in accordance with the findings of Sasse (1971), Muylle and Oyaert (1973) and Willoughby and McDonnell (1979).

The pulmonary function values for asymptomatic COPD

TABLE 3: Statistical correlation between remission time (ie, time taken for COPD-affected horses to become asymptomatic in a controlled environment) with their age, bwt, duration of illness and some symptomatic pulmonary function measurements

Parameter	Correlation coefficient	Significance
Age	r(18) = 0.713	P<0.01
Body weight	r(18) = -0.260	P>0.05 ^{NS}
Duration of illness	r(18) = 0.674	P<0.01
Max Δ Ppl	r(18) = 0.854	P<0.01
Tidal volume	r(18) = 0.135	P>0.05 ^{NS}
Non-elastic work per breath	r(18) = 0.846	P<0.01
Non-elastic work per inspired litre	r(18) = 0.819	P<0.01
Dynamic compliance	r(18) = -0.452	P<0.05
PaO ₂	r(18) = -0.289	P>0.05 ^{NS}

^{NS} Not significant

horses did not differ significantly from those of normal horses which indicates that the respiratory function changes occurring in COPD are reversible. However it has been suggested that such pulmonary function tests are only capable of identifying gross respiratory disease and are insufficiently sensitive to detect low grade of localised disease (Spiers, Tschudi and Gerber 1981). Because some of the more sensitive pulmonary function tests used in human medicine, eg, forced expiratory volume over 1 sec, require patient cooperation, they are not applicable to veterinary medicine. Human patients with mild lung disease show more evidence of impaired pulmonary function on exercise than at rest (West 1977). It may, therefore, be of value to perform exercise studies on asymptomatic COPD horses. However, this would necessitate fast treadmill or telemetric techniques.

The use of bronchodilator drugs in symptomatic COPD horses has been found to markedly decrease the max ΔP_{pl} and non-elastic work of breathing (Muylle and Oyaert 1973; Sasse and Hajer 1977; Denac and Pfister 1981). These changes, which were temporary, indicate that airway spasm constitutes a major component of the airway obstruction occurring in COPD. However, at peak response to bronchodilator treatment, the pulmonary function values of COPD-affected horses remained significantly different from those of normal horses (Murphy, McPherson and Dixon 1980). This was thought to be because of the anatomical changes occurring in COPD, ie, diffuse bronchiolitis, and the presence of mucopurulent exudate in the airways (Thurlbeck and Lowell 1964; Nicholls 1978).

From this present study, it would appear that environmental control brings about reversal of these changes thereby allowing pulmonary function and blood gas values to attain normal levels. It also supports the pathological findings of Thurlbeck and Lowell (1964) and Nicholls (1978) that destructive emphysema is not a major feature of this disease. Should structural emphysema occur as part of the pathology in advanced cases (Alexander 1959; Gillespie and Tyler 1969) the degree of reversibility would be proportionately reduced. If such anatomical changes were widespread, the condition would be completely irreversible. However, the authors consider the latter phenomenon to be very uncommon.

The remission time varied greatly and correlated most significantly with age, duration of illness and severity of disease as adjudged by the non-elastic work of breathing. It seems likely that animals in which the disease has been long standing would have more advanced, albeit reversible, pulmonary pathology which would take longer to regress. The finding that two horses which had been affected for more than four years required the longest remission time, ie, 16 and 24 days, suggests that the pathological changes were advanced in those cases. It is also possible that these horses were extremely sensitive to the aetiological antigens of COPD and that the level of such antigen in the environment was sufficient to act as a challenge.

In conclusion, this study suggests that environmental control brings about satisfactory remission of COPD in affected horses and is, therefore, of major importance therapeutically.

Acknowledgements

Thanks to the technical staff of the Department of Veterinary medicine, Royal (Dick) School of Veterinary

Studies, for their assistance with blood gas analyses, Mr J. Pettie for his help in the care and handling of the horses and Mrs S. Oliff for typing the manuscript.

References

- Alexander, A. F. (1959) Chronic alveolar emphysema in the horse. *Am. Rev. Resp. Dis.* **80**, 141-146.
- Bliss, C. I. (1967) *Statistics in Biology* vol 1. McGraw-Hill Book Co. New York. pp 253-257.
- Bliss, C. I. (1970) *Statistics in Biology* vol 2. McGraw-Hill Book Co. New York. pp 499-501.
- Cook, W. R. (1976) Chronic bronchitis and alveolar emphysema in the horse. *Vet. Rec.* **99**, 448-451.
- Denac, M. and Pfister, R. (1981) Der Einfluss des Beta-2-Rezeptoren-stimulierenden Sympathomimeticums Ventipulmin (R) auf die Atmungsmechanik des Pferdes. *Tierärztl. Umschau* **3b**, 188-194.
- Dixon, P. M. (1979) Some blood pressure studies in normal horses and in horses affected with chronic obstructive pulmonary disease. PhD thesis, University of Edinburgh.
- Downie, N. M. and Heath, R. W. (1974) *Basic Statistical Methods*, 4th edn. Harper & Row, New York. pp 206-215.
- Eyre, P. (1972) Equine pulmonary emphysema: A bronchopulmonary mould allergy. *Vet. Rec.* **91**, 134-140.
- Gillespie, J. R. and Tyler, W. S. (1969) Chronic alveolar emphysema in the horse. *Adv. vet. Sci.* **13**, 59-99.
- McPherson, E. A., Lawson, G. H. K., Murphy, J. R., Nicholson, J. M., Fraser, J. A., Breeze, R. G. and Pirie, H. M. (1978) Chronic obstructive pulmonary disease (COPD): Identification of affected horses. *Equine vet. J.* **10**, 47-53.
- Meister, U., Gerber, H. and Tschudi, P. (1976) Die arterielle Blutgasanalyse in der Diagnostik und Überwägung chronisch lungenkranker Pferde. *Schw. Arch. Tier.* **118**, 99-104.
- Murphy, J. R., McPherson, E. A. and Dixon, P. M. (1980) Chronic obstructive pulmonary disease (COPD): Effects of bronchodilator drugs on normal and affected horses. *Equine vet. J.* **12**, 10-14.
- Muylle, E. and Oyaert, W. (1973) Lung function tests in obstructive pulmonary disease in horses. *Equine vet. J.* **5**, 37-44.
- Nicholls, J. M. (1978) A pathological study of chronic pulmonary disease in the horse. PhD thesis, University of Glasgow.
- Sasse, H. H. L. (1971) Some pulmonary function tests in horses. An aid to the diagnosis of chronic obstructive pulmonary disease (heaves) in horses. PhD thesis, University of Utrecht, Holland.
- Sasse, H. H. L. and Hajer, R. (1977) Enkele veterinaire klinische ervaringen met het gebruik van een B2-receptoren stimulerend sympaticomimeticum NAB 365 (R) bij paarden met longaandoeningen. *Tijds. Dier.* **102**, 1233-1238.
- Schatzmann, U., Straub, R., Gerber, H., Lazary, S., Meister, U. and Spörri, H. (1974) Die Elimination von Heu und Stroh als Therapie chronischer Lungenerkrankungen des Pferdes. *Tier. Prax* **2**, 207-214.
- Snedecor, G. W. and Cochran, W. G. (1967) *Statistical Methods*, 6th edn. Iowa State College Press, Ames, Iowa, USA. pp 246-248.
- Spiers, V. C., Tschudi, P. R. and Gerber, H. (1981) Pressure and air flow in the upper respiratory tract of horses with partial obstructions. *Schw. Arch. Tier.* **123**, 293-304.
- Thurlbeck, W. M. and Lowell, F. C. (1964) Heaves in horses. *Am. Rev. resp. Dis.* **89**, 82-88.
- West, J. B. (1977) *Pulmonary Pathophysiology — The Essentials*. Williams & Wilkins Company, Baltimore. pp 102-103.
- Willoughby, R. A. and McDonnell, W. N. (1979) Pulmonary function testing in horses. *Vet. Clin. North Am., Large Anim. Pract.* **1**, 171-196.

TRACHEOBRONCHIAL SECRETION FROM NORMAL HORSES AND HORSES WITH
RESPIRATORY DISEASES.

SUSAN L. MAXWELL

B.V.M.S. (UNIVERSITY OF GLASGOW)

M.R.C.V.S.

Degree of Master of Philosophy
Department of Veterinary Medicine
Royal (Dick) School of Veterinary Studies
University of Edinburgh.

September 1984.

SUMMARY

Some parameters of equine tracheobronchial secretion (TBS) from 9 normal horses were studied and compared with TBS from 25 horses suffering from various chronic respiratory diseases. Specimens were collected using a transnasal route and lavage in 20ml of sterile normal saline.

TBS from normal horses contained fewer cells than from diseased horses and these comprised predominantly ciliated columnar epithelial cells with some squamous epithelial cells and macrophages. Inflammatory cells were scarce or absent. The specimens from 12 horses with chronic obstructive pulmonary disease (COPD) contained neutrophils predominantly. Small to moderate numbers of eosinophils were present in some samples. Curshman's spirals and "foamy macrophages" were also identified. In TBS from 8 COPD affected horses which had been rendered asymptomatic by environmental control, fewer neutrophils and eosinophils were present, as compared with specimens collected in the symptomatic phase. TBS from 6 horses suspected of having chronic bacterial bronchopneumonia contained moderate to large numbers of neutrophils with occasional eosinophils in some specimens. The cytological pattern in TBS from 7 horses with lungworm infestation (Dictyocaulus arnfieldi) was dominated by pronounced eosinophilia with mild to moderate neutrophilia.

Electrophoresis (EP) and immunoelectrophoresis (IEP) on agarose and single radial immunodiffusion (SRID) were performed on the sol phase of TBS separated by routine centrifugation, from normal and COPD affected horses. EP and IEP suggested that the predominant proteins in equine TBS were albumin and immunoglobulins. In specimens from COPD affected horses which were symptomatic at the time of collection, the proportions of protein which migrated in the α_2 , β_1 and γ_2 zones were significantly higher than in specimens from normal horses. TBS from COPD affected horses which were asymptomatic at the time of collection had an electrophoretic protein profile intermediate between that of the symptomatic group and the normal horses. IEP and SRID analyses revealed the presence of IgA in each specimen. IgG and IgT were infrequently present in detectable levels in specimens from normal horses and IgM was not detected. In comparison, IgG and IgT were detected in nearly all of the samples from COPD affected horses. The ratio of IgA/Total Protein was significantly elevated in TBS specimens from COPD affected horses.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN THE HORSE — A REVIEW

JILL R. THOMSON, Veterinary Investigation Centre, Penicuik, Midlothian and
E.A. McPHERSON, Department of Veterinary Medicine, Royal (Dick) School of Veterinary Studies,
Easter Bush, Midlothian.

Chronic obstructive pulmonary disease (COPD) is also known by a number of other terms such as "heaves", "broken wind", "alveolar emphysema", "equine asthma" and more recently the name "recurrent airway obstruction" was introduced. It is probably the most common cause of chronic coughing in horses in the temperate parts of the Northern Hemisphere and its importance is recognised widely. Horses from two years old and over may be affected and the prevalence increases with age. There is no breed or sex predisposition for COPD.

In the United Kingdom, the condition is most frequently seen in the stabled horse and is associated with dusty atmospheres and "mould" in hay and straw. Studies carried out over the past 10 years have shown equine COPD to be a pulmonary hypersensitivity to organic dust antigens in the environment. *Micropolyspora faeni* (a thermophilic actinomycete) and *Aspergillus fumigatus* (a fungus) appear to be the most predominant aetiological agents in northern Britain. The principle antigens may vary in different parts of the world. Thermophilic actinomycetes and fungi occur in large numbers (5 million/g) even in very good quality hay and straw and in amounts of up to 1 million/g in visibly contaminated material (Lacey 1974). Baling fodder with a high moisture content results in heat being generated within the bale, thus creating optimum conditions for multiplication of the thermophilic organisms. Occasionally, the problem may be encountered when horses are left out-of-doors during the summer months in the United Kingdom. These cases are thought to be associated with pollen from grass or trees. Undoubtedly there are many other as yet unidentified agents which may be involved in some cases of COPD. Recently we have succeeded in demonstrating respiratory hypersensitivity to forage mites (*Tyroglyphus* sp.) in COPD affected horses. Multiple hypersensitivity is not uncommon.

The reason for this apparent hypersensitivity in certain horses is not at all clear though a respiratory allergy seems most likely.

CLINICAL SIGNS

The disease may manifest itself suddenly with horses becoming acutely dyspnoeic. More commonly, the condition develops gradually, the horse showing reduced exercise tolerance and a chronic cough i.e. present for more than 3 months. Coughing for over a year is highly suggestive of COPD. Nasal discharge is usually scanty, watery or mucoid. Mucopurulent material may be expelled during coughing resulting in deposits of such material against stable walls or on the floor.

The clinical signs vary considerably in degree, from occasional coughing with a slight increase in expiratory effort to dyspnoea with marked increase in inspiratory and expiratory effort, increased respiratory rate, flaring of the nostrils and frequent coughing. On auscultation, the respiratory sounds vary according to the severity of the condition. This ranges from slightly increased and harsh inspiratory sounds, mostly over the dorsal areas of the chest to a range of pronounced inspiratory and expiratory sounds including crepitant sounds and wheezing being heard all over the chest.

PATHOLOGY AND PATHOPHYSIOLOGY

If a COPD-affected horse has been symptomatic of long standing, at autopsy the lungs fill the freshly open chest and often have rib indentations on the surface. The lungs retain their over-inflated appearance for some time, are pale pink in colour and may have puffy-looking peripheral areas to the lobes. In an asymptomatic horse or symptomatic horses in which the current attack has been of short duration (i.e. less than 7-14 days), the lungs usually appear macroscopically normal.

Nicholls (1978) autopsied 25 COPD-affected horses and summarised her findings as follows: 'the principal lesion

in all 25 horses examined was chronic bronchiolitis consisting of a hyperplastic bronchiolar epithelium, goblet cell metaplasia, peribronchial cellular infiltration and exudation of mucous or pus into the lumen. This combination produced narrowing of the airway and resultant alveolar over-inflation. Emphysema estimated by examining slices of inflated lung was infrequent, occurring only in the cranial lobe or the periphery of the caudal lobe and seemed to be a late development in the course of the disease'. This author stated also that all the small airways (less than 2mm in diameter) was affected and the lesion was characterised as quoted above. More than half the cases examined had a pulmonary eosinophilia. It was also noted that the number and type of goblet cells in the bronchial epithelium were similar in COPD-affected horses and in normal horses. In addition there was no hyperplasia of the bronchial submucous glands and the author concluded therefore that the disease in the horse bears no pathological resemblance to chronic bronchitis and emphysema of man.

During the past 10 years, many groups of workers have been concerned with measuring the pathophysiological changes which occur in COPD-affected horses. It has been shown that horses suffer an increase in the work of breathing and that this is mainly caused by an increased maximum change in intrathoracic pressure during each breath. Pulmonary resistance is increased and dynamic compliance decreased. Hypoxaemia and pulmonary artery hypertension exists in such cases. These changes appear to be reversible in the majority of cases if suitable management changes are introduced.

McPherson et al (1979) showed that inhalation of extracts of *M. faeni* and *A. fumigatus* cultures may exacerbate the clinical disease in many affected horses. It may also provoke asymptomatic horses to become symptomatic. This effect may occur within 1 to 2 hours of inhalation challenge; however, more commonly, the re-

action culminates 4 to 10 hours after challenge. The mechanisms giving rise to this reaction are not known but this is an important area for further study. A functional spasm of the airway smooth muscle is thought to occur as part of the disease process. This has been deduced from studies showing that administration of bronchodilator drugs will alleviate (totally or partially) the signs temporarily.

The addition of forceful expiration to the normal expulsive effort tends to exaggerate the normal collapse of the non-cartilaginous airways and partially collapses even the cartilaginous ones as demonstrated endoscopically by Fischer (1980). This, in addition to airway spasm and the above mentioned pathological changes, causes a reduction of air in the alveoli with each breath because the residual alveolar volume is higher than normal. This leads to a reduction in the alveolar oxygen content (PAO_2) which in turn results in reduced oxygen uptake by the blood. The oxygen level in arterial blood (the partial arterial oxygen pressure- PaO_2), is therefore subnormal. This hypoxaemia causes pulmonary hypertension and consequently an increased right ventricular workload. In extremely advanced cases cor pulmonale is said to occur (Salutini 1959) although Dixon et al (1982) found that this was uncommon even in animals with long standing COPD.

It has been suggested that COPD affected horses suffer from non-specific hyper-reactivity of the airways and that this mechanism rather than allergy is involved in the pathogenesis of COPD. Recent studies by Derkson et al (1985 a and b) have shown that such horses are hyperreactive to aerosol and intravenous administration of histamine whilst symptomatic but during disease remission, the response to histamine is no different from that of control ponies.

DIAGNOSIS

If a horse is showing marked clinical signs of COPD, a diagnosis can be made on these grounds and confirmed by intravenous administration of a bronchodilator drug (e.g. atropine sulphate 0.02 mg/kg bodyweight, clenbuterol (Ventipulmin; Boehringer) 0.8 µg/kg bodyweight or etamiphylline camsylate (Millophylline-V; Dales Pharmaceuticals) 3mg/kg bodyweight). An improvement should be evident within 10 minutes of treatment. A retrospective diagnosis may be made by assessing the horse's response to a change in environment.

If horses are asymptomatic or in the early stages of developing the disease,

the diagnosis can be very difficult and relies on a combination of the history plus attempts to either exacerbate or improve the condition through environmental changes. Such cases may be referred to veterinary schools or equine research centres for assistance with diagnosis. If facilities are available, pulmonary function testing in combination with antigen inhalation challenge may be used. Exposure to mouldy hay or to pollen or, more specifically, to nebulised culture extracts has been described by McPherson et al (1979). A crude natural inhalation challenge can be carried out in practice by exposing horses to mould-contaminated hay or straw for 12 to 24 hours. Should a horse be sensitive to that environment, a distinct worsening of clinical signs should be evident within that time. The owner should observe the horse hourly during the first 8 hours of the challenge in case it precipitates marked respiratory distress. If this occurs, the horse should be removed from the challenge environment immediately. As previously mentioned, horses may be sensitive to a range of different antigens, including pollens, and this should be borne in mind in the event of a horse failing to respond to challenge with mouldy hay or straw.

The use of intradermal testing for antigen sensitivity and examining for serum precipitating antibody have been investigated but found to be unreliable as diagnostic tools. A positive antigen intra-dermal reaction is evidence that a horse has had previous exposure to that antigen and developed skin hypersensitivity. It does not necessarily indicate respiratory hypersensitivity in the horse. Similarly, serum precipitins to antigens are commonly found in both normal and COPD-affected horses and are simply an indication of previous exposure.

Traditionally, an increase in the area of expansion of the lungs was used as a diagnostic test, but in general, this is now considered too subjective and unreliable to be of diagnostic value. Additionally, considerable advancement of lung changes would need to have taken place before a measureable difference in lung area could be detected. Thus, it is of little help to veterinarians called to examine a horse in the early stages of the disease.

In recent years, there have been many studies of the cytology of tracheal aspirate from COPD affected horses and these have all yielded similar results. Neutrophils are the predominant cell type in symptomatic horses comprising 60-70% of the total cell count. They may be found in very large numbers in some cases. As the total

cell count of the aspirate increases, the percentage of neutrophils tends to increase in a linear fashion (Nuytten et al 1983). Macrophages comprise 10-20% of the aspirate with small numbers of epithelial cells, monocytes, eosinophils and lymphocytes also being present. Plugs of inspissated mucus which have adopted the shape of small airways (Kurchman's spirals) may also be seen and indicate a small airways disease. However, these cytological features are not specific enough to be diagnostic for COPD.

Immunological studies on the tracheobronchial secretion of COPD-affected horses are in their infancy. Maxwell (1984) has demonstrated an increase in the total protein content of tracheobronchial mucus of symptomatic COPD horses as compared with normal horses. In particular, the ratio of IgA/total protein was significantly elevated and IgG and IgT were detected in nearly all samples from symptomatic COPD-affected horses. The latter two immunoglobulins were infrequently present in detectable levels in normal horses. Further immunological studies on the tracheobronchial secretion of COPD affected horses may ultimately yield a reliable diagnostic test.

THERAPY

The therapy of COPD involves the introduction of environmental control measures (a so-called minimal dust environment) which may be used in conjunction with chemotherapy.

ENVIRONMENTAL CONTROL

As most cases of COPD are associated with stabling and exposure to the aetiological antigens occurring in hay and straw, the most favourable long term method of controlling the disease is to introduce changes to the environment. This can be achieved most readily by substituting for straw any of the following forms of stable bedding — shredded paper, hardwood shavings or peat with soiled material removed on a daily basis and not 'deep littered'. The horse should be fed on a complete cubed diet and no hay. An alternative suitable feeding regime consists of vacuum-packed hay (Horsehage; Westway, Torbay) in combination with a type of cube appropriate to the horse's energy needs. The stable should, preferably, be at least 46m (50 yards) to the windward side of the hay store, according to the direction of the prevailing wind. However, it is futile to take such precautions with the individual's environment if the dust generated in the maintenance of other horses on the premises gains admission to the airspace in which the

allergic respiratory disease in man. It has no direct bronchodilatory or anti-inflammatory effects. The mechanism of action is not completely understood, but it is believed to stabilise sensitised membranes of pulmonary mast cells. This prevents degranulation of the mast cells after challenge with the aetiological antigens, thus inhibiting the release of pharmacologically active substances, e.g. histamine, slow-reacting substance of anaphylaxis and 5-hydroxytryptamine (Cox 1976). This drug should be administered prophylactically and is not intended for therapy once the patient is showing clinical signs of disease.

Inhaled sodium cromoglycate (Cromovet; Fisons) has been found to be effective for the treatment of equine COPD when administered prophylactically to asymptomatic horses. The duration of the protective period increases linearly with the number of successive days of treatment with the drug, e.g. the protective period varies from about three days after a single sodium cromoglycate treatment to as long as 24 days after four successive days' treatment. Twice-weekly sodium cromoglycate inhalations have been found to be effective in preventing the onset of COPD when affected horses are housed in a challenge environment (i.e. on hay and straw) for longer periods. In this respect, a four day treatment schedule may be preferred because this would allow longer inter-

vals, e.g. 21 days, between treatments.

This therapy, although of proven efficacy, should not, if possible, be substituted for the management of cases by means of environmental control. However, there are some cases where exposure to the aetiological antigens may be unavoidable, e.g. during transport or when horses are temporarily housed away from their home environment. In such instances, prophylactic sodium cromoglycate treatment could facilitate the management of a horse kept at livery or in large stables where the provision of special environmental control measures may prove difficult to institute.

REFERENCES

- Cox, J.S.G. (1976). *Disodium cromoglycate (cromolyn sodium) in bronchial asthma*. Eds E.B. Weiss and M.S. Segal. In: *Bronchial Asthma: Mechanisms and Therapeutics*. Little, Brown & Co., Boston. pp. 805-836.
- Deegan, E., Lieske, R. and Fischer, J. (1980). *Eine neue Methode der sekretolytischen Therapie bei Pferden mit chronisch obstruktiver Bronchitis*. *Deutsche Vet. Gese.* 1-3 October 1980, pp 63-73.
- Derksen, F.J., Robinson, N.E., Armstrong, P.J., Stick, J.A. and Slocombe, R.F. (1985a). *Airway reactivity in ponies with recurrent airway obstruction (heaves)*. *J. Appl. Physiol.* 58, 598-604.
- Derksen, F.J., Scott, D., Robinson, N.E., Slocombe, R.F. and Armstrong, P.J. (1985b). *Intravenous histamine administration in ponies with recurrent airway obstruction (heaves)*. *Am.J.Vet.Res.*, 46, 774-777.
- Dixon, P.M., Nicholls, J.M., McPherson, E.A., Lawson, G.H.K., Thomson, J.R., Pirie, H.M. and Breeze, R.G. (1982). *Chronic obstructive pulmonary disease, anatomical cardiac studies*. *Equine Vet. J.* 14, 80-82.
- Fischer, J. (1980). *Bronchokopische untersuchungen als beitrage zur klinischen und atiologicalen diagnostik bei pferden mit atemwegserkrankungen*. Doctoral thesis, Hanover.
- Lacey, J. (1974). *The microbiology of hay and straw*. In: *Aspergillosis and Farmer's Lung in man and in animal*. Eds R. De Haller and F. Suter. Hans Huber, Berne, pp 16-23.
- McPherson, E.A., Lawson, G.H.K., Murphy, J.R., Nicholson, J.M., Breeze, R.G. and Pirie, H.M. (1979). *Chronic obstructive pulmonary disease (COPD) in horses: Aetiological studies: Responses to intradermal and inhalation antigenic challenge*. *Equine Vet. J.* 11, 159-166.
- Maxwell, S.L. (1984). *Tracheobronchial secretion from normal horses and horses with respiratory disease*. M. Phil thesis, University of Edinburgh.
- Nicholls, J.M. (1978). *A pathological study of chronic pulmonary disease in the horse*. PhD thesis, University of Glasgow.
- Nuytten, J., Muylle, E., Oyaert, W., Van Den Hende, C., Vlaminck, K. and De Keersmaecker, F. (1983). *Cytology, bacteriology and phagocytic capacity of tracheobronchial aspirates in healthy horses and horses with chronic obstructive pulmonary disease (COPD)*. *Zbl. Vet. Med. A.*, 30, 114-120.
- Salutini (1959). *Cuore polmonare cronico e bolsaggine*. *Nuova Vet.* 35, 101-104.
- Thomson, Jill, R. (1982). *Studies of the therapy of equine chronic obstructive pulmonary disease*. PhD thesis, University of Edinburgh.