STUDIES ON THE THERAPY OF EQUINE CHRONIC OBSTRUCTIVE PULMONARY DISEASE.

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I declare that the contents of this thesis are my own work and have not been presented to any University other than the University of Edinburgh.

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To my husband Iain and to my family.

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SUMMARY

The review of the literature indicated that although there have been several recent studies into the aetiology and pathology of equine chronic obstructive pulmonary disease (COPD) as well as into the functional disturbances occurring in this disease, very few objective studies have been carried out into the management and therapy of affected horses. The effects of environmental control and several therapeutic agents in COPD affected horses are therefore investigated in this thesis.

The use of a controlled environment, i.e. minimising exposure to the aetiological antigens which are contained in hay and straw, by bedding horses on peat or shredded paper and feeding a complete cubed diet, allowed symptomatic COPD affected horses to become asymptomatic within 4 to 32 days (mean ($\pm$ S.D.) 9.0 $\pm$ 4.8 days). When asymptomatic, their respiratory function values did not differ significantly from those of normal horses. This indicates that the pathophysiological changes occurring in equine COPD are reversible and that most COPD affected horses are capable of regaining normal pulmonary function when contact with the aetiological antigens is minimised.

Symptomatic COPD affected horses are those showing clinical signs of COPD with abnormal pulmonary function values, as described by McPherson *et al.*, 1978, i.e. maximum change in intrathoracic pressure (max. Δ Ppl) >

6 mm Hg and partial pressure of arterial oxygen (PaO_2)< 82 mm Hg. Asymptomatic COPD affected horses are those previously shown to be affected with COPD by the above-mentioned criteria which, at the time of the present examination, are clinically normal and their pulmonary function values within normal ranges, i.e. max. Δ Ppl < 6 mm Hg and PaO_2 > 82 mm Hg.

Inhalation or intravenous administration of bronchodilator drugs (atropine, isoprenaline, terbutaline, clenbuterol and etamiphylline camsylate) to symptomatic COPD affected horses brought about a temporary, marked improvement in clinical signs, accompanied by significant decreases in max. Δ Ppl and significant increases in PaO_2. These findings show that airway spasm is involved in the pathogenesis of equine COPD. Although the therapeutic use of parenterally administered bronchodilator drugs in this disease is subject to many limitations including partial effectiveness, short duration of action and side effects, this form of therapy could be of value as a temporary measure in the treatment of acute or severe attacks.

Studies into the efficacy of orally administered bronchodilator drugs for equine COPD proved disappointing. When horses were housed in the natural antigen challenge environment, i.e. exposed to poor quality hay and straw bedding which was dusty and visibly contaminated with moulds, and treated with oral clenbuterol or

etamiphylline camsylate, they remained symptomatic. Apart from the significant decreases in respiratory rate and max. ΔPpl recorded on 2 days during the clenbuterol trials, there were no significant changes in their pulmonary function values from those recorded when horses were untreated and housed in similar conditions. 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.

In contrast to the results of oral bronchodilator treatment, studies on the prophylactic treatment of asymptomatic COPD affected horses with inhaled sodium cromoglycate proved hopeful. In preliminary studies, prophylactic sodium cromoglycate inhalation in 2 affected horses prevented the exacerbation of respiratory disease, normally observed in COPD affected horses 4 to 8 hours after experimental Micropolyspora faeni inhalation challenge. These studies were followed by a clinical trial with 56 COPD affected horses in which it was shown that a linear response existed between the number of successive days treatment with this drug and the duration of remission of COPD, while horses were exposed to natural challenge. The protective period was 3.6 $\pm$ 1.1 days (mean $\pm$ S.D.) after a single sodium cromoglycate treatment, 8.0 $\pm$ 3.4 days after 2 days treatment, 11.9 $\pm$ 2.9 days after 3 days treatment and

24.3 $\pm$ 13.4 days after 4 days treatment.

In a 28 day trial, two successive days sodium cromoglycate treatment administered at weekly intervals was effective in preventing the onset of COPD in 6 out of 8 affected horses housed in the natural challenge environment. These experiments show that prophylactic treatment of asymptomatic COPD affected horses with inhaled sodium cromoglycate is an effective method of controlling this disease. As sodium cromoglycate is believed to act by stabilising mast cell membranes, these results suggest that pulmonary mast cell degranulation is involved in the pathogenesis of equine COPD.

Sodium cromoglycate treatment would be useful when unavoidable exposure to the aetiological antigens is anticipated, for instance, during transportation or when horses are moved temporarily away from the home environment. 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 to individual animals may prove difficult to institute.

ABBREVIATIONS.

COPD.	chronic obstructive pulmonary disease.
M. faeni	*Micropolyspora faeni*.
A. fumigatus	*Aspergillus fumigatus*.
max. ΔPpl	maximum change in intrathoracic pressure.
PaO_2	partial pressure of arterial oxygen.
$PaCO_2$	partial pressure of arterial carbon dioxide.
cyclic 3',5'-AMP	cyclic 3', 5'- adenosine mono-phosphate.
COMT	catechol-O-methyltransferase.
SRS-A	slow releasing substance of anaphylaxis.
5-HT	5-hydroxytryptamine.
SCG	sodium cromoglycate.
NSAIDS	non-steroidal anti-inflammatory drugs.

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CHAPTER 1

GENERAL INTRODUCTION

Chronic obstructive pulmonary disease (COPD) formerly known as heaves, broken wind or alveolar emphysema is probably the commonest chronic equine respiratory disease occurring in the United Kingdom and Western Europe. The disease was recognised as early as 333 B.C. by Aristotle who described the characteristic "drawing in of the flank" or "heave". Although the prevalence of the disease appears to fluctuate from year to year, COPD was regarded by Gerber (1973) as the most common cause of premature retirement in horses through disease.

COPD which affects horses 2 years old and over has been shown to be a respiratory hypersensitivity to thermophyllic actinomycetes and fungal organisms (hereafter loosely termed "moulds") occurring in hay, straw and organic dust and/or to pollens. Although the aetiological antigens may vary with geographical location Micropolyspora faeni (M. faeni) and Aspergillus fumigatus (A. fumigatus) have been identified as common causes of the disease in Northern Britain (McPherson et al., 1979b). Very large numbers of these moulds occur in hay and straw which has been baled with a high moisture content and subsequently become heated giving rise to ideal conditions for growth of these thermophyllic actinomycetes (Lacey, 1974).

The pathogenesis of COPD is poorly understood. Upon exposure to the aetiological antigens, affected horses may show signs of disease within 30 minutes to

1 hour, however, the response is more frequently observed 4 to 8 hours thereafter. The major pathological anatomical changes occurring in equine COPD are a diffuse exudative bronchiolitis and alveolar overinflation (Nicholls, 1978). Despite the disease's previous nomenclature i.e.: alveolar emphysema and despite the long standing but unproven analogy between this syndrome and human pulmonary emphysema, in which the pulmonary changes are largely irreversible, structural emphysema is not a major feature of equine COPD and when it does occur, is limited to small areas of the lungs (Nicholls, 1978).

Although equine COPD has been recognised for many centuries, few definitive studies have been carried out on the treatment of the disease. Early authors advocated good nutrition, good ventilation, clean stables and rest combined with the empirical systemic administration of agents such as arsenic or strychnine (Huytra and Marek, 1926) and later, the parasympatholytic drug atropine or the feeding of atropine-containing plants (Alegren and Carlström, 1940) despite the attendant side effects which were frequently quite severe.

Management of the disease by means of environmental control has been favoured by more recent authors (Cook, 1965; Eyre, 1972) who reported clinical improvement in horses turned out to grass or housed on peat moss or wood shavings instead of straw and fed a pelleted diet or dampened hay. Chemotherapeutic agents which have been used for the treatment of COPD include

corticosteroids, bronchodilators, bromhexine hydrochloride and antihistamines.

The corticosteroids act by suppressing the allergic response and will partially alleviate clinical dyspnoea in affected horses (Gerber, 1973; Beech, 1979b). However, the resulting improvement only lasts for the duration of the corticosteroid therapy and due to their side effects, the corticosteroids are not favoured for long term therapy of this condition.

Bronchodilator drugs including atropine and the sympathomimetic drugs, adrenaline and noradrenaline have been reported to bring about a very rapid and marked clinical improvement in affected horses (Obel and Schmiterlöw, 1948; Schatzmann, Straub and Gerber, 1972) but they have not been widely used due to their very short duration of action (less than 2 hours) and their untoward cardiac stimulating effects. More recently, a longer acting beta 2 sympathomimetic bronchodilator, clenbuterol, has been used with more success in a limited number of horses (Sasse and Hajer, 1977).

Neither the antihistamines nor bromhexine hydrochloride have been found to have any lasting beneficial effects in COPD affected horses (Beech, 1979a).

As yet, none of the above measures have been shown to adequately control the disease. More work is therefore required on the therapy of equine COPD, regarding both the effects of environmental control and the newer chemotherapeutic agents.

CHAPTER 2

REVIEW OF THE LITERATURE

HISTORICAL REVIEW OF THE LITERATURE

COPD was recognised as early as 333 B.C. when Aristotle, in "The History of Animals" described a disease of horses characterised by "drawing in of the flank" (Smith, 1919). In the 13th Century, a German, Conrad Heresbach reported that under the existing laws, any horse which developed "broken wind" subsequent to purchase could be returned to the original owner. The disease was thought to be chronic, incurable and of unknown aetiology (Smith, 1919).

In 1523, Fitzherbert considered "broken wind" to be a possible sequel to fast work especially after recently drinking water (Smith, 1919). Fitzherbert observed that the condition was least likely to occur in horses kept at grass. Clifford, in 1585, is believed to be the first author to advocate good ventilation for the prevention of "broken wind" (Smith, 1919).

In 1664, Solleysel associated "broken wind" with the feeding of hay, particularly to older animals. Many later authors ascribed the disease to poor quality feed (Gibson, 1751; Law, 1896; Baker, 1900; Malkmus, 1912).

Solleysel considered the disease to be hereditary (Smith, 1919), as did Gibson (1751) and Axe (1906), the latter author observing the disease more frequently in coarse-bred horses and ponies than in thoroughbreds.

Floyer (1698) was the first to record the

pathological changes in the lungs of a horse with "broken wind". He described "....rupture or dilation of the bladders of the lungs". Gibson (1751) considered the lungs to have lost their elasticity. In 1837, Bracy Clark performed postmortem examinations on a few affected horses and found pulmonary emphysema which he thought resulted from "rupture of the air cells" (Smith, 1924). Delafond (1844) found emphysema in 45 out of 54 horses with "broken wind", however Percivall (1853) reported that emphysema was not necessarily present in all cases, an opinion shared by Law (1896).

The importance of "good stable ventilation and hygiene" in the prevention of respiratory disorders, and in particular "broken wind" was stressed by Clark (1788), by Coleman and by many other early authors (Smith, 1905; Fitzwygram, 1911).

Malkmus (1912) was the first to perform pulmonary function studies in horses and use such tests to quantify equine pulmonary dysfunction. By means of a kymograph, he recorded the intrapleural pressure during the respiratory cycle of a horse with "heaves" and compared it with that of a normal horse. He was thus able to demonstrate an increased expiratory effort in the affected horse.

DISEASE DEFINITION

The problem of disease definition and in particular that of defining the equine syndrome known as "heaves",

"broken wind", chronic alveolar emphysema, chronic pulmonary disease, pulmonary emphysema, chronic obstructive pulmonary disease (COPD), "dampfigkeit" in the German literature and "pousse" in the French literature has been reviewed by Breeze (1979). This author outlined the main features upon which disease definitions are usually based, namely; clinical-descriptive, morbid-anatomical, functional or aetiological. A term such as "heaves" is clinical - descriptive but unfortunately, there is incomplete agreement on what is understood by this term (Sasse, 1971).

Although there is unanimous agreement over the respiratory involvement in this syndrome, there has been a tendency particularly in the German literature to also include certain cardiac conditions under the term "heaves" (Malkmus and Oppermann, 1935; Bolz and Bieniek, 1961; Gerber, 1969). Neither "heaves" nor "broken wind" give any exact indication as to the underlying pathology. This was demonstrated by Breeze (1979) who classified 9 pathological conditions all of which he claimed could give the clinical signs of "heaves".

On the basis of lung function tests, Sasse (1971) introduced COPD into the veterinary literature and this functional-descriptive term has been adopted by most subsequent authors. Littlejohn (1978) and Breeze (1979) felt a need for further clarification of the

term COPD, as they claimed there were a number of different pathological entities potentially capable of being classed as COPD. However, rigidly sub-classifying COPD on a pathological basis could re-introduce confusion as detailed pathological sub-divisions may be impossible to differentiate clinically. Furthermore, it is possible that differing pathological changes may be manifestations of the same disease process depending on the degree and duration of the illness.

On the basis of post-mortem examinations on 25 COPD affected horses, in the only thorough pathological study of this syndrome to date, Nicholls (1978) suggested that the term COPD should be replaced by chronic bronchiolitis as this was the major pathological feature present in all the horses.

Currently, COPD appears to be the most appropriate term available for this syndrome and it is probable that a more definitive term will only arise when there is universal agreement on the aetiology, pathogenesis and pathology of this disease.

EPIDEMIOLOGY

OCCURRENCE AND PREVALENCE.

The occurrence of equine COPD appears to be world-wide. There are many reports in the literature from European countries (Sasse, 1971), Great Britain (McPherson *et al.*, 1978), U.S.A. (Halliwell *et al.*, 1979), Canada (Eyre, 1972) and Southern Africa (Littlejohn, 1978). COPD is reported to occur very

rarely in Australia and New Zealand (Rose, R.J., 1981; Goulden, B.E., 1981, both personal communications).

The current prevalence of COPD in Great Britain is unknown, but Cook (1976) considered it to be the most common chronic equine pulmonary disease. A survey carried out by the British Equine Veterinary Association in 1962-63 (BEVA, 1965) indicated that 0.3% of horses examined by veterinary surgeons in Great Britain suffered from this disease. In Sweden, more accurate statistics of its incidence are available owing to the practice of insuring against this disease. Over a 10 year period from 1968-1977, COPD accounted for 428 claims out of 509,400 horses insured with a company which insures over half of the horses in Sweden (Bergsten, G., 1979, personal communication). This gives a mean incidence of 0.08% in that country.

In general, COPD is a disease of individual animals (Eyre, 1972), but there have been reports where a large proportion of horses in a stable were affected (Morgan, 1940; McPherson et al., 1979a).

AGE DISTRIBUTION

Up to the 1960's COPD was generally regarded to affect horses 8 years old and above (Udall, 1954; Alexander, 1959). However, Bolz and Bienick (1961) recorded the disease in horses 3 and 4 years of age, as

did Gerber (1973) who noted the disease in horses from 3 to 19 years of age. Sasse (1971) found a mean (± S.D.) age of 8.5 ± 4.1 years in 38 COPD affected animals and this group included 2 foals, 1 two-year-old, 1 three-year-old and 5 four-year-old horses. Littlejohn's (1978) series of 20 COPD affected horses ranged in age from 3 to 19 years old, mean (± S.D.) 9.9 ± 4.5 years. A group of 37 affected horses examined by McPherson et al., (1979a) included horses from 2 years old upwards, the prevalence of the disease increasing with age. Most horses were in the 6 to 10 year old age group, but the authors suggested that this may have been a reflection of the age grouping of the horse population as a whole.

BREED TYPE AND WORK PERFORMED.

There does not appear to be any recorded breed predisposition for COPD. Udall (1954) and Alegren and Carlström (1940) observed the disease more commonly in heavier breeds and draught horses, than in thoroughbreds. The disease was frequently seen in ponies and hunters but was rare in the British racehorse (Cook and Rossdale, 1963; Cook, 1965). A wide representation of different breeds was recorded by Bolz and Bieniek (1961) Sasse (1971) and Eyre (1972). McPherson et al., (1979a) detected COPD most commonly in showjumping and hacking horses. Of the horses in their non-random sample of the population, thoroughbred horses were affected least and ponies most often.

SEX.

Alexander (1959) considered COPD to affect both sexes equally and Sasse (1971) and McPherson et al., (1979a) found no significant difference in the sex distribution between affected and non-affected horses.

ENVIRONMENTAL CONDITIONS AND SEASONAL FACTORS.

Many authors have associated COPD with the stabling of horses and with the feeding of hay, particularly poor quality hay (Alexander, 1959; Cook and Rossdale, 1963; Eyre, 1972; Gerber, 1973; McPherson et al., 1979a). However, there are a few reports of the disease commencing or being exacerbated in horses at pasture, in the absence of hay feeding (Foley & Lowell, 1966; Halliwell, R. 1979, personal communication).

McPherson et al., (1979a) found that stabling horses did not, in itself, significantly influence the incidence of COPD ($P > 0.05$) but poor stable ventilation did ($P < 0.05$). Contact with 'poor quality' hay or straw (visibly heavily contaminated by moulds, very dusty or showing marked evidence of dampness or over-heating) was significantly greater in their affected animals ($P < 0.05$). The authors concluded, "Thus the more frequent the exposure to moulds in the 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".

Whereas Gerber (1973) believed a seasonal incidence existed depending on seasonal differences in feeding practices, neither Gillespie and Tyler (1969) nor Littlejohn, Schlegemilch and Le Roux (1977) or McPherson et al., (1979a) were able to record any significant seasonal distribution of COPD.

AETIOLOGY

THE ROLE OF ALLERGY

As large numbers of horses subjected to poor environmental conditions do not contract COPD, McPherson et al., (1979a) postulated that intrinsic factors, possibly related to the immunological reactivity of individuals, play a role in the pathogenesis of equine COPD. Many earlier authors considered the disease to be allergic in nature (Alström and Lauritzson, 1953; Cook and Rossdale, 1963; Lowell, 1964; Eyre, 1972; Gerber, 1973; McPherson and Lawson, 1974; Cook, 1976). Furthermore, some authors likened the disease to allergic respiratory disease in man and in particular, to bronchial asthma (Alexander, 1959; Hoyt, 1963; Thurlbeck and Lowell, 1964; Cook, 1965; Gerber, 1973).

Lowell (1964) hypothesised that the disease was induced by contact with one or more of the components of hay. Cook (1965) and Eyre (1972) believed that moulds and dust from hay and straw constituted the aetiological allergens, whilst Cook (1976) also incrimated fungal spores.

In studies on the pathogenesis of COPD and on the possible antigenic agents involved, researchers carried out intradermal, inhalation and serum precipitin tests, the results of which are discussed on pages 15, 16, and 17 respectively. To the same ends, Eyre (1972), using isolated pulmonary venous strips from 3 affected horses, demonstrated positive Shultz-Dale reactions to specific fungal antigens in 2 animals which had previously elicited positive intradermal reactions to these same antigens in vivo. The contraction of the pulmonary venous strips in the presence of antigen was taken to indicate pulmonary vascular hypersensitivity in the donor animal.

Antigen Intradermal Tests

Larsson (1936) appears to have been the first to perform equine antigen intradermal tests and he found cutaneous hypersensitivity in COPD affected horses, to extracts of mouldy hay as also did Sertić (1968). However, Lowell (1964) reported that hay dust, house dust and mould extracts elicited similar intradermal reactions in 6 affected and 6 normal horses. He, therefore, thought it possible that many hay-fed horses develop skin hypersensitivity to allergenic components in hay without developing pulmonary hypersensitivity, i.e. COPD. Eyre (1972) recorded marked intradermal reactions in 10 out of 15 COPD affected horses against moulds commonly found in Canadian stables, whilst 6 out of 10 clinically normal horses showed relatively weak

skin reactions to the same antigens. A. fumigatus was found to elicit the most positive reactions in affected horses. Eyre (1972) also demonstrated the presence of reagin-like skin-sensitising antibodies in the sera of 8 affected horses by means of the Prausnitz-Küstner passive cutaneous anaphylactic test.

Schatzmann and Gerber (1972) performed intradermal tests on horses in Switzerland using a wide range of commercially available allergy testing solutions, but were unable to demonstrate any allergen causing significantly more positive reactions in affected horses as compared with the controls. McPherson et al., (1979b) recorded positive intradermal reactions in 25 out of 35 affected horses to M. faeni and in 17 out of 34 affected horses to A. fumigatus. Significantly more COPD affected horses showed skin sensitivity to these agents than did those in the control group ($P<0.001$). Many of their horses responded positively to mixed moulds, actinomycetes and fewer to pollens, but there was no significant difference in the incidence of positive responses to these antigens between the affected and non-affected horses.

Using a wide range of allergens including moulds, Halliwell et al., (1979) carried out intradermal tests on 25 COPD affected and 25 normal horses. Positive reactions were seen more frequently and the mean reaction size was greater in diseased horses than in normal horses (both $P < 0.001$). M. faeni elicited the

greatest number of positive reactions.

From the literature, it is apparent that COPD affected horses show cutaneous hypersensitivity to certain environmental antigens more frequently than do normal horses. Moulds appear to be involved most frequently and although the most prevalent antigens may differ from one country to another, M. faeni and A. fumigatus have each been implicated by two groups of workers on different continents.

To evaluate the accuracy of intradermal tests in the diagnosis of respiratory hypersensitivity as assessed by antigen inhalation tests, McPherson et al., (1979b) compared the results of these tests in 35 COPD affected horses. Using M. faeni antigen, the agreement between the tests was 92%, so the M. faeni intradermal test could therefore be used as an indicator of the cause of respiratory hypersensitivity in COPD affected animals. The agreement between the two tests using A. fumigatus was low, possibly resulting from the two antigens being prepared differently from different strains.

Antigen Inhalation Tests

Alström and Lauritzson (1953), Lowell (1964), Sertić (1968), Eyre (1972) and Nicholls (1978) performed crude antigen inhalation tests on COPD affected horses by exposing them to dusty hay and provoked signs of COPD in this way. All these authors thus concluded that the disease was allergic in nature and that the

causal allergic agents were airborne.

McPherson et al., (1979b) performed antigen inhalation tests on normal and affected horses. Maximum intrapleural pressure changes and arterial oxygen partial pressures were used in addition to clinical examinations, to assess the response to inhalation challenge. 25 out of 32 COPD affected horses responded positively to M. faeni, 12 out of 29 to A. fumigatus, 6 out of 8 to mouldy hay and 5 out of 12 to Rye grass pollen. 2 COPD affected horses failed to respond to inhalation challenge with available antigens. Multiple hypersensitivity was not uncommon. 6 and 4 normal horses were challenged with M. faeni and A. fumigatus respectively. None of these horses gave a positive response to inhalation challenge.

Antigen inhalation tests using specific antigens confirmed the hypothesis that respiratory hypersensitivity exists in COPD affected horses and studies by McPherson et al., (1979b) identified M. faeni and A. fumigatus as common allergens involved in COPD in Northern Britain.

Serological Tests

Serum precipitating antibodies to the aetiological antigens are found in humans and cattle suffering from certain allergic pulmonary diseases (Pepys et al., 1963; Longbottom and Pepys, 1964; Wiseman et al., 1973; Hollingdale, 1974) and they are thought to indicate previous exposure to the relevant antigen.

Serum precipitating antibodies to M. faeni and A. fumigatus were isolated in COPD affected horses by Schatzmann and Gerber (1972), Nicholls (1978) and Lawson et al., (1979). Lawson et al., (1979) reported that whilst precipitins to both antigens were shown in normal horses, they occurred more frequently in affected animals and their findings indicate that M. faeni is a major allergen involved in equine COPD in Great Britain. It is, however, interesting to note that the serum precipitating antibody responses in COPD affected horses following M. faeni and A. fumigatus inhalation challenge are quite different (Lawson et al., 1979). It seems likely that the varying responses reflect differences in the nature of the antigens and the behaviour of these micro-organisms following inhalation.

THE ROLE OF INFECTIOUS AGENTS.

A number of authors considered infectious agents to be a primary cause of COPD (Huytra and Marek, 1926; Wester, 1935; Cook and Rossdale, 1963; Lowell, 1964; Gerber, 1973; Fischer, 1980). Gerber (1973) recorded acute influenza prior to developing the syndrome in 25 out of 33 affected horses whilst Streptococcal infections were believed to be secondary or aggravating factors in COPD by Erasmus (1965) and Gerber (1973). McPherson and Lawson (1974) reported that many affected

horses had a history of continuing coughing for many months following bacterial e.g.: Streptococcus zooepidemicus or viral e.g.: Influenza A/equi type 1 infections. The precise role of infectious agents in the aetiology of equine COPD is unclear. McPherson and Lawson (1974) postulated that damage to the respiratory mucous membrane resulting from such infections may allow antigens to make contact with immunologically active tissues resulting in the development of respiratory allergy in susceptible horses.

In man, stressful conditions or viral or bacterial infections have been shown to be predisposing factors in the development of hypersensitivity (Szentivanyi, 1968). Following many viral respiratory infections in man, Picken, Niewohner and Chester (1972) found that there could be prolonged changes in the small airway dynamics which were not clinically apparent and Laitinen et al., (1976) found decreases, possibly permanent, in the respiratory irritant receptor threshold. Similarly, Empey and co-workers (1976) reported that viral upper respiratory infections in otherwise healthy human subjects cause a transient but sometimes striking increase in the bronchial response to chemical and pharmacological stimuli. This bronchial hyperreactivity persists for 4 to 6 weeks after symptomatic recovery and is thought to be caused by damage to the airway epithelium.

THE ROLE OF IRRITANT MATERIALS.

There was a view held among Dutch and German authors that stable dust could initiate COPD in horses by direct irritation of respiratory mucous membranes (Hajer, 1979; Fischer, 1980). There were, however, no studies to substantiate this hypothesis. To establish the effects of inhalation of non-allergic foreign material by affected horses, McPherson, E.A. (1980, personal communication) challenged horses with kaolin dust. He was unable to elicit any response in any of the horses as a result of dust inhalation.

Bannerman and Nicolet (1976), investigating the components of the M. faeni antigen, discovered a chymotrypsin-like enzyme produced by M. faeni as a metabolic by-product of its growth process and postulated that this enzyme, through its irritant nature, may be responsible for respiratory diseases in man and animals associated with M. faeni. Thomson, J.R. (personal observations) measured the chymotrypsin content of different batches of M. faeni antigen used for inhalation challenge of affected horses. There was no significant correlation between the antigen's chymotrypsin content and the degree of response to challenge in COPD affected horses and the author deduced therefore, that this enzyme does not play a significant role in the onset of the disease.

GENETIC FACTORS.

Neither Gillespie and Tyler (1969) nor Sasse (1971) found any evidence of a hereditary predisposition to COPD in horses. However, Littlejohn, Schlegemilch and Le Roux (1977), surveying a horse population of about 14,000 in Southern Africa found that the onset of COPD in some cases may be associated with genetic factors also linked to the coat colour. All reported COPD cases were chestnut in colour and the probability of such a colour linkage was highly significant ($P < 0.001$).

In man, some cases of chronic alveolar emphysema have been shown to be associated with a hereditary alpha-1-antitrypsin deficiency caused by a single autosomal recessive gene (Eriksson, 1965). Breeze et al., (1977) investigated the role of antitrypsin deficiency in the aetiology of equine COPD by studying the serum trypsin inhibitory capacity in 19 affected horses and ponies. These authors found no significant diminution of this factor in COPD cases. Matthews (1979) identified and characterised 2 major antiprotease components in equine serum and investigated their role in the onset of COPD. The author found no definite association of reduced levels of the Pr antiprotease, the homologue of human alpha-1-antitrypsin, with COPD in the horse. Furthermore, Ek and Braend (1980) have shown that no known allele product of the Pr locus in

the horse is associated with significantly low serum antiprotease protein levels. Thus, it is apparent that a hereditary antiprotease deficiency does not play a role in the aetiology of equine COPD.

OTHER FACTORS.

Gillespie and Tyler (1967a and b) postulated that loss of pulmonary elastic tissue plus an increase in the amount of surfactant was responsible for the emphysema described in their cases of COPD. These authors found type II epithelial cells, which are believed to produce surfactant, to be present more frequently in the alveoli and to be larger in size in horses with COPD than in normal horses.

Emphysema and other structural pulmonary changes have been artificially induced in normal horses through administration of substances by routes other than inhalation. McLaughlin and Edwards (1966) injected chlorpromazine into the proximal bronchial artery of normal horses and this resulted in an obliterative pulmonary endarteritis and emphysema in those animals. Pathologically, the induced changes did not resemble the major lesions occurring in equine COPD, as described by Nicholls (1978). Breeze (1979) reported that oral administration of 3-methylindole (an end-product in the metabolism of L-tryptophan by Lactobacillus skatoli) to normal horses consistently produced severe obstructive pulmonary disease with similar pathological changes to COPD. He, therefore, postulated that exposure routes

other than inhalation may be involved in the pathogenesis of equine COPD.

McPherson, E.A. and Thomson, J.R. (personal observations) have recorded two cases of COPD in which clinical signs on some occasions were excitement-induced. Two horses in which signs of COPD appeared to be exercise-induced have been reported (Robinson and Sorenson, 1978 ; Hillidge, C.J., 1979, personal communication).

Thus the question as to why some horses develop COPD whereas others, ostensibly exposed to similar environmental conditions do not, still remains. Although the aetiology may be complex with factors such as a previous bacterial or viral infection predisposing to the onset of the disease, most workers appear to agree that COPD is primarily allergic in origin. The aetiological allergens may vary in different parts of the world but thermophyllic actinomycetes have been widely implicated and in Northern Britain, M. faeni and A. fumigatus have been identified as playing a major aetiological role.

CLINICAL SIGNS

Horses affected with COPD were frequently presented with a history of poor work performance (Gillespie and Tyler, 1969; McPherson et al., 1978; Littlejohn, 1978).

The disease was found to be non-febrile (Mahaffey,

1962; Cook, 1965; Sasse, 1971) and the heart rate in affected horses was reported to be slightly but not significantly increased (Gillespie and Tyler, 1969; Sasse, 1971; Littlejohn, 1978). Gillespie, Tyler and Eberly (1966), Gillespie and Tyler (1969) and Sasse (1971) found no significant increase in resting respiratory rates of affected horses, however, Eyre (1972), Gerber (1973) and Littlejohn (1978) all recorded increased respiratory rates in affected animals.

Most authors agreed that the disease is invariably associated with coughing of more than 3 months duration (Sasse, 1971; Cook, 1976; McPherson et al., 1978; Littlejohn, 1978). The nature of the cough was frequently described as dry or non-productive (Gerber, 1973), but a nasal discharge has been reported in many affected horses. This is usually slight and watery (Boddie, 1962; McPherson et al., 1978; Littlejohn, 1978) but may occasionally be mucoid or mucopurulent (Hoyt, 1963; Eyre, 1972; McPherson et al., 1978).

Dyspnoea was reported to be present in some COPD affected horses (Gillespie and Tyler, 1969; Sasse, 1971; McPherson et al., 1978) but there was universal agreement that a forced expiratory effort is present in the vast majority of cases. The normal abdominal phase of expiration is followed by a very marked secondary contraction of the abdominal muscles variously described as a "double expiratory effort", "abdominal

lift" or "heave" (Figure 2.1) (Hoyt, 1963; Sasse, 1971; Cook, 1976; McPherson et al., 1978). Littlejohn (1969) and Robinson and Sorenson (1978) pointed out that, as normal resting horses show an abdominal phase of expiration, the clinical significance of the double expiratory effort is one of degree. McPherson et al., (1978) also recorded a double inspiratory effort in some cases.

On chest auscultation the respiratory sounds, particularly inspiratory, are increased in volume and are harsh (Boddie, 1962; Hoyt, 1963; Gillespie and Tyler, 1969; McPherson et al., 1978). In more advanced cases, crepitant and/or wheezing sounds occur (Cook, 1965; Gillespie and Tyler, 1969; Eyre, 1972; McPherson et al., 1978).

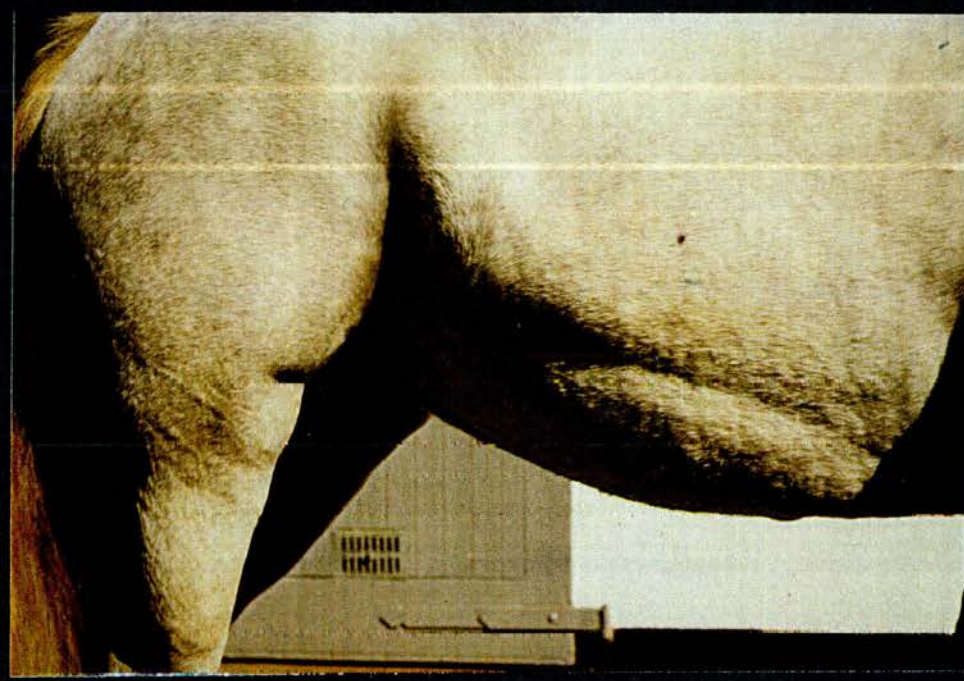
Many authors described an enlarged area of resonance on chest percussion (Alegren and Carlström, 1940; Hoyt, 1963), but Sasse (1971) considered the results of this technique to be too unreliable to be of diagnostic value.

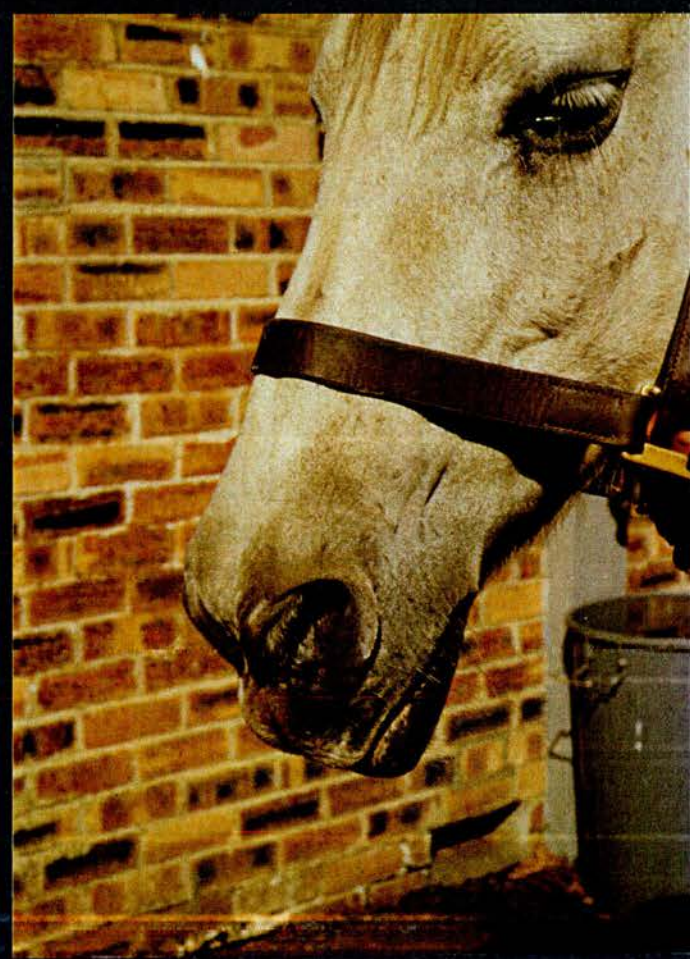
Additional signs described in advanced COPD cases include elbow abduction, extension and lowering of the head, an anxious expression, nostrils continually dilated (Figure 2.2), wheezing audible at the nostril and protrusion of the rectum during expiration (Hoyt, 1963; Cook, 1965; Gillespie and Tyler, 1969).

The clinical signs vary considerably according to

Figure 2.1. The chest and abdomen of a COPD affected horse showing the characteristic "heave line".

Figure 2.2. A COPD affected horse showing flaring of the nostrils.





the severity of the disease. While most or all of the signs could be present in severely affected horses, few of these signs may be evident in early cases. This can make the clinical diagnosis in early cases difficult, as similar clinical signs may be observed in horses with other equine pulmonary diseases.

PATHOLOGY

Although several authors have reported on the pathology of equine COPD, many of these studies were performed on small numbers of cases or gave very limited descriptions of the pathological changes. Many authors reported emphysema to be the major pathological lesion in equine COPD, possibly due to the long standing but unproven analogy between equine COPD and human pulmonary emphysema (Alexander, 1959; Cook and Rossdale, 1963; Gillespie and Tyler, 1969; Jubb and Kennedy, 1970). However, more objective studies by Thurlbeck and Lowell (1964), Sasse (1971) and Nicholls (1978) have shown that diffuse bronchiolitis and alveolar overinflation (or alveolar emphysema) are the main lesions in this disease. Breeze (1979) found that the term "alveolar emphysema" had led to confusion in the veterinary literature, as some authors understood it to mean alveolar overinflation whilst others considered it to indicate destruction of alveolar walls. He suggested that the definition for "emphysema", provided by the World Health Organisation (1963) which includes"...

destructive alterations of the alveolar walls" should be adopted into the veterinary literature, whilst alveolar overinflation should indicate "...an increase beyond normal in the size of the airspaces distal to the terminal bronchiole without destructive changes in the interalveolar septa". The terminology recommended by Breeze (1979) will be used in this work.

Other pathological changes described in equine COPD are:- excess mucus in the airways (Figure 2.3) (Hug, 1937; Roost, 1950; Hoyt, 1963; Thurlbeck and Lowell, 1964; Nicholls, 1978), pulmonary eosinophilia (Thurlbeck and Lowell, 1964; Gerber, 1973; Nicholls, 1978), bronchiolar smooth muscle hyperplasia (Wester, 1935; Gillespie and Tyler, 1969; Gerber, 1973; Nicholls, 1978) and pulmonary vasculitis (Gillespie and Tyler, 1969; Gerber, 1973).

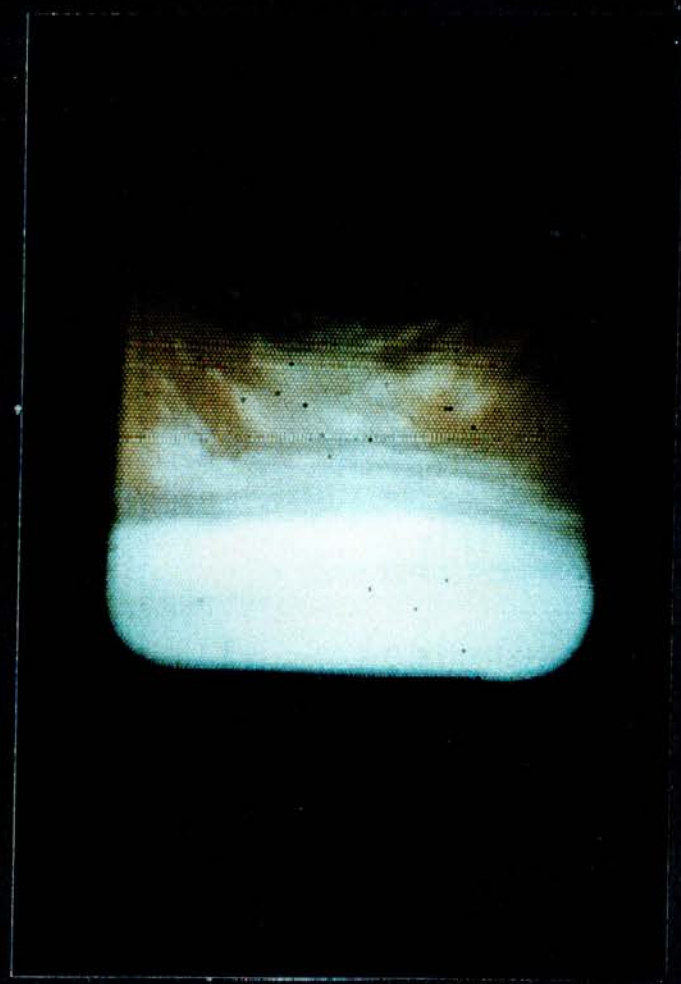
Nicholls (1978) performed the most extensive and objective pathological study of equine COPD using 25 affected horses, which included cases confirmed by pulmonary function studies at the Royal 'Dick' School Of Veterinary Studies, Edinburgh. The main lesions found in diseased horses were:-

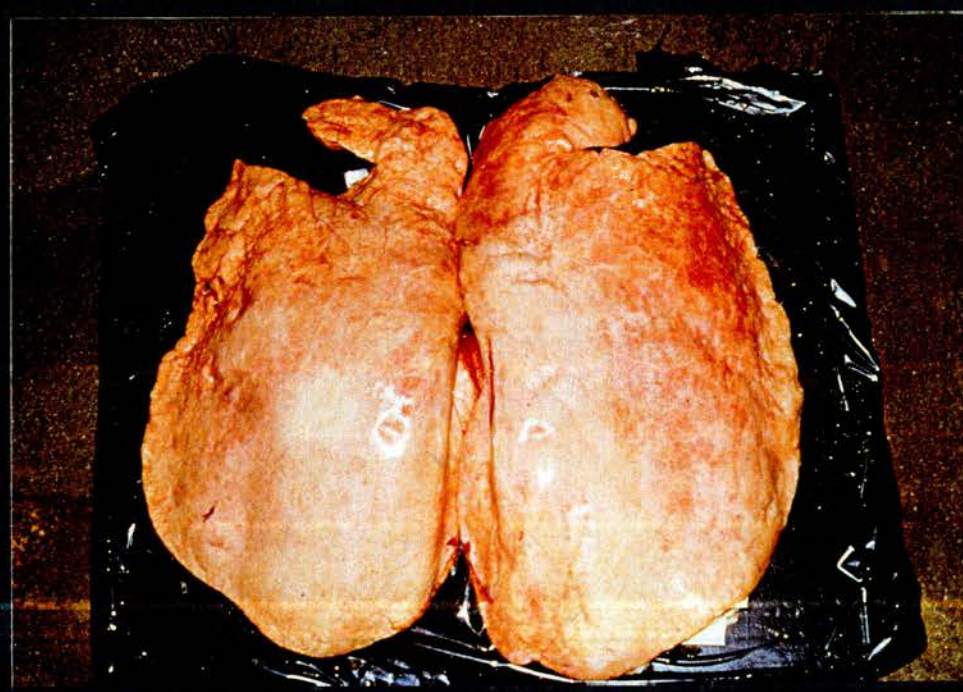
(1) alveolar overinflation

(2) bronchiolitis (a lesion affecting all the small airways less than 2 mm in diameter), characterised by epithelial hyperplasia, epithelial metaplasia with appearance of goblet cells in the

Figure 2.3. Endoscopic view of the tracheal lumen of a COPD affected horse (at the level of the thoracic inlet) showing a large volume of pooled mucus.

Figure 2.4. Lungs from a COPD affected horse showing overinflation of the diaphragmatic lobes and small areas of emphysema in the apical lobes.





bronchiolar epithelium and by peri-bronchiolar cellular infiltration.

(3) mucous and cellular exudation in the bronchiolar lumina.

Emphysema occurred infrequently and was confined to small areas in the apical lobe and periphery of the diaphragmatic lobes. Several horses with a long standing history of COPD but asymptomatic at the time of death as a result of environmental control, showed little or no pathological changes confirming therefore, that the major pathological lesions are reversible.

Lungs from a COPD affected horse which was symptomatic at the time of death are shown in Figure 2.4.

PATHOGENESIS.

As the majority of early authors believed emphysema to occur in COPD affected horses, most of the hypotheses on the pathogenesis of equine COPD were concerned with the development of structural emphysema. Many authors favoured the theory introduced by Laennec (1819) that partial bronchial obstruction, possibly due to chronic bronchitis, led to overinflation and to increased intra-alveolar pressure distal to the obstructed airways resulting in eventual destruction of the alveolar walls (Wester, 1935; Hoyt, 1963; Cook and Rossdale, 1963; Lowell, 1964; Cook, 1965; Gerber, 1973). However, Alexander (1959) and McLaughlin and Edwards (1966) postulated that primary intra-

pulmonary vascular lesions could give rise to regional hypoxia in the lung resulting in emphysema in those areas.

Cook and Rossdale (1963) clinically recognised two forms of the disease, firstly, a so-called "functional emphysema" which occurred in the initial stages of the disease and was believed to be reversible and secondly, emphysema with progressive, structural lung damage. Other authors who believed the disease to be progressive were Alexander (1959), Foley and Lowell (1966), Gerber (1969), Gillespie and Tyler (1969).

Lowell (1964) and Gerber (1973) considered that emphysema might be the end result of recurrent or protracted attacks of allergic respiratory disease. Cook (1976) supporting an allergic pathogenesis of the disease, suggested two means whereby the airways might react to the allergens: (1) a simple inflammatory response and (2) an allergic response producing bronchospasm. He suggested that both mechanisms operated together to produce "distal air trapping". Other authors who believed bronchospasm to occur as a result of an allergic reaction were Lowell (1964) and McPherson and Lawson (1974).

Several authors described signs of COPD in horses developing within one hour of exposure to certain materials and postulated that this reaction was analogous to the immediate allergic reaction (Type I reaction) which occurs in human bronchial asthma

(Alström and Lauritzson, 1953; Cook and Rossdale, 1963; Eyre, 1972). However, McPherson et al., (1979b) described the response to inhalation challenge as culminating at 4 to 8 hours after challenge and believed the reaction was essentially Arthus-like (Type III allergic reaction). The authors postulated that both Type I and Type III reactions may be involved in the pathogenesis of equine COPD.

Andberg, Boyd and Code (1941) and Obel and Schmiterlöw (1948) believed that the pathogenesis consisted of an allergic reaction in the lungs involving histamine and reproduced clinical signs of the disease in normal horses by injecting histamine intravenously. Obel and Schmiterlöw (1948) reported that COPD affected horses were more susceptible to histamine than normal horses. Eyre (1972), however, measuring blood histamine concentrations in eleven affected horses, could only demonstrate elevated levels in one animal. This author recorded increased plasma 5-hydroxytryptamine concentrations in affected horses and postulated that this mediator may play a role in the pathogenesis of the disease. As yet, little is known about the role of chemical mediators in equine pulmonary hypersensitivity.

Hajer (1979) and Fischer (1980) postulated that bronchoconstriction in horses could be vagally-induced due to stimulation of the airway irritant receptors

by dust or mucus. This phenomenon is recognised in man (Nadel, 1968; Boushey et al., 1980). Hajer (1979) and Fischer (1980) also postulated that changes in the chemical composition and increased viscosity of the sputum leads to direct chemical or mechanical airway irritation which, in turn, initiates the formation of more abnormal sputum resulting in a vicious circle, giving rise to COPD.

In conclusion, it appears that, although the pathogenesis of COPD is unclear, the reaction gives rise to airway obstruction, both mechanical (e.g. bronchiolitis and mucus in the airways) and possibly functional (e.g. airway spasm).

PULMONARY FUNCTION TESTS.

In view of the variability in the clinical signs of equine COPD and the consequent difficulty in establishing a diagnosis in early cases, equine pulmonary function testing was introduced by Spörri and Leeman (1964) and Spörri and Zerobin (1964) to physiologically classify and to aid in the diagnosis of equine respiratory disorders. In the past, many reports differed regarding, for instance, the clinical signs and pathology of COPD, and this may have resulted from authors describing different disease entities. However, the use of pulmonary function tests provides a more objective assessment of equine respiratory function and allows a more accurate comparison of results between authors. Pulmonary function tests are now widely used in COPD cases both for diagnostic and research

purposes. However, as patient co-operation is required for many of these tests, only a restricted range of those used in human medicine can be used in veterinary medicine. Summaries of some respiratory function values obtained from normal and COPD affected horses by previous authors are shown in Tables 2.1 and 2.2 respectively.

INTRATHORACIC PRESSURE CHANGES

Intrathoracic (intrapleural) pressures in horses have been measured by two methods i.e. intrapleural cannulation and by the intraoesophageal balloon. The latter method is described on page 49. The intrapleural cannulation technique gives a more accurate intrapleural pressure reading as the intraoesophageal balloon method measures not only intrathoracic pressure but also pressures exerted by oesophageal wall contractions. The properties of the balloon itself and its position in the oesophagus also affect readings (Banchero, Rutishauser, Tsakiris and Wood, 1967; Dixon, 1979; Derksen and Robinson, 1980). The intraoesophageal balloon technique is, however, non-invasive and is therefore more suitable for routine diagnostic use and for repeated measurements on the same subject.

Fry et al., (1952) and Mead and Gaensler (1959) recorded intrathoracic pressures in man using both methods simultaneously and found a close correlation between the values obtained by the two techniques. Similar findings have been shown in the dog (Banchero,

TABLE 2.1.

Summary of respiratory function measurements obtained from normal horses by previous authors.

Author	Number of horses	* Resp. Rate/ min.	Intrapleural pressure Measurements (mm Hg)				Tidal Volume (litres)	Minute Volume (litres/ min.)	✕ Insp. Flow Rate (litres/ min.)	Ø Exp. Flow Rate (litres/ min.)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	Arterial pH
			Technique of Measurement	Min. Ppl Insp.	Max. Ppl Exp.	Max. Δ Ppl							
Gillespie et al (1964)	9	-	-	-	-	-	-	-	-	-	103 ±5	43 ±4	-
Gillespie et al (1966)	15	10 ±1.2	Oesophageal balloon	-	-	4.6 ±0.5	7.3 ±0.34	79 ±10.4	217 ±23.9	200 ±24.6	-	-	-
De Moor (1968)	22	-	-	-	-	-	-	-	-	-	96	45	-
Gillespie & Tyler (1969)	40	13.8 ±9.4	Oesophageal balloon	-	-	2.6 ±1.6	5.98 ±0.74	74.7 ±38.1	212 ±84.3	208 ±95.6	-	-	-
Littlejohn (1969)	14	15.1 ±2.9	-	-	-	-	-	-	-	-	95.6 ±5.4	44.9 ±2.5	7.409 ±0.022
Sasse (1971)	24	16 ±6.0	Thoracic cannulation	-10 ±2.5	-2.5 ±1.5	7.6 ±2.1	5.8 ±1	89.2 ±26.0	204 ±52	160 ±60	100 ±5.3	44.1 ±5.1	7.399 ±0.037
Muylle & Oyaert (1973)	10	-	-	-	-	-	6.67 ±1.62	-	190.2 ±36.77	151.9 ±21.54	-	-	-
Bergsten (1974)	17	-	-	-	-	-	-	-	-	-	94 ±14	34 ±3	-
Meister et al (1976)	16	-	-	-	-	-	-	-	-	-	95.5 ±4.3	35.3 ±4.8	7.430 ±0.022
Littlejohn (1978)	38	16.7 ±5.1	Thoracic cannulation	-10.6 ±4.1	-4.8 ±3.2	5.8 ±2.4	-	-	-	-	77.1 ±5.6	38.3 ±3.17	7.384 ±0.034
McPherson et al (1978)	34	-	Oesophageal balloon	-	-	3.5 ±1.0	-	-	-	-	91.5 ±8.8	-	-
Dixon (1979)	39	-	-	-	-	-	-	-	-	-	92.0 ±5.1	37.5 ±2.8	7.403 ±0.036

* Respiratory
✕ Inspiratory
Ø Expiratory

TABLE 2.2.

Summary of respiratory function measurements obtained from COPD affected horses by previous authors.

Author	Number of horses	*Resp. Rate/min.	Intrapleural pressure Measurements (mm Hg) Technique of Measurement	Min. Ppl Insp.	Max. Ppl Exp.	Max. ΔPpl	Tidal Volume (litres)	Minute Volume (litres/min.)	X Insp. Flow Rate (litres/min.)	Ø Exp. Flow Rate (litres/min.)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	Arterial pH
Gillespie et al (1964)	9	-	-	-	-	-	-	-	-	-	80.0 ***	46.0 NS	-
Gillespie et al (1966)	11	13 NS ±1.8	Oesophageal balloon	-	-	7.9 * ±1.3	5.7 * ±0.5	76 NS ±4.3	315 * ±36.2	213 NS ±23.1	-	-	-
Gillespie & Tyler (1969)	17	16.8 NS ±7.35	Oesophageal balloon	-	-	7.1 *** ±4.7	5.29 * ±1.65	84.6 NS ±41.9	293 ** ±103.6	235 NS ±95.9	-	-	-
Sasse (1971) (Groups 2 & 3)	43	16 NS ±6.25	Thoracic cannulation	-15.5 *** ±3.3	+3.8 *** ±4.2	19.3 *** ±5.5	6.9 ** ±1.4	109.3 NS ±42.7	252.8 * ±84.8	195.9 NS ±95.3	82 *** ±15.2	45.5 NS ±6.4	7.389 NS ±0.037
Muylle & Oyaert (1973)	15	-	-	-	-	-	5.96 NS ±1.3	-	266.5 ** ±81.1	201.8 * ±66.8	-	-	-
Bergsten (1974)	11	-	-	-	-	-	-	-	-	-	68 *** ±8	-	-
Meister et al (1976)	13	-	-	-	-	-	-	-	-	-	64.3 *** ±11.2	41.3 ** ±6.8	7.423 NS ±0.028
Littlejohn (1978)	20	25.4 *** ±8.2	Thoracic cannulation	-11.5 NS	+1.9 ** ±8.5	13.0 ** ±10.2	-	-	-	-	60.8 *** ±9.1	42.3 ** ±7.1	7.369 NS ±0.052
McPherson et al (1978)	38	-	Oesophageal balloon	-	-	14.1 *** ±8.6	-	-	-	-	77.0 *** ±9.1	-	-
Dixon (1979)	50	-	-	-	-	-	-	-	-	-	70.0 *** ±7.4	38.9 NS ±4.2	7.408 NS ±0.029

Statistical difference from corresponding values in normal horses. (Table 1:1)

NS = not significant
* = $P < 0.05$
** = $P < 0.01$
*** = $P < 0.001$

* Respiratory
X Inspiratory
Ø Expiratory

Schwartz, Tsakaris and Wood, 1967). Gillespie, Tyler and Eberly (1966) recorded intrapleural pressures in horses using both techniques simultaneously and found that the shape of the recorded curves were very similar and the pressure changes measured by the two methods did not vary by more than 0.4 mm Hg when the maximum change in intrathoracic pressure (max.ΔPpl) was 7.4 mm Hg or less. McPherson and Lawson (1974), McPherson *et al.*, (1978) and Willoughby and McDonell (1979) also demonstrated a good correlation between the two methods in horses. More recently, detailed studies have confirmed that intrathoracic pressure measurement using a balloon in the middle or caudal thoracic oesophagus accurately reflects the local changes in intrapleural pressure, in the standing horse (Derksen and Robinson, 1980).

Several authors have demonstrated significantly raised max.ΔPpl levels in COPD affected horses and concluded that max.ΔPpl determination is a very important diagnostic aid (Sasse, 1971; McPherson *et al.*, 1978; Littlejohn, 1978). The increased max.ΔPpl in COPD affected horses results from the pulmonary pathophysiological changes occurring in this disease, i.e. diffuse bronchiolitis, excess mucus secretion, and possibly airway spasm.

VISCOUS (PULMONARY) RESISTANCE, VISCOUS WORK OF BREATHING AND DYNAMIC COMPLIANCE.

The viscous resistance (Rossier, Bühlmann and Wiesinger (1958)), also termed pulmonary resistance (Comroe et al., 1962) designates the sum of; the airway resistance (the resistance which must be overcome by the air flowing in the bronchi and bronchioli), the deformation resistance, the frictional resistance and the resistance due to inertness of the lung. Narrowing of the airways can lead to increased viscous resistance (Lambertsen, 1961) as recorded in COPD affected horses by Spörri and Leeman, (1964), Spörri and Zerobin (1964), Gillespie, Tyler and Eberly (1966), Spörri and Denac (1967) and Gillespie and Tyler (1969).

Sasse (1971) and Muylle and Oyaert (1973) calculated the viscous work of breathing by constructing pressure-volume diagrams and found that viscous work was significantly greater ($P < 0.01$) in COPD affected horses than in normal horses. Great importance was attached by Sasse (1971) to the minute viscous work of breathing (work = Δ pressure x Δ volume/minute). However, because minute volume did not differ significantly between his normal and affected horses, it seems likely that the increased max. Δ Ppl accounted for the significantly greater minute viscous work of breathing in the affected horses. Marschall, Stone and Christie (1954) and Gilbert and Auchincloss (1969) both

demonstrated a good correlation between max. $\triangle$ Ppl and viscous work of breathing in human patients.

Dynamic compliance is calculated from the ratio of the tidal volume to the difference in intrathoracic pressure between the points of zero airflow corresponding to the beginning of inspiration and expiration. Gillespie, Tyler and Eberly (1966) and Muylle and Oyaert (1973) recorded a significantly lower mean dynamic compliance in COPD affected horses than in normal horses.

TIDAL VOLUME AND AIR FLOW RATES.

Gillespie, Tyler and Eberly (1966) and Gillespie and Tyler (1969) recorded significantly lower ($P < 0.05$) inspiratory tidal volumes in COPD affected horses than in normal horses. This was in contrast to the findings of Muylle and Oyaert (1973) who were unable to demonstrate any significant difference in inspiratory tidal volume between normal and affected horses and Sasse (1971) who found inspiratory tidal volumes to be significantly greater ($P < 0.01$) in COPD affected horses than in the control group. Muylle and Oyaert (1973) believed that temperament, excitement and respiratory rate could greatly influence the tidal volume and considered this parameter too inconsistent to be of diagnostic use. None of the above authors recorded any significant difference in the minute volume between normal and affected horses.

A significant increase ($P < 0.01$) in mean inspiratory flow rate in COPD affected horses above that of normal horses has been reported by Gillespie, Tyler and Eberly (1966), Gillespie and Tyler (1969), Sasse (1971) and Muylle and Oyaert (1973). Sasse (1971) believed that the increased inspiratory tidal volume in his affected group accounted for the increased inspiratory flow rate.

Neither Gillespie, Tyler and Eberly (1966) nor Sasse (1971) recorded any significant difference in mean expiratory flow rate between normal and COPD affected horses. However, Muylle and Oyaert (1973) found the mean expiratory flow rate in their group of 15 COPD affected horses to be significantly higher ($P < 0.05$) than that in normal horses.

ARTERIAL BLOOD GAS AND pH ANALYSES

Arterial blood gas (i.e. partial pressure of arterial oxygen (PaO_2), partial pressure of arterial carbon dioxide ($PaCO_2$)) and arterial pH analyses are frequently used as diagnostic aids in respiratory diseases in man and animals as they reflect the degree of respiratory dysfunction present in the patient. They are consequently also used to assess the response to therapy and for prognosis (Palmer and Flenley, 1976; Cumming and Semple, 1980).

A number of authors have recorded PaO_2, $PaCO_2$ and arterial pH values in normal and COPD affected horses,

shown in Tables 2.1 and 2.2 respectively.

When comparing the results of blood gas analyses by various authors, it is important that the altitude of the laboratory be taken into consideration. For instance, Littlejohn (1978), at 1,300 metres above sea level recorded far lower PaO_2 levels in normal and affected horses than did other authors, which would appear to be an altitude-related hypoxaemia. It is also important that normal values for blood gas analyses are established for each laboratory as variations in technique and equipment can account for major differences in results (Barnett, 1971). The blood gases should preferably be measured at normal equine body temperature i.e. 37.7°C. However, this is not always possible as many blood gas analysers operate at human body temperature only, i.e. 37.0°C. There is a 5% difference in values obtained from blood analysed at these two temperatures, so the blood gas analysing temperature should, therefore, always be noted.

In comparison with normal horses, COPD affected horses are hypoxaemic (Tables 2.1 and 2.2) but there appears to be no evidence of hypercapnia nor respiratory acidosis. This suggests that the pulmonary dysfunction in equine COPD is a perfusion:ventilation disorder rather than a generalised alveolar hypoventilation, where the hypoxaemia would be accompanied by hypercapnia and possibly by respiratory acidosis (World Health Organisation, 1963).

Although several sites have been described for collection of arterial blood samples from horses (Rose and Rossdale, 1981), the two most commonly used are the common carotid artery (Littlejohn and Mitchell, 1969; McPherson and Lawson, 1974; McPherson et al., 1978; Littlejohn, 1978; Dixon, 1978) and the brachial artery (Fisher, 1959; Sasse, 1971).

CONCLUSIONS OF THE USE OF PULMONARY FUNCTION TESTS IN COPD AFFECTED HORSES.

There is universal agreement between authors that COPD affected horses have a significantly higher max. Δ Ppl, viscous resistance, viscous work of breathing, mean inspiratory flow rate and a significantly lower dynamic compliance and PaO_2 than normal horses. There is, however, no overall agreement as to whether the tidal volume or mean expiratory flow rate differ between normal and COPD affected horses.

Sasse (1971) regarded the minute viscous work of breathing and PaO_2 to be the most useful tests for diagnosing COPD. However, as previously noted, Sasse (1971) failed to record any significant difference in minute volume between normal and COPD affected horses and it is therefore likely that the increased max.ΔPpl gave rise to the increased minute viscous work of breathing in his affected horses. For this reason, and because of the good correlation between the viscous

work of breathing and max.ΔPpl recorded in man, the main ancillary tests currently used by the Edinburgh workers are max. Δ Ppl and PaO_2 measurements. Taking the mean $\pm$ 2 standard deviations of max. Δ Ppl and PaO_2 for 11 normal horses, McPherson et al., (1978) suggested that values for max. ΔPpl greater than or equal to 6 mm Hg and PaO_2 less than or equal to 82 mm Hg were abnormal (for their laboratory and apparatus) and used these parameters in their COPD diagnostic criteria.

REVERSIBILITY OF RESPIRATORY FUNCTION DISTURBANCES.

The respiratory function disturbances in COPD, being erroneously likened to human emphysema, were formerly believed to be irreversible. However, following atropine administration to affected horses Obel and Schmiterlöw (1948) recorded a temporary decrease in max. ΔPpl whilst Spörri and Leeman (1964) and Muylle and Oyaert (1973) reported a temporary decrease in the work of breathing which indicates that the airway obstruction in COPD can be partially reversed by parasympatholytic agents.

Using environmental control, i.e. removing hay and straw from the stable and surrounding area, Meister, Gerber and Tschudi (1976) recorded a marked clinical improvement and a highly significant ($P< 0.001$) increase in PaO_2 from 65.8 $\pm$ 11.1 mm Hg to 86.0 $\pm$ 5.4 mm Hg in

17 affected horses after 11 days. Similarly Dixon (1978) rendered 10 COPD affected horses asymptomatic and recorded a mean PaO_2 value of 86.5 $\pm$ 6.4 mm Hg in asymptomatic horses which is significantly higher ($P < 0.001$) than that for his affected horses (66.1 $\pm$ 6.4 mm Hg). This shows that COPD affected horses, under certain conditions, are capable of improving their ventilation.

MANAGEMENT AND TREATMENT.

Although equine COPD has been recognised for many centuries, few definitive studies have been carried out on the treatment of the disease. Early authors advocated good nutrition, good ventilation, clean stables and rest combined with the empirical systemic administration of agents such as arsenic, strychnine, vergotinin, fibrolysin, calcium chloride (Huytra and Marek, 1926). However, as expected, none of these agents were found to have a lasting beneficial effect.

The feeding of atropine-containing plants (Atropa belladonna, Datura stramonium, Hyoscyamus niger) or twice daily subcutaneous atropine sulphate injections temporarily reduced the dyspnoea and respiratory rate, the latter method proving more successful (Raitsits, 1923; Bürger, 1926; Alegren and Carlström, 1940). Despite the untoward effects of systemic parasympatholytic agents, especially on the gastro-intestinal system, frequently resulting in severe colic, this form of

treatment was advocated for many years.

Management of the disease by means of environmental control has been favoured by more recent authors. Cook and Rossdale (1963), Lowell (1964), Cook (1965) and Eyre (1972) reported an improvement in horses turned out to grass, or housed on peat moss or wood shavings instead of straw, and fed a pelleted diet or dampened hay. None of these authors quantitatively recorded the improvement which occurred in affected horses through use of these measures.

Gerber (1973) injecting 25 mg dexamethasone intramuscularly every second day, reported that "coughing may stop, dyspnoea may be reduced or disappear as do the enlargement of the lung percussion field and abnormal auscultatory sounds in a number of cases". However, horses have been found to revert to their dyspnoeic state on cessation of corticosteroid treatment (Gregg, 1969; Gerber, 1973). Due to the side effects of corticosteroids, these drugs are not generally favoured for long-term therapy of this condition.

The use of a bronchial/bronchiolar secretolytic drug, bromhexine hydrochloride for the treatment of equine COPD has been reported by Schatzmann, Bergi and Straub (1973), Cook (1976), and Nicholls (1978), but none of these authors found any lasting beneficial effects as a result of treatment.

Calverley (1977) administered levamisole hydrochloride, an anthelmintic, to 40 COPD affected horses.

He claimed that one-third of the horses showed little or no improvement, whilst the remaining two-thirds were said to be cured after treatment although there was no objective evidence to support the claim. This author postulated that this drug's property of stimulating the immunity was responsible for the alleged success in treating COPD. However, in human medicine, levamisole hydrochloride has only proved beneficial in patients who have a primary immunodeficiency and it does not stimulate immunity beyond the normal level in patients who are immunocompetent (Symoens and Rosenthal, 1977). Since immunodeficiency has not been demonstrated in horses with COPD, the alleged mechanisms of action of levamisole hydrochloride in COPD affected horses is unclear.

Sympathomimetic agents which are frequently used for the treatment of human bronchial asthma have also been used for the treatment of equine COPD. Sasse and Hajer (1977) injected NAB 365CL (clenbuterol) a beta 2 sympathomimetic bronchodilator intravenously into 5 affected horses. They reported a marked decrease in the viscous work of breathing similar to that obtained after intravenous atropine administration and clinical improvement lasting 5 to 8 hours. Additionally, 14 COPD affected horses were treated with clenbuterol orally twice daily for 14 days and the response assessed by the owners. The horses were all thought

to improve as a result of treatment (Sasse and Hajer, 1977).

From the literature review, it appears that more information is required on the management and treatment of equine COPD. Several questions remain unanswered, in particular, on the degree of reversibility obtainable through environmental control and on the time required to obtain this improvement. Pertaining to chemotherapy, more information is required on the effects of bronchodilator drugs, particularly on the longer acting oral bronchodilators. Additionally, the possible use of the newer prophylactic anti-allergy compounds for the therapy of equine COPD needs to be investigated.

CHAPTER 3

GENERAL MATERIALS AND METHODS

HORSES.

The control horses (Appendix 3.1) were mostly adult hunters and thoroughbreds. They had no history of recent respiratory illness and their respiratory systems were normal on clinical and pulmonary function examinations.

The COPD affected horses (Appendix 3.2) were of similar age and breed-type. They coughed intermittently, were dyspnoeic, manifested a double expiratory effort and had harsh inspiratory chest sounds, including wheezing and crepitation in some cases. Additionally, all horses showed a max. Δ Ppl of greater than or equal to 6 mm Hg and a resting PaO_2 of less than or equal to 82 mm Hg (McPherson et al., 1978).

MONITORING TECHNIQUES.

During all recordings, the animals were standing, untranquilised, loosely restrained by a halter only and were handled quietly to prevent any excitement - induced respiratory or cardiac changes.

INTRATHORACIC PRESSURE MEASUREMENTS.

Intrathoracic pressures including maximum expiratory pressure, minimum inspiratory pressure and max. ΔPpl were measured using the intraoesophageal balloon technique (McPherson et al., 1978). A medium-sized plastic horse stomach tube with an internal diameter of 13 mm (Portex, London) had 4 lateral openings cut in the distal tip and a 2.5 cm length of rubber

tubing slipped over and securely stitched to the plastic tube 12 cm from the distal end. This end including the lateral eyes was covered with a condom (Durex Gossamer, LR Industries Ltd., London) which was bound to the rubber tubing with linen thread (Figure 3.1).

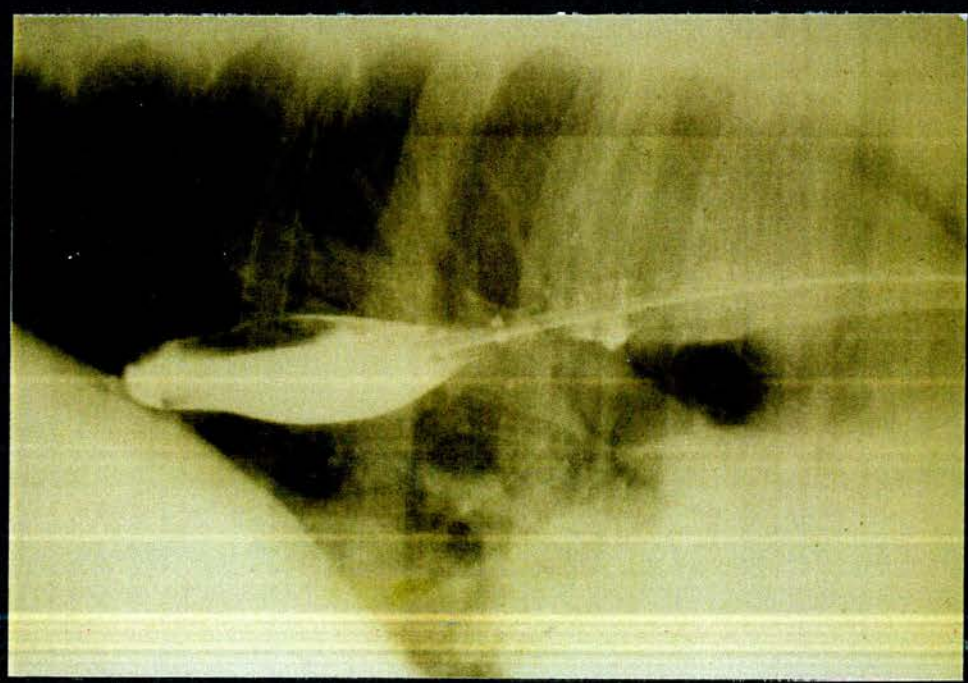
The tube was passed down the oesophagus in the conventional manner, until the balloon was in the mid-thoracic position opposite the 9th and 10th ribs (Figure 3.2), as adjudged by the length of stomach tube passed. The proximal end of the stomach tube was connected by a rigid-walled plastic tube 260 cm long, of internal diameter 3 mm, to a strain guage pressure transducer (L22, Devices Ltd., London), an amplifier and a heated pen recorder (M19, Devices Ltd., London). The intrathoracic pressure changes were recorded in mm Hg. The recording equipment was allowed to heat up for 30 minutes before use and prior to each recording was electronically calibrated with a 10 mm Hg signal. Atmospheric pressure was used as zero baseline. The position of the balloon within the thoracic oesophagus was adjusted until maximal intrathoracic pressure fluctuations were recorded. The max. Δ Ppl was calculated from the mean of ten consecutive and representative respiratory tracings.

TIDAL VOLUME AND AIR FLOW RATES.

The tidal volume, minute volume, mean inspiratory

Figure 3.1. The intraoesophageal tube and balloon used for intrathoracic pressure measurement in horses.

Figure 3.2. Radiograph showing the position of the intraoesophageal balloon in the horse's chest during intrapleural pressure recording. The balloon has been filled with barium for demonstration purposes.



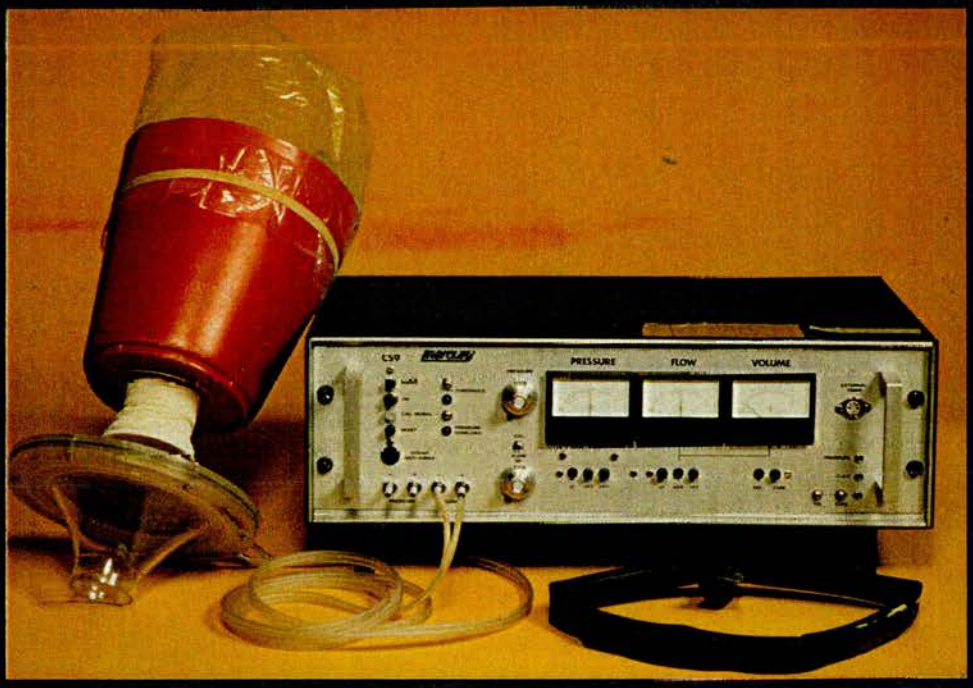
flow rate and mean expiratory flow rates were measured using a face mask, a pneumotachograph (F3000L Flowhead, Mercury Electronics (Scotland) Ltd., Glasgow) and an electronic spirometer (CS9 Electrospirometer, Mercury Electronics (Scotland) Ltd., Glasgow) (Figure 3.3).

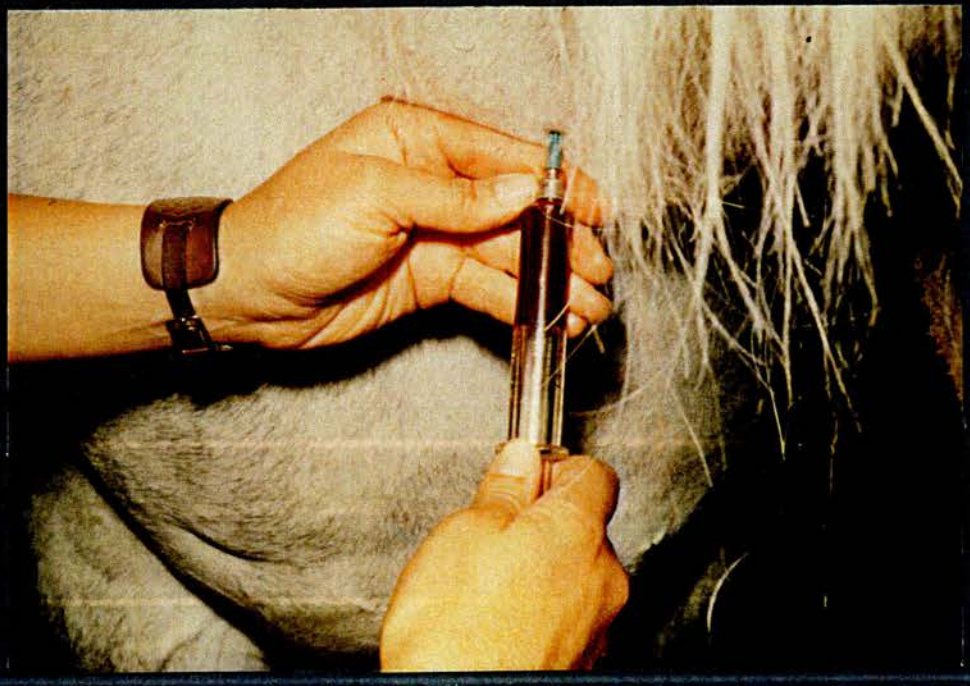
The face mask which covered the horse's mouth and nose was constructed from a rigid polythene container (length 20 cm, top diameter 20 cm, bottom diameter 12 cm) with a 15 cm wide flexible plastic sleeve attached to its proximal end by two 15 cm diameter, 1.25 cm wide rubber bands. A circular hole 5 cm diameter was cut in the centre of the base of the mask into which a rigid plastic cuff was fitted and fully sealed in place with perspex cement. This cuff protruded 3 cm from the base of the mask. The tapering entrance to the flowhead was fitted securely into the cuff and the connection sealed by 7.5 cm wide adhesive bandage (Elastoplast, T.J. Smith and Nephew Ltd., Hull).

The mask was held in position on the horse's head by a nylon strap from both sides of the mask over the poll region. The proximal end of the mask was sealed with a double rubber strap 66 cm long, 3 cm wide. A foam pad 3 x 6 x 2 cm attached to the rubber strap was positioned in the intermandibular space, the rubber strap was tightened and secured with self-adhesive nylon strip. Care was taken to ensure that the strap provided an airtight closure. The pneumotachograph was connected by twin tubing 2.5 m long, inner

Figure 3.3. The face mask, pneumotachograph and electronic spirometer used for measuring tidal volume and air flow rates in horses.

Figure 3.4. Anaerobic collection of arterial blood from the common carotid artery of a horse.


PRESSURE
FLOW
VOLUME



diameter 3 mm to the spirometer.

The spirometer was allowed to heat up for 30 minutes prior to recording and was calibrated using a Rotameter Float Guage (G.E.C. - Elliott, Process Instruments Ltd., Croydon). A vacuum pump (Electrolux Ltd., Luton) provided a positive air-flow through the pneumotachograph and the float guage and 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 measured 3 litre syringe.

The tidal volume, expiratory and inspiratory flow rates were calculated from a mean of ten consecutive and representative respiratory tracings. The cumulative volume was measured over 1 minute, i.e. the minute volume.

ARTERIAL BLOOD GAS AND pH MEASUREMENTS.

Arterial blood samples for PaO_2, $PaCO_2$ and pH analyses were obtained by percutaneous carotid puncture in the lower neck region using a 21 guage, 4 cm hypodermic needle, after swabbing the site with 1% chlorohexidine BP solution. The blood was collected anaerobically into a 10 ml glass syringe (Rocket Ltd., London) which contained a brass washer to facilitate mixing (Figure 3.4). The solid barrel was coated in vaseline to prevent leakage of air into the syringe

during sampling. The syringe was flushed with sterile heparin 25,000 I.U/L (Pularin Heparin, Evan's Medical Ltd., Liverpool), both to act as an anticoagulant and to eliminate airspace within the syringe. Approximately 10 ml of blood was collected over a 30 to 60 second period to ensure several respiratory cycles during collection. The syringe was sealed with a plastic cap and shaken to mix the blood with the heparin.

After withdrawing the needle, digital pressure was applied to the puncture site for approximately two minutes to reduce haematoma formation.

Samples were stored in iced water until analysed, which was always within 1 hour of collection. Blood gas estimations were carried out using a Corning pH/ blood gas 161 analyser (Corning Medical Ltd., Halstead, Essex) by the technical staff of the Department of Veterinary Medicine. All blood gas estimations were measured and reported at 37°C. The equipment was calibrated as instructed by the makers. The altitude of the laboratory is 200 metres.

ANTIGEN INHALATION CHALLENGE.

NATURAL ANTIGEN INHALATION CHALLENGE.

Horses were housed in well-ventilated loose boxes measuring 5 x 4 x 3 m and bedded on wheat straw which was dusty and visibly contaminated with mould (Figure 3.5). The diet consisted of poor quality hay *ad libitum* and oats (appropriate to the needs of the animal).

Figure 3.5. A sample of mould-contaminated straw used for natural antigen inhalation challenge (left) compared to good quality straw (right).

Although the straw quality varied considerably, every attempt was made to provide similar challenge conditions with high mould levels for all horses.

ARTIFICIAL ANTIGEN INHALATION CHALLENGE.

M. faeni and A. fumigatus antigens were prepared by the Department of Veterinary Pathology as described by Lawson et al., (1979). The challenge dose of each antigen was approximately 12 mg of antigen extract (estimated as dry matter) suspended in 5 ml normal saline.

The antigens were nebulised over a period of 20 minutes using a Wright's nebuliser (Aerosol Products, London) connected by a polythene tube, 2.5 m long, 10 mm internal diameter to a face mask similar to that described for tidal volume measurement (Figure 3.6). The tube from the nebuliser passed through the face seal into the mask. The attendant wore a nontoxic particle face mask (No. 8800, Minnesota Mining and Manufacturing Co., St. Paul, Minnesota) and the administration was carried out in a well-ventilated room. The Wright's nebuliser produces a droplet size of less than 8 microns.

THE CONTROLLED ENVIRONMENT.

Exposure to the aetiological agents was minimised using a controlled environment. The loose boxes were bedded with shredded paper (Shredabed Ltd., Exeter)

Figure 3.6. Method of artificial antigen inhalation challenge using a Wright's nebuliser, delivery tube and face mask.

and the horses were fed a complete cubed diet (Spillers Agriculture Ltd., London). The loose boxes which were washed regularly to minimise dust levels were upwind from and 40 m away from the hay store. Care was taken to ensure that stables in the immediate vicinity were not bedded with straw and the hay barrows and hay nets did not pass in front of the horses.

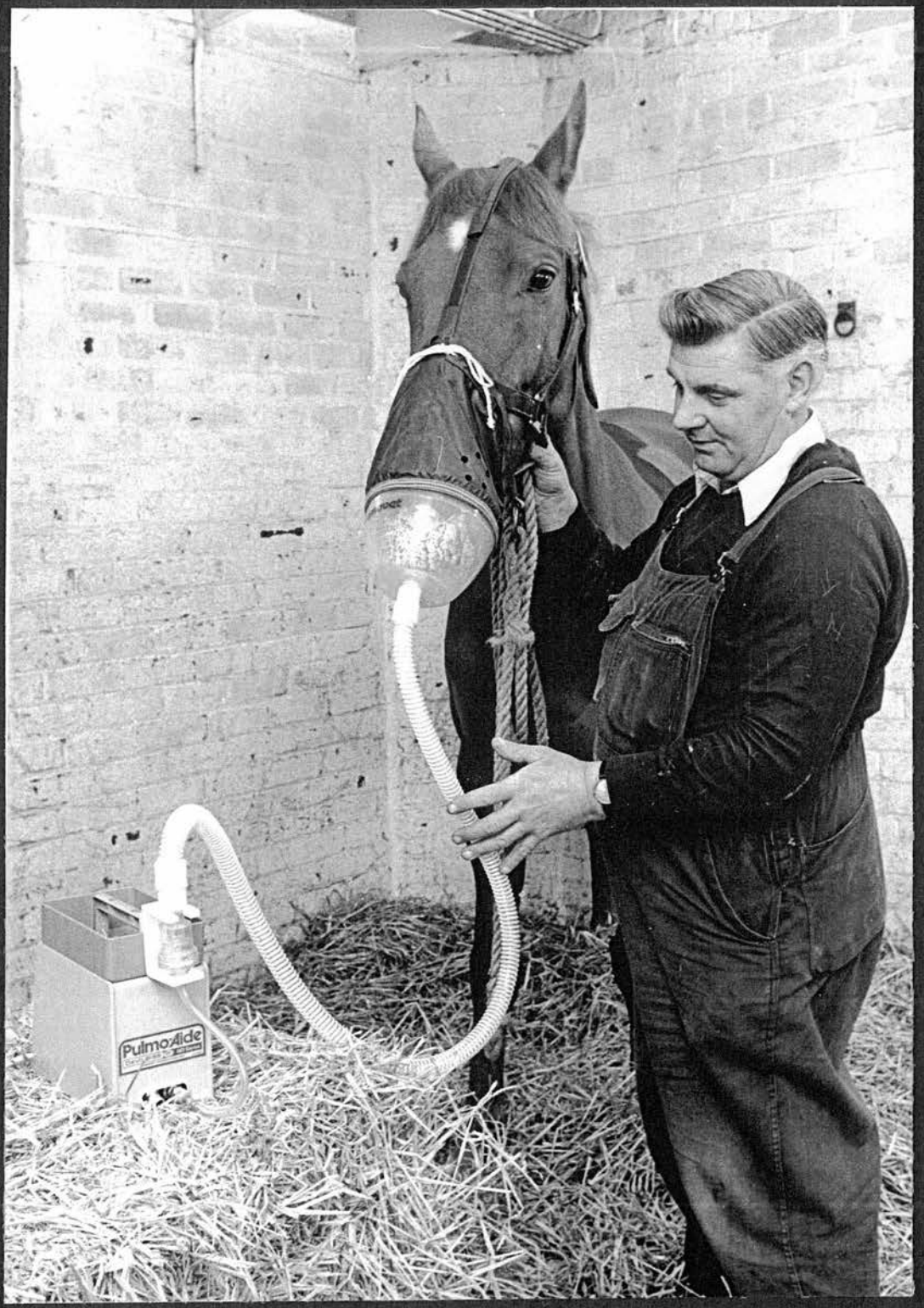
DRUGS AND ADMINISTRATION METHODS.

As different drugs and different routes and methods of administration were used in each trial, they will be described in the materials and methods of the appropriate chapters.

THE CROMOVET INHALATION SYSTEM.

The Cromovet inhalation system (Figure 3.7) comprises a Pulmo-Aide series 561 Portable Compressor (Devilbiss, Pennsylvania) a Cromovet nebuliser and face mask and a delivery tube (Fisons Ltd., Loughborough). The Cromovet face mask, the dimensions of which are shown in Figure 3.8, is constructed from a rigid polythene inhalation chamber and a flexible PVC/nylon sleeve which fits over the horse's mouth and nose. The face mask is attached to the poll piece of the headstall by velcro strip and is sealed around the nose at the proximal end by a draw string. The face mask is connected to the Cromovet nebuliser by a polythene delivery tube, 1.5 m in length and of 2.5 cm internal diameter. The droplet size produced by the Cromovet inhalation system is less than 8 microns.

Figure 3.7. The Cromovet inhalation system comprising a Pulmo-Aide portable compressor, a Cromovet nebuliser, face mask and a delivery tube.


Pulmo-Aide

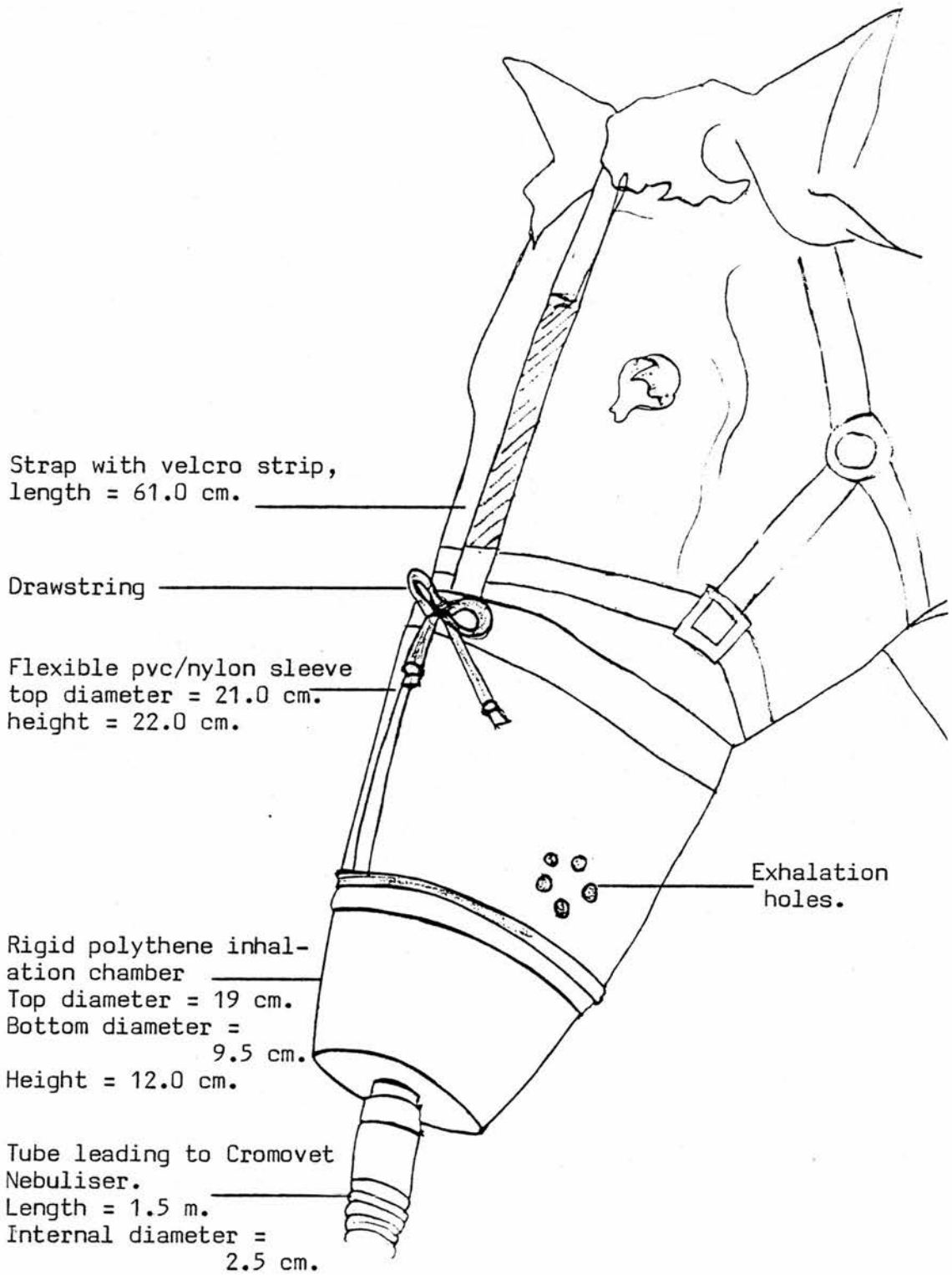


Figure 3.8. Diagram illustrating the Cromovet face mask and its dimensions.

CHAPTER 4.

EFFECTS OF ENVIRONMENTAL CONTROL ON HORSES AFFECTED WITH COPD.

INTRODUCTION

Several authors have noted clinical improvement in COPD affected horses as a result of environmental control and thus advocated minimal dust regimes for the management of this disease (Thurlbeck and Lowell, 1964; Eyre, 1972; Cook, 1976). Such measures aim to prevent exposure to the aetiological agents which are usually moulds e.g. M. faeni found in hay and straw. Minimal dust regimes include, for example, 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 upwind and as far away as possible from the hay store and other horses bedded on straw and fed hay.

Although clinical improvement has been noted by several authors as a result of environmental control, there have been very few studies into the effects of such measures on the respiratory function of COPD affected horses. In this respect, arterial blood gases appear to be the only parameters previously reported. Meister, Gerber and Tschudi (1976) and Dixon (1979) recorded significant PaO_2 increases in COPD affected horses which had shown marked clinical improvement following removal of hay and straw from their environment. Further studies are, therefore, required on the reversibility of the COPD pulmonary function disturbances through the use of environmental

control measures.

In this section, respiratory function measurements are obtained from affected horses in the symptomatic and asymptomatic phases of COPD along with values from normal horses. The time taken for horses to become asymptomatic and some factors playing a role therein are also investigated.

MATERIALS AND METHODS.

92 COPD affected horses (horses Nos. B1-20, B93-164; Appendix 3.2) (Group A) exposed to the natural challenge environment (page 55) were examined clinically and their max. Δ Ppl, PaO_2, $PaCO_2$ and arterial blood pH values recorded. Additionally, tidal volume, minute volume, maximum inspiratory and expiratory flow rates were measured in 20 of these horses (horses Nos. B1-20; Appendix 3.2). These horses were then moved to the controlled environment (page 57) and given daily clinical examinations. When clinically asymptomatic, the above-named parameters were re-measured in the same horses i.e. max. Δ Ppl, PaO_2, $PaCO_2$ and arterial pH in all animals (horses Nos. B1-20, B93-164; Appendix 4.1) (Group B); tidal volume, minute volume, maximum inspiratory and expiratory flow rates (horses Nos. B1-20; Appendix 4.1).

Max. Δ Ppl, PaO_2, $PaCO_2$ and carotid blood pH values were recorded in 68 normal horses also housed

in the natural challenge environment (horses Nos. A1-68; Appendix 3.1) (Group C) 20 of which also had tidal volume, minute volume, maximum inspiratory and expiratory flow rates measured (horses Nos. A1-20; Appendix 3.1).

The results from the asymptomatic COPD affected horses were statistically compared with values for symptomatic and normal horses by analysis of variance (Downie and Heath, 1974) and the Duncan multiple-range test (Bliss, 1967, 1970). The Students' t-test was used to compare results between symptomatically affected COPD horses and normal horses. The paired t-test was used to compare findings between symptomatic and asymptomatic COPD affected horses. The significance of the correlation between the time taken for horses to become asymptomatic and age, bodyweight, duration of illness, symptomatic max. ΔPpl, PaO_2, $PaCO_2$, arterial pH, tidal volume, minute volume, maximum inspiratory and expiratory flow rates, was tested by linear regression (Snedecor and Cochran, 1967).

RESULTS

Individual respiratory function measurements (max. ΔPpl, tidal volume, minute volume, maximum inspiratory and expiratory flow rates, PaO_2, $PaCO_2$, and arterial pH values) for normal horses, symptomatic COPD affected horses and asymptomatic COPD affected horses are shown in Appendices 3.1, 3.2 and 4.1

respectively. The mean ($\pm$ S.D.) value for each parameter in each group and the statistical differences between groups are presented in Table 4.1.

Compared to normal horses, symptomatic COPD affected horses (whilst in the natural challenge environment) had significantly increased max. Δ Ppl ($P < 0.001$), respiratory rate ($P < 0.001$), minute volume ($P < 0.05$), maximum inspiratory flow rate ($P < 0.05$) and $PaCO_2$ ($P < 0.001$) and significant decreases in PaO_2 ($P < 0.001$) and pH ($P < 0.001$) but no significant differences ($P > 0.05$) in tidal volume or maximum expiratory flow rate.

When housed in the controlled environment, the affected horses became clinically asymptomatic in 4 to 32 days, mean ($\pm$ S.D.): 9.0 ($\pm$ 4.8) days. When this occurred, there were significant decreases in max. Δ Ppl ($P < 0.001$), respiratory rate ($P < 0.001$), maximum inspiratory flow rate ($P < 0.05$) and $PaCO_2$ ($P < 0.001$), significant increases in PaO_2 ($P < 0.001$) and arterial pH ($P < 0.001$) but no significant changes ($P > 0.05$) in tidal volume, minute volume or maximum expiratory flow rate.

On testing the results by analysis of variance, the 3 groups (i.e. Group A = symptomatic COPD horses, Group B = asymptomatic COPD horses, Group C = normal horses) fell into two subsets (\A/ \BC/) for all parameters except minute volume (\A \B/ C/), tidal volume

TABLE 4.1.

Respiratory function values (mean ± S.D.) for symptomatic COPD affected horses, asymptomatic COPD affected horses and normal horses.

Horses	Number of horses	Respiratory rate (breaths/ minute)	Max. Δ Ppl (mm Hg)	Ø Tidal volume (litres)	Ø Minute volume (litres/ minute)	Ø Maximum inspiratory flow rate (litres/ minute)	Ø Maximum expiratory flow rate (litres/ minute)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	Arterial pH
Symptomatic COPD horses (Group A)	92	15.49 ± 3.61	13.92 ±6.23	6.66 ±1.61	93.14 ±33.56	261.34 ±58.76	201.90 ±31.78	70.90 ±6.56	39.28 ±3.42	7.420 ±0.016
Asymptomatic COPD horses (Group B)	92	11.49 ±2.07	3.71 ±0.63	6.78 ±1.52	78.68 ±13.81	219.86 ±36.87	198.02 ±27.24	87.99 ±2.91	37.01 ±2.40	7.428 ±0.010
Normal horses (Group C)	68	12.07 ±3.34	3.59 ±0.66	6.32 ±1.53	72.25 ±15.93	224.25 ±39.00	207.92 ±41.40	89.82 ±3.77	36.34 ±3.10	7.433 ±0.018
Statistical Differences between Groups.	F Value	44.81**	212.83**	NS 0.50	4.37*	4.92*	NS 0.43	414.56**	22.23**	19.23**
	Sub-sets	A / BC	A / BC	-	A B C	A / BC	-	A / BC	A / BC	A / BC

Ø = Parameter recorded in 20 horses in groups A, B and C.
NS = not significant
* = $P < 0.05$
** = $P < 0.01$

and maximum expiratory flow rate. (In the latter two parameters there were no significant differences between groups, as shown in Table 4.1). This indicates that there were no significant differences in respiratory rate, max.$\triangle$Ppl, tidal volume, minute volume, maximum inspiratory and expiratory flow rates, PaO_2, $PaCO_2$ or carotid arterial pH between normal and asymptomatic COPD affected horses.

The individual times taken for horses to become asymptomatic are shown in Appendix 4.1. Significant linear and positive correlations existed between this time and four parameters, namely age, symptomatic max.$\triangle$Ppl, respiratory rate and duration of illness. Significant linear and negative correlations occurred with symptomatic PaO_2 and arterial pH (Table 4.2). There were no significant correlations with bodyweight, symptomatic tidal volume, minute volume, maximum inspiratory and expiratory flow rates or $PaCO_2$. Significant, linear and positive correlations existed between duration of illness and age ($r_{(90)} = +0.79$, $P< 0.01$) and symptomatic max.$\triangle$Ppl ($r_{(90)} = +0.50$, $P< 0.01$). A significant linear and negative correlation existed between duration of illness and symptomatic PaO_2 ($r_{(90)} = -0.23$, $P< 0.05$).

DISCUSSION

The mean values of the recorded parameters for symptomatic COPD affected horses and normal horses are

TABLE 4.2.

Statistical correlation of the time taken for COPD affected horses to become asymptomatic in the controlled environment with their age, bodyweight, duration of illness and respiratory function measurements when symptomatic.

Factor	Correlation Coefficient	Significance
Age	$r(90) = 0.458$	$P < 0.01^{**}$
Bodyweight	$r(90) = -0.119$	$P > 0.05^{NS}$
Respiratory rate	$r(90) = 0.214$	$P < 0.05^{*}$
Max. ΔPpl	$r(90) = 0.648$	$P < 0.01^{**}$
Tidal volume	$r(18) = 0.135$	$P > 0.05^{NS}$
Minute volume	$r(18) = -0.082$	$P > 0.05^{NS}$
Inspiratory flow rate	$r(18) = 0.155$	$P > 0.05^{NS}$
Expiratory flow rate	$r(18) = 0.129$	$P > 0.05^{NS}$
PaO_2	$r(90) = -0.466$	$P < 0.01^{**}$
$PaCO_2$	$r(90) = 0.165$	$P > 0.05^{NS}$
Arterial pH	$r(90) = -0.217$	$P < 0.05^{*}$
Duration of illness	$r(90) = 0.398$	$P < 0.01^{**}$

NS = not significant

* = $P < 0.05$

** = $P < 0.01$

similar to those recorded by previous authors (Tables 2.1. and 2.2). The great differences in max.ΔPpl and PaO_2 between normal and symptomatically affected horses are in accordance with the findings of Gillespie and Tyler (1969), Sasse (1971), Bergsten (1974), Meister, Gerber and Tschudi (1976), Littlejohn (1978), McPherson et al., (1978) and Dixon (1979). The mean PaO_2 values reported by Littlejohn (1978) are lower than those determined in this study (and by other authors) probably owing to the higher altitude (1300m) at which Littlejohn's work was performed.

There appears to be little agreement in the literature as to whether tidal volume changes occur in COPD affected horses. Gillespie, Tyler and Eberly (1966) and Gillespie and Tyler (1969) reported significantly lower mean tidal volumes in affected horses than in normal horses whereas Sasse (1971) found the mean tidal volume in affected horses to be significantly higher. Like Muylle and Oyaert (1973), the present author failed to record any significant difference in mean tidal volume between normal and symptomatically affected horses. The reason for the variation between authors is unknown but it may have resulted from the COPD syndrome differing with geographical location or different horse populations. It is also possible that some animals examined by other authors were suffering from different forms of chronic pulmonary disease as not all authors used the same identification criteria.

As no significant change in tidal volume was observed in affected horses, the increased minute volume in this group is due to the elevated respiratory rate, which possibly also gave rise to the increased maximum inspiratory flow rate. Gillespie, Tyler and Eberly (1966), Gillespie and Tyler (1969), Sasse (1971) and Littlejohn (1978) all recorded higher respiratory rates in affected horses, however, only Littlejohn (1978) found this increase to be significant. When studying the interactions of PaO_2 and $PaCO_2$ on the ventilatory response in normal horses, Muir, Moore and Hamlin (1975) found that the respiratory rate increased with lowering of the PaO_2. The increased respiratory rate in the affected horses in this study might therefore be related to the hypoxaemia.

Minimising exposure of COPD affected horses to moulds in hay and straw enabled them to become clinically asymptomatic, with mean values for the respiratory function measurements not significantly differing from normal horses.

There was a significant ($P < 0.001$) increase in PaO_2 (mean $\pm$ S.D.) from 70.9 $\pm$ 6.6 mm Hg (symptomatic value) to 88.0 $\pm$ 2.9 mm Hg when horses were asymptomatic. This is similar to the findings of Meister, Gerber and Tschudi (1976) who reported a significant ($P < 0.001$) increase in (mean $\pm$ S.D.) PaO_2 from 65.8 $\pm$ 11.1 mm Hg to 86.0 $\pm$ 5.4 mm Hg in 17 COPD affected horses after withdrawal of hay and straw for 11 days.

However, the mean PaO_2 tension remained significantly lower than their values for normal horses (95.5 $\pm$ 4.3 mm Hg). Similarly, Dixon (1979) recorded a PaO_2 (mean $\pm$ S.D.) value of 87.2 $\pm$ 5.1 mm Hg in 10 asymptomatic COPD affected horses which is significantly higher ($P < 0.001$) than that for this author's symptomatically affected group (70.0 $\pm$ 7.4 mm Hg) but significantly lower ($P < 0.001$) than that for his normal horses (92.0 $\pm$ 5.1 mm Hg).

In human asthma, several studies have demonstrated that complete reversal of all respiratory functional abnormalities will occur (McFadden and Lyons, 1969; Tooley, Demuth and Nadel, 1965) but hypoxaemia may persist for long periods of time following the recovery from an acute attack (Rees, Millar and Donald, 1968). The mechanism that most commonly produces hypoxaemia in asthma is poor distribution of inspired air leading to mismatched ventilation - perfusion ratios (Heckscher et al., 1968; Levine et al., 1967; McFadden and Lyons, 1968). A degree of mismatching is thought to persist in patients for some time after apparent full clinical recovery. Although ventilation - perfusion studies have not been performed on COPD affected horses, it is probable that a similar mechanism occurs as symptomatically affected horses suffer a normocapnoeic hypoxaemia which indicates a perfusion - ventilation imbalance. This could explain

the recorded PaO_2 differences between normal and asymptomatic COPD affected horses recently recovered from the disease (Meister, Gerber and Tscudi, 1976; Dixon, 1979) and it is possible that further increases in PaO_2 may occur with prolonged environmental control.

The time taken for horses to show remission of COPD signs varied greatly and correlated most significantly with the severity of the condition (as adjudged by the symptomatic max. $\triangle$ Ppl and PaO_2 values) and the duration of illness. It seems likely that the degree of pathological change in the lungs would increase with increasing severity of disease and duration of illness. However, there have been no studies correlating the severity of equine COPD pathology with these two factors. In human asthma, it has been found that the more prolonged the attack is, the longer it takes to reverse the respiratory impairment (McFadden, 1976). This is thought to result in part from mucous plugging of the bronchi and the formation of mucous casts which tend to remain in the airways for prolonged periods of time (Dunnill, 1965). Although, as explained in chapter 2, equine COPD and human asthma differ functionally and anatomically, hypersecretion of mucus and mucous plugging of the bronchi and bronchioles are also commonly found in equine COPD and this, as well as bronchiolar cellular infiltration and fibrotic changes described by Nicholls (1978) could all prolong the resolution of clinical signs.

CONCLUSIONS

From this study, it is apparent that the pathophysiological changes occurring in equine COPD are largely reversible and that many affected horses are capable of regaining normal pulmonary functions when removed from contact with the aetiological agents.

CHAPTER 5.

EFFECTS OF BRONCHODILATOR DRUGS IN NORMAL AND COPD AFFECTED HORSES.

INTRODUCTION

Although the pathogenesis of equine COPD is not fully understood, many authors have postulated that it is allergic in nature. Additionally, McPherson et al., (1979b)have demonstrated respiratory hypersensitivity in COPD affected horses to a number of allergens, predominantly M. faeni. Equine COPD has been likened to human bronchial asthma on account of the predictable onset, clinical appearance and spontaneous remission with environmental control. Airway spasm is a major functional change in bronchial asthma and it has, therefore, been postulated that airway spasm also plays a role in the pathogenesis of equine COPD (Alegren and Carlström, 1940; Obel and Schmiterlöw, 1948; Cook and Rossdale, 1963; Lowell, 1964; Eyre, 1972; Gerber, 1973; McPherson and Lawson, 1974; Cook, 1976) in addition to the well recognised exudative bronchiolitis occurring in this disease.

A further reason for the hypothesis that airway spasm is involved in the pathogenesis of equine COPD is derived from results of in vitro studies on isolated pulmonary tissues from COPD affected horses. Eyre (1972), using pulmonary venous strips from 3 COPD affected horses which had previously elicited positive intradermal reactions to A. fumigatus, demonstrated Schultz-Dale reactions in two of the horses, which indicated pulmonary vascular hypersensitivity in these animals. In a pilot experiment, it was found that

isolated airway tissues (bronchial strips and lung parenchymal strips) from eight out of ten COPD affected horses which showed *in vivo* respiratory hypersensitivity to *M. faeni*, contracted *in vitro* when exposed to *M. faeni* antigen. Similar tissues from nine out of eleven clinically normal horses failed to respond to *M. faeni* antigen. (Details of this pilot study are presented in Appendix 5.1). This confirms that airway tissue in COPD affected horses is hypersensitive *in vitro* to the causal antigens and suggests that airway spasm occurs when horses are exposed to these antigens *in vivo*. It was, therefore, decided to study the effects of bronchodilator drugs on clinically affected COPD horses *in vivo*.

There are 3 main classes of bronchodilator drugs; sympathomimetic amines, parasympatholytic agents and xanthine derivatives.

SYMPATHOMIMETIC AMINES

The sympathomimetic bronchodilators have been widely used in the treatment of human bronchial asthma over the past 30 years and extensive research into these agents has resulted in their actions becoming increasingly more selective for bronchial asthma therapy. The concept of selective adrenergic receptors, alpha and beta, was introduced by Ahlquist (1948) to account for the different responses to sympathomimetic agents at various sites. In general the effects of alpha

receptors in smooth muscle are mainly excitatory, including vasoconstriction and constriction of the sphincters of the gastro-intestinal tract and those of beta receptors at such sites are mainly inhibitory causing airway dilation, vasodilation and cardiac stimulation. The beta receptors were further subdivided by Lands et al.,(1967) into beta 1 receptors which are situated predominantly in the heart and small intestine and beta 2 receptors found mainly in the airways, vascular beds and uterus. The primary cardiopulmonary response to beta 2 receptor stimulation is bronchodilation whilst tachycardia, increased cardiac output and a slight rise in blood pressure occurs with beta 1 receptor stimulation (Gaddie et al., 1972). Thus, the specific beta 2 stimulants e.g. terbutaline and salbutamol are preferred as bronchospasmolytic agents as they reduce airway resistance with fewer side effects than the less specific sympathomimetic amines e.g. isoprenaline (Formgren, 1977; McFadden, 1981; Williams and Shim, 1976).

At a cellular level, the activation of beta receptors results in stimulation of the enzyme adenylcylase which causes increased production of cyclic 3', 5'- adenosine monophosphate (cyclic 3', 5'- AMP). Cyclic 3', 5'- AMP stimulates calcium binding to cell membranes and cytoplasmic reticulum thereby reducing the myoplasmic calcium concentration which, in turn, gives

rise to smooth muscle relaxation (Anderson and Nilsson, 1977).

PARASYMPATHOLYTIC AGENTS

Parasympatholytic drugs e.g. atropine act by blocking the effects of acetylcholine at postganglionic parasympathetic nerve endings. In the airways, atropine causes smooth muscle relaxation and reduces respiratory secretions. Other effects of atropine include tachycardia, reduced gastrointestinal motility, mydriasis and central nervous system stimulation. These side effects preclude the widespread therapeutic use of atropine as a bronchodilator in man.

XANTHINE DERIVATIVES

The xanthine derivatives have a number of pharmacological actions including central nervous system stimulation, myocardial stimulation, and smooth muscle relaxation, notably in the airways (Ritchie, 1970). Because the various xanthine derivatives differ markedly in the intensity of their actions on various structures, one particular xanthine derivative is usually more suitable than another for any specific therapeutic effect. Theophylline and its derivatives are potent bronchodilators and are widely used in the treatment of bronchial asthma.

Biochemically, the xanthine derivatives, particularly theophylline, act as competitive inhibitors of cyclic nucleotide phosphodiesterase, an enzyme that catalyzes

the conversion of cyclic 3', 5'-AMP to cyclic 5'-AMP (Butcher and Sutherland, 1962). This results in elevated cyclic 3', 5'-AMP concentrations in certain tissues and consequently leads to relaxation of airway smooth muscle similarly to that initiated by sympathomimetic stimulation of the beta receptors, as previously described.

Bronchodilator drugs, including atropine, have been found to induce temporary relief of clinical dyspnoea in COPD affected horses (Raitsits, 1923; Obel and Schmiterlöw, 1948; Lowell, 1964; Muylle and Oyaert, 1973; Sasse and Hajer, 1977). Marked clinical improvement has also been reported in affected horses with the use of sympathomimetic bronchodilators, including adrenaline, noradrenaline, scopolamine hydrobromide and clenbuterol (Obel and Schmiterlöw, 1948; Schatzmann, Straub and Gerber, 1972; Corbella, 1978). In more detailed studies on five COPD affected horses, intravenous clenbuterol administration caused a significant but temporary decrease in the pulmonary viscous resistance and clinical improvement lasting six hours (Sasse and Hajer, 1977). However, values recorded at the time of peak response were not compared with those of normal horses, thus the authors provided no quantitative indication of the efficacy of clenbuterol. In contrast to the above-mentioned marked clinical improvements following treatment of COPD affected horses with bronchodilator drugs, Corbella (1978) reported only

slight and transitory clinical improvement with the sympathomimetic drugs, ephidrine, orciprenaline and salbutamol. Many of these studies were carried out on animals diagnosed as suffering from COPD on clinical grounds only and the response to treatment assessed by clinical observation alone. Such subjective assessments are possibly the cause of the disparity in results obtained with bronchodilator therapy by different authors.

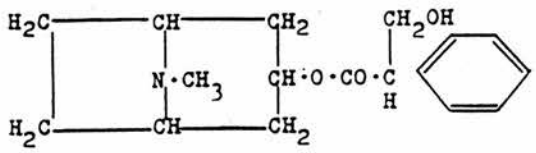
Studies on the effects of five bronchodilator drugs, namely atropine (a parasympatholytic drug), isoprenaline, terbutaline and clenbuterol (all sympathomimetics) and etamiphylline camsylate (a theophylline derivative) on some pulmonary functions and clinical parameters in normal and COPD affected horses are described here.

MATERIALS AND METHODS.

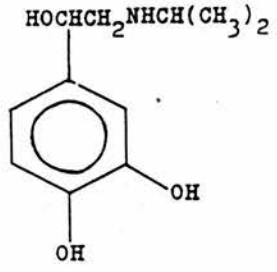
The structural formulae of the bronchodilator drugs used in these studies are shown in Figure 5.1.

DRUGS ADMINISTERED BY INHALATION

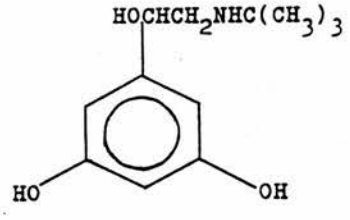
The following drugs were each administered by inhalation to 8 normal and 12 COPD affected horses; isoprenaline sulphate (Thornton and Ross Ltd., Huddersfield) (horses Nos. A21-28, Appendix 3.1 and Nos. B21-32, Appendix 3.2), atropine sulphate (Macfarlane Smith Ltd., Edinburgh) horses Nos. A29-36, Appendix 3.1 and Nos. B33-44, Appendix 3.2) and terbutaline (Bricanyl



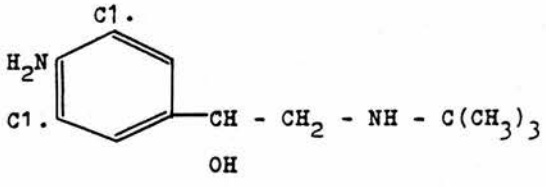
ATROPINE



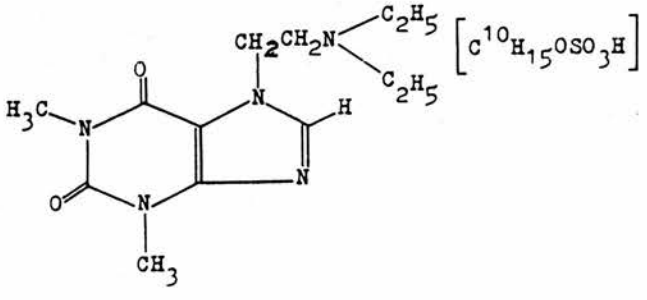
ISOPRENALINE



TERBUTALINE



CLENBUTEROL



ETAMIPHYLLINE CAMSYLATE

Figure 5.1. Structural formulae of the bronchodilator drugs used in these studies.

respirator solution, Astra Chemicals Ltd., Watford) (horses Nos. A37-44, Appendix 3.1 and Nos. B45-56 Appendix 3.2). Dosage levels which would provide the maximum therapeutic effect without severe side effects were established by previous pilot experiments and for all drugs, a dose of 0.02 mg/kg was found suitable. For inhalation administration, the drugs were dissolved in physiological saline, to a total volume of 4 ml, to standardise the time taken for drug nebulisation. The drugs were aerosolised using two Wright's nebuliser pumps and were administered via plastic tubing to a face mask, Figure 3.6, page 58. Nebulisation took 7 minutes.

DRUGS ADMINISTERED BY INTRAVENOUS INJECTION

The following drugs were each administered by slow intravenous injection over 2 minutes to normal and COPD affected horses; atropine sulphate (Bimeda U.K. Ltd., Liverpool) (horses Nos. A45-52, Appendix 3.1 and Nos.B57-68, Appendix 3.2) at a dose of 0.02 mg/kg, clenbuterol (Ventipulmin, Boehringer, Sohn, Ingleheim, BRD) (horses Nos. A53-60, Appendix 3.1 and B69-80, Appendix 3.2) at a dose of 0.8 μg/kg and etamyphilline camsylate (Millophyline, Dales Pharmaceuticals Ltd., Skipton)(horses Nos. A61-68, Appendix 3.1 and B81-92, Appendix 3.2) at a dose of 3.0 mg/kg, as recommended by the manufacturer.

MONITORING TECHNIQUES

Prior to drug administration and during all measurements, the animals were standing, untranquilised and were handled quietly to prevent any excitement-induced respiratory or cardiac changes. Max. ΔPpl, measured using an intraoesophageal balloon, p. 49, was monitored for 10 minutes, prior to treatment to establish baseline values, for 30 minutes 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 PaO_2 and $PaCO_2$ estimations were collected and analysed as previously described, p. 54. Samples were obtained at rest and at 10, 20, 60, 120, 240 and 360 minutes after treatment.

The heart rate was measured from a standard lead I electrocardiogram recording (M19, Devices Ltd., London).

Other clinical parameters observed before and after drug administration were the presence and degree of double expiratory effort, dyspnoea, flaring of the nostrils and wheezing.

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. Values for

respiratory rate, max.ΔPpl, PaO_2, $PaCO_2$ and heart rate in normal and COPD affected horses were compared at rest and after bronchodilator treatment by Student's t-test.

RESULTS

RESPIRATORY RATE

Horses affected with COPD showed a decrease in respiratory rate after drug administration with significant changes occurring 30 minutes after isoprenaline inhalation, for up to 2 hours after atropine and etamiphylline camsylate and for up to 4 hours after terbutaline and clenbuterol administration (Figures 5.2 and 5.3). During these times, the mean respiratory rates of the affected horses did not differ significantly from those of the normal horses (Appendices 5.2 to 5.7).

There were slight transient increases in mean respiratory rates in normal horses after bronchodilator treatment (Figures 5.2 and 5.3) but they were not statistically significant ($P > 0.05$).

INTRATHORACIC PRESSURE CHANGES

Max.ΔPpl decreased significantly ($P < 0.001$) in COPD affected horses after administration of all 5 drugs.

Examples of max.ΔPpl changes occurring after bronchodilator treatment are illustrated in Figure 5.4 where max.ΔPpl in an affected horse (B28) dropped from

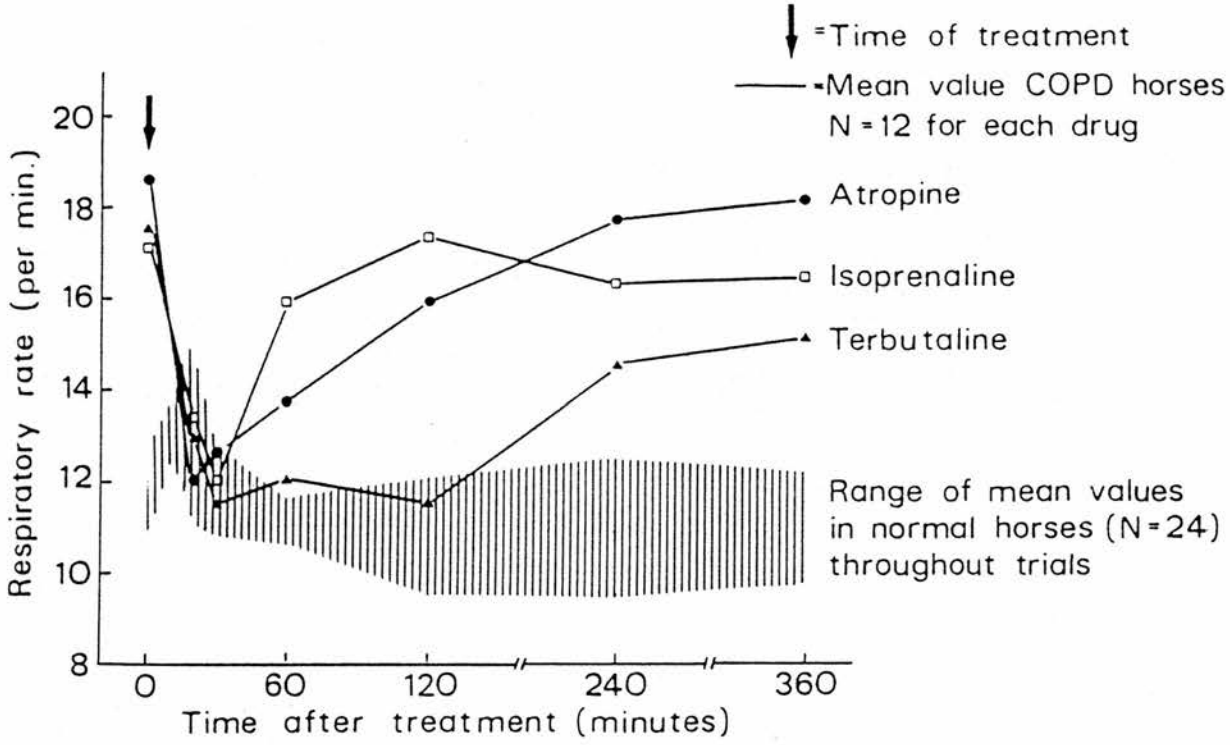


Figure 5.2. Effects of inhaled bronchodilator drugs on respiratory rate in normal and COPD affected horses.

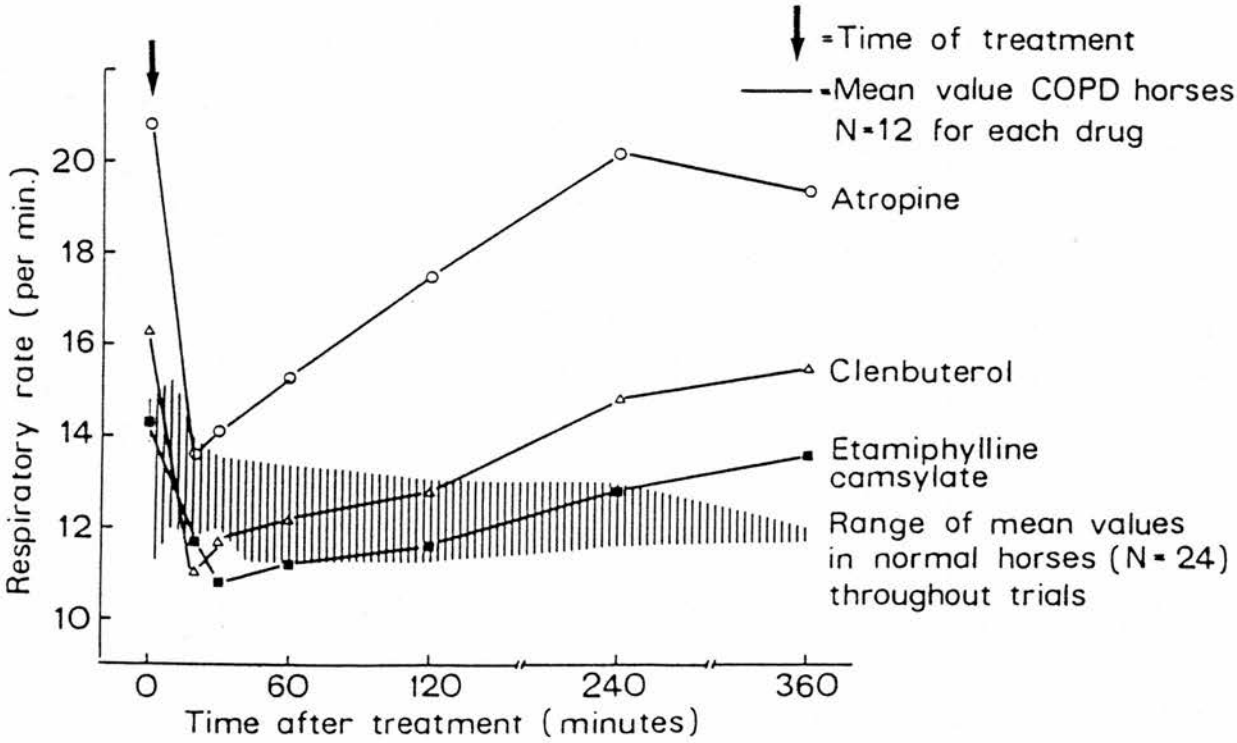


Figure 5.3. Effects of intravenous bronchodilator drugs on respiratory rate in normal and COPD affected horses.

26.4 mm Hg before treatment to 6.0 mm Hg 10 minutes after isoprenaline inhalation and Figure 5.5 where, in another affected horse (B45), max. ΔPpl prior to terbutaline treatment was 19.5 mm Hg and fell to 5.2 mm Hg 30 minutes after drug inhalation.

At peak response to inhaled isoprenaline, atropine and terbutaline the mean max. ΔPpl in COPD affected horses was reduced by 70%, 62% and 63% respectively (Figure 5.6) and after intravenous atropine, clenbuterol and etamiphylline camsylate, the mean max. ΔPpl values were decreased by 66%, 62% and 57% respectively (Figure 5.7). The intrathoracic pressure decreases remained significant for 1 hour following isoprenaline, 2 hours following atropine and etamiphylline camsylate, 4 hours following clenbuterol and 6 hours following terbutaline administration (Appendices 5.2 to 5.7). Despite the large decreases in max. ΔPpl after all drugs the values for COPD affected horses at times of maximum response still remained significantly higher ($P < 0.01$) than those of the resting control horses.

No significant max. ΔPpl changes were recorded in the normal horses after bronchodilator treatment (Figures 5.6 and 5.7).

ARTERIAL BLOOD GASES.

The effects of the bronchodilator drugs on PaO_2 levels in COPD affected and normal horses are shown in

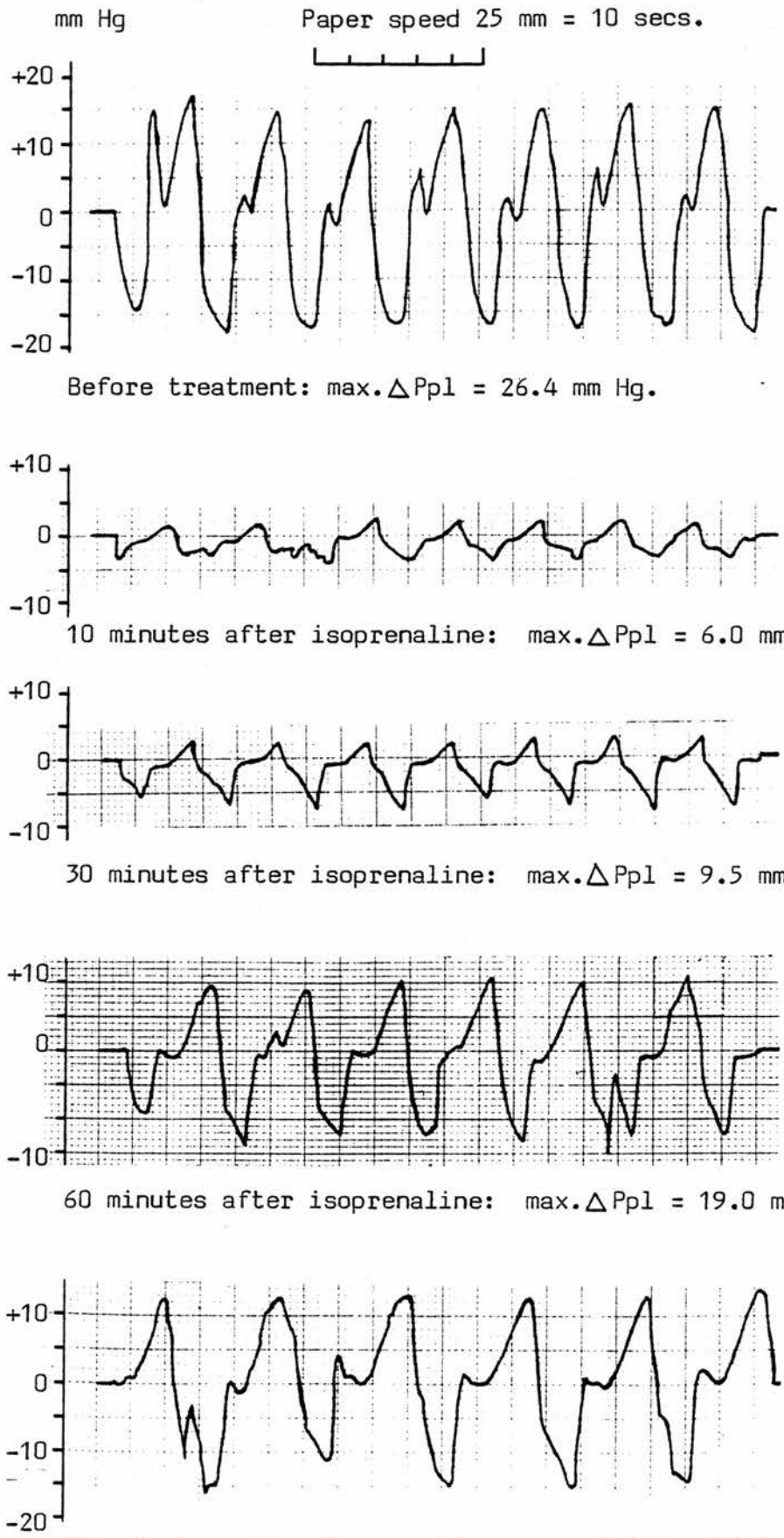


Figure 5.4. Max.ΔPpl recordings from a COPD affected horse (No. B28) at rest and at intervals after isoprenaline inhalation.

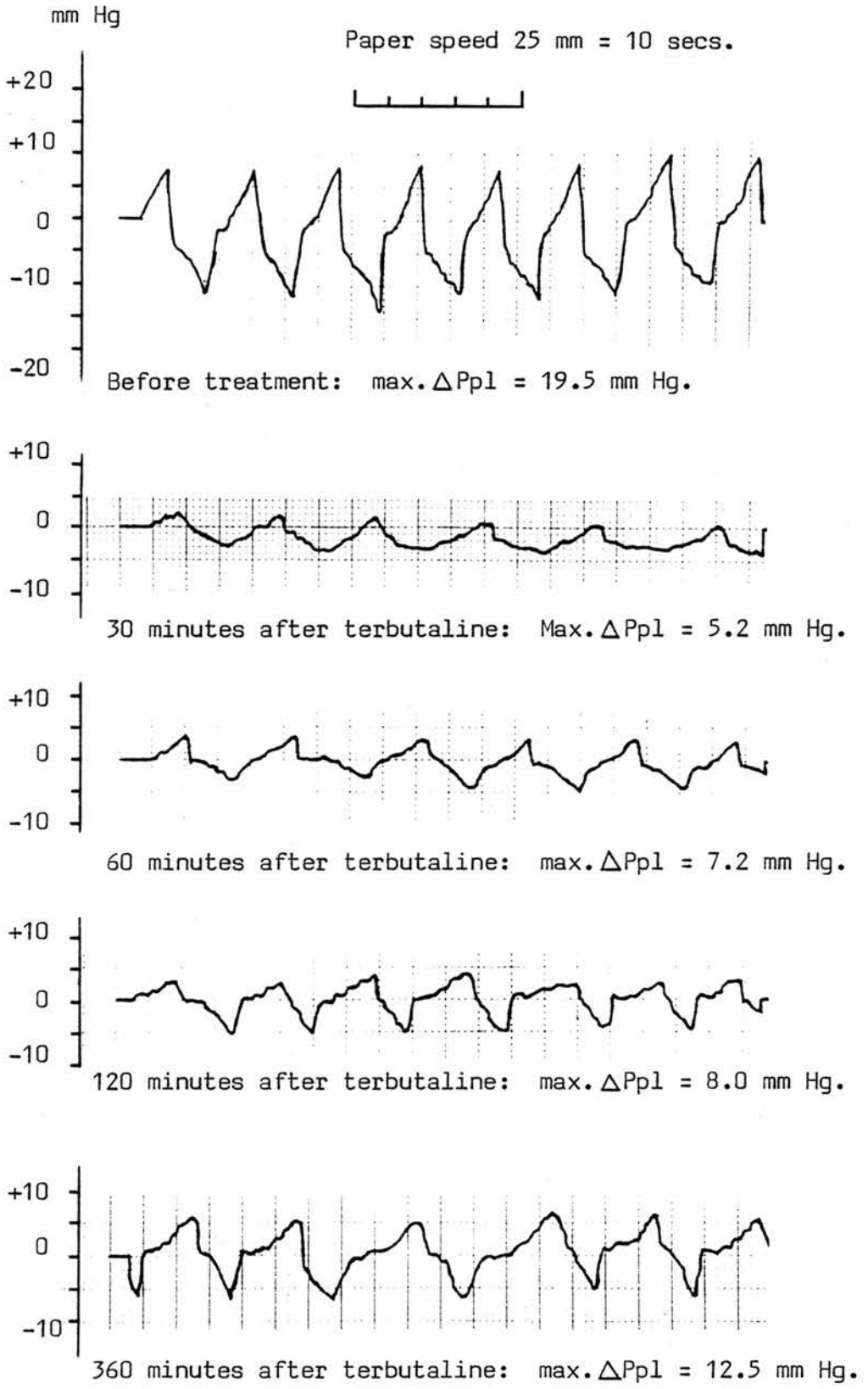


Figure 5.5. Max. ΔPpl recordings from a COPD affected horse (No. B45) at rest and at intervals after terbutaline inhalation.

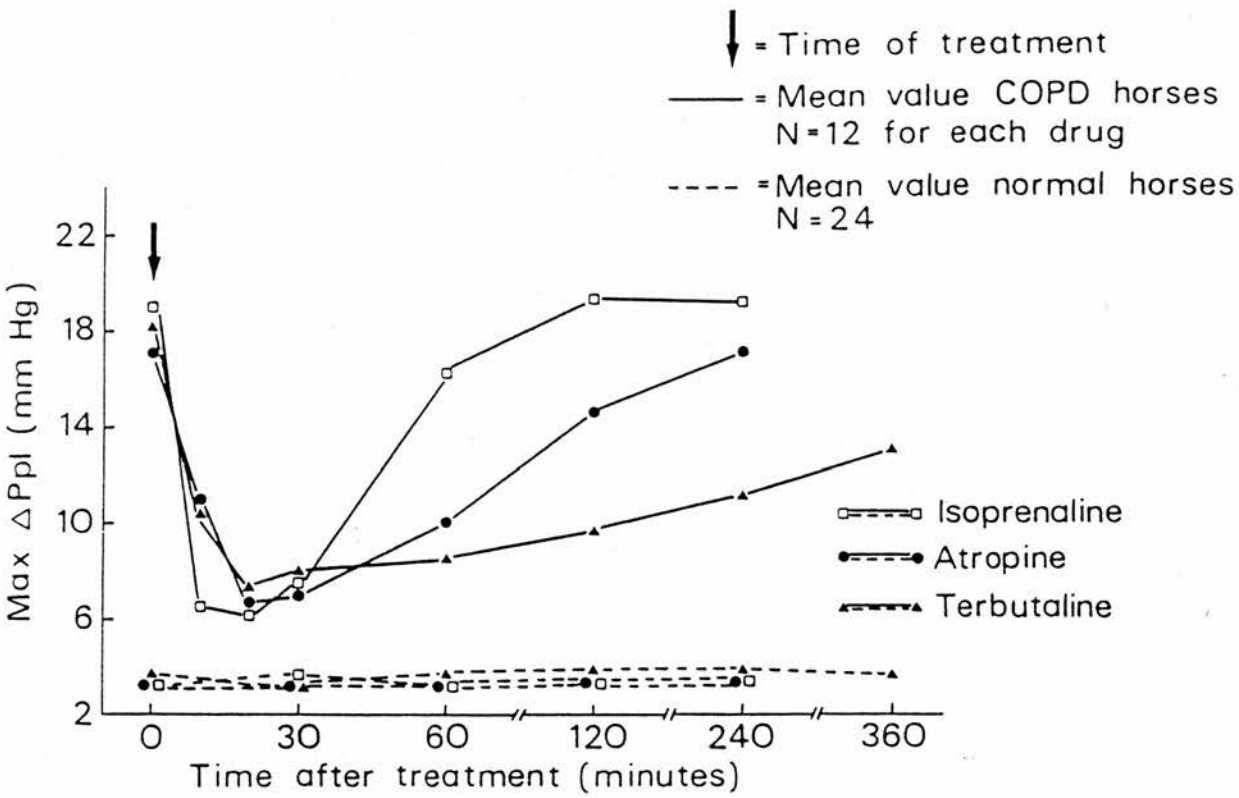


Figure 5.6. Effects of inhaled bronchodilator drugs on max. ΔPpl in normal and COPD affected horses.

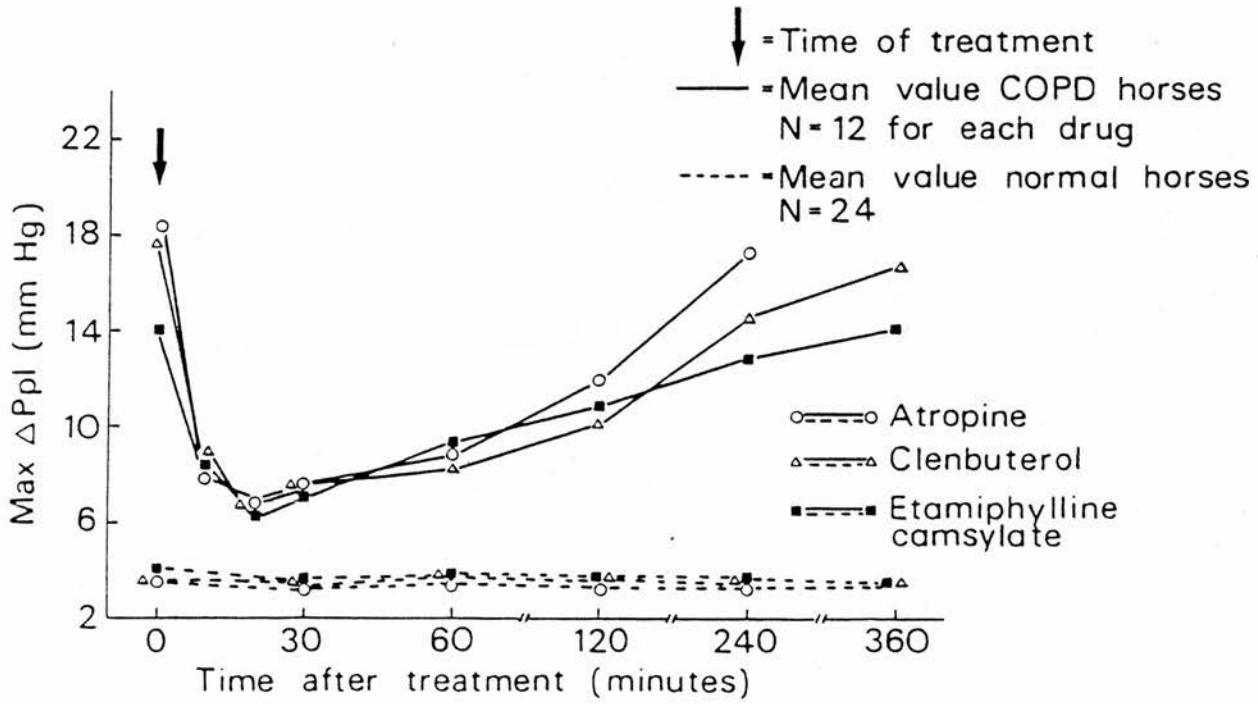


Figure 5.7. Effects of intravenous bronchodilator drugs on max.ΔPpl in normal and COPD affected horses.

Figures 5.8 and 5.9. All drugs caused an initial temporary drop in PaO_2 levels in both COPD affected and normal horses which was followed by increases in PaO_2 to above resting levels in COPD affected horses. Significant increases in PaO_2 levels were present up to 2 hours after isoprenaline, 4 hours after atropine and clenbuterol and for 6 hours after terbutaline administration (Appendices 5.2 to 5.6). A non-significant ($P > 0.05$) increase in mean PaO_2 levels occurred from 2 to 6 hours after etamiphylline camsylate treatment in the affected horses.

Despite the PaO_2 increases in COPD horses after treatment, their mean PaO_2 levels remained significantly lower ($P < 0.001$) than those of the control horses. The only significant PaO_2 increase recorded in the normal horses after bronchodilator treatment occurred at 6 hours after etamiphylline camsylate administration. The mean PaO_2 rose from 88.06 mm Hg to 91.51 mm Hg ($P < 0.05$).

COPD affected horses showed a slight decrease (1 to 3 mm Hg) in $PaCO_2$ levels from 1 to 6 hours after treatment, corresponding to the times at which increased PaO_2 levels occurred. No significant $PaCO_2$ changes were recorded in the control horses.

HEART RATE.

There was a significant ($P < 0.001$) increase in heart rate in both COPD and normal horses within 10 to 20 minutes of administration of all 5 drugs (Figures

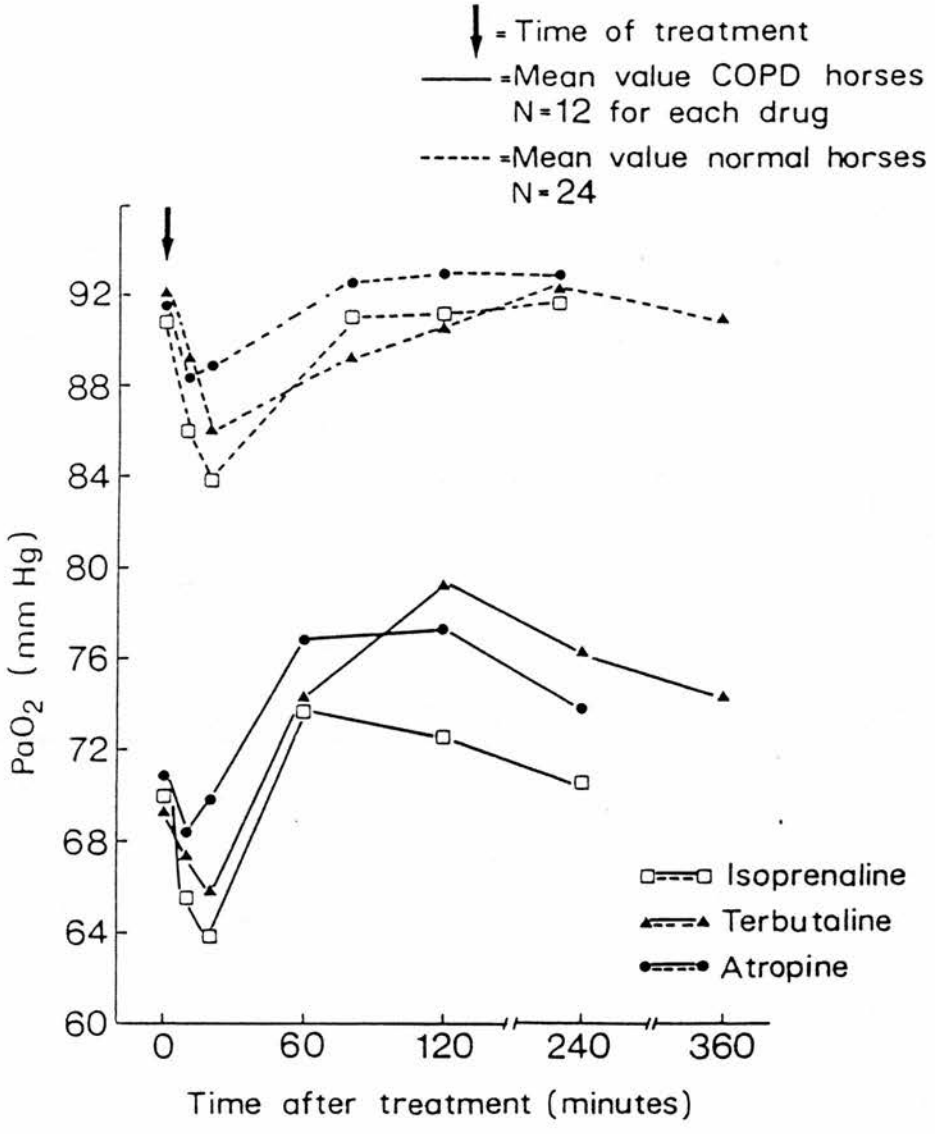


Figure 5.8. Effects of inhaled bronchodilator drugs on PaO_2 in normal and COPD affected horses.

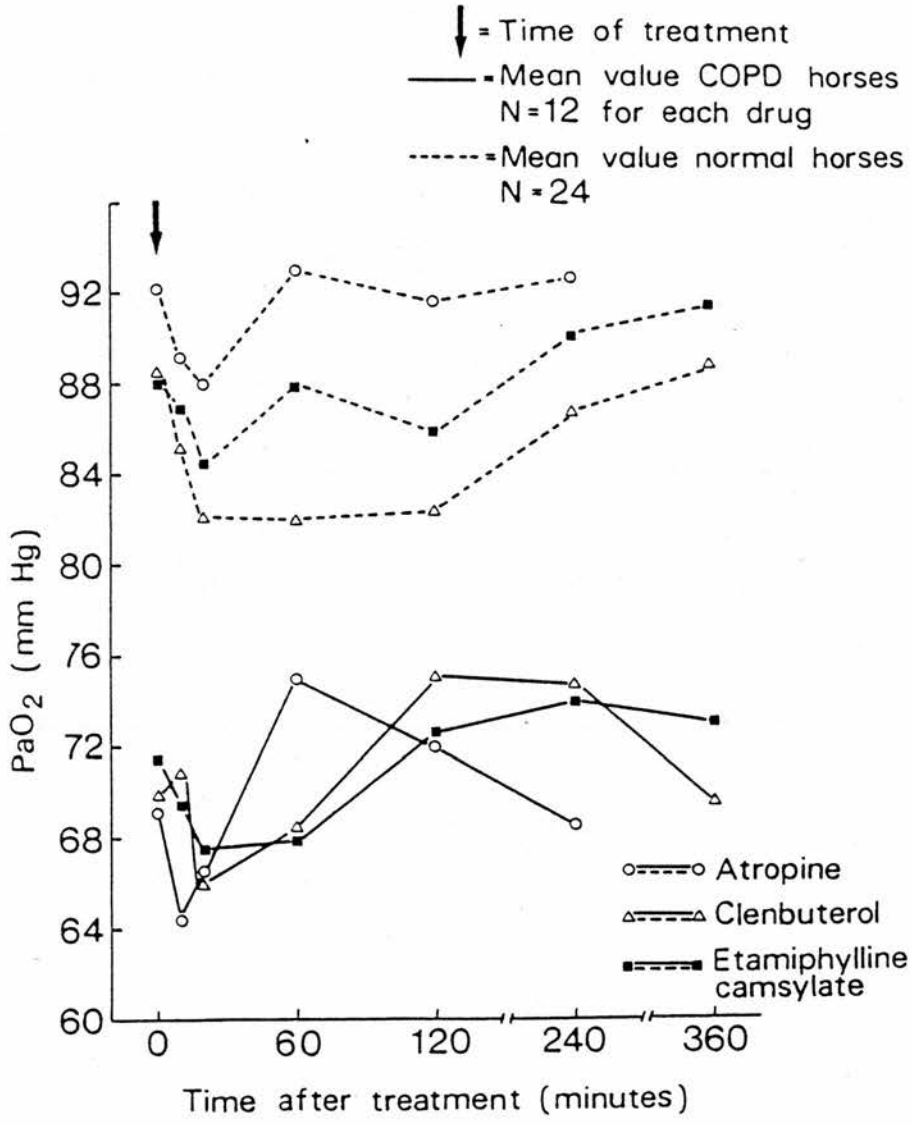


Figure 5.9. Effects of intravenous bronchodilator drugs on PaO_2 in normal and COPD affected horses.

5.10 and 5.11). Tachycardia was most pronounced after atropine injection and isoprenaline inhalation, when the mean heart rates increased twofold over the resting rates. The heart rates returned to resting levels within 1 to 2 hours after all treatments.

CLINICAL OBSERVATIONS.

After drug administration, COPD affected horses showed a temporary reduction in their expiratory effort, dyspnoea and flaring of the nostrils. All drugs temporarily 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, clenbuterol and etamiphylline camsylate treatment.

Atropine administration caused mydriasis in all animals which persisted for 12 to 24 hours. Mild sweating and muscle tremor occurred in 4 normal and 5 affected horses 10 to 20 minutes after isoprenaline inhalation and continued for approximately 30 minutes. A similar phenomenon occurred in 2 normal and 2 affected horses after terbutaline inhalation and in 4 normal and 6 affected horses following clenbuterol treatment. The sweating and tremor were more pronounced after clenbuterol administration and persisted up to 3 hours in some cases.

Individual values for respiratory rate, max. Δ Ppl, PaO_2, $PaCO_2$ and heart rate in normal and COPD affected

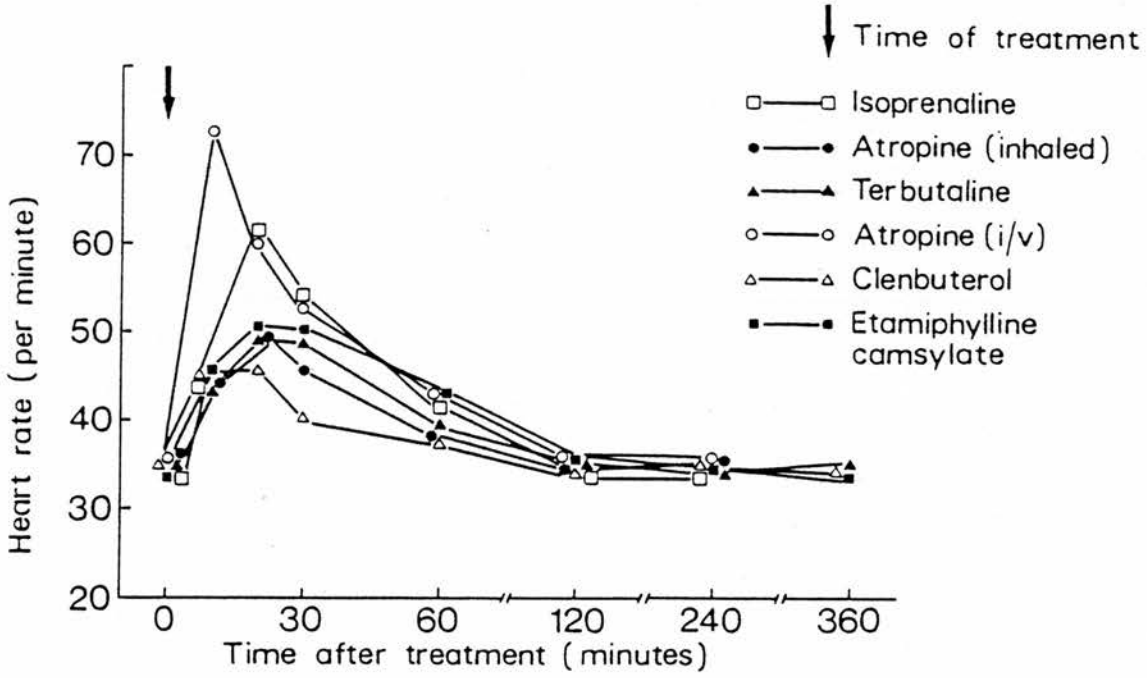


Figure 5.10. Effects of bronchodilator drugs on heart rate in COPD affected horses.

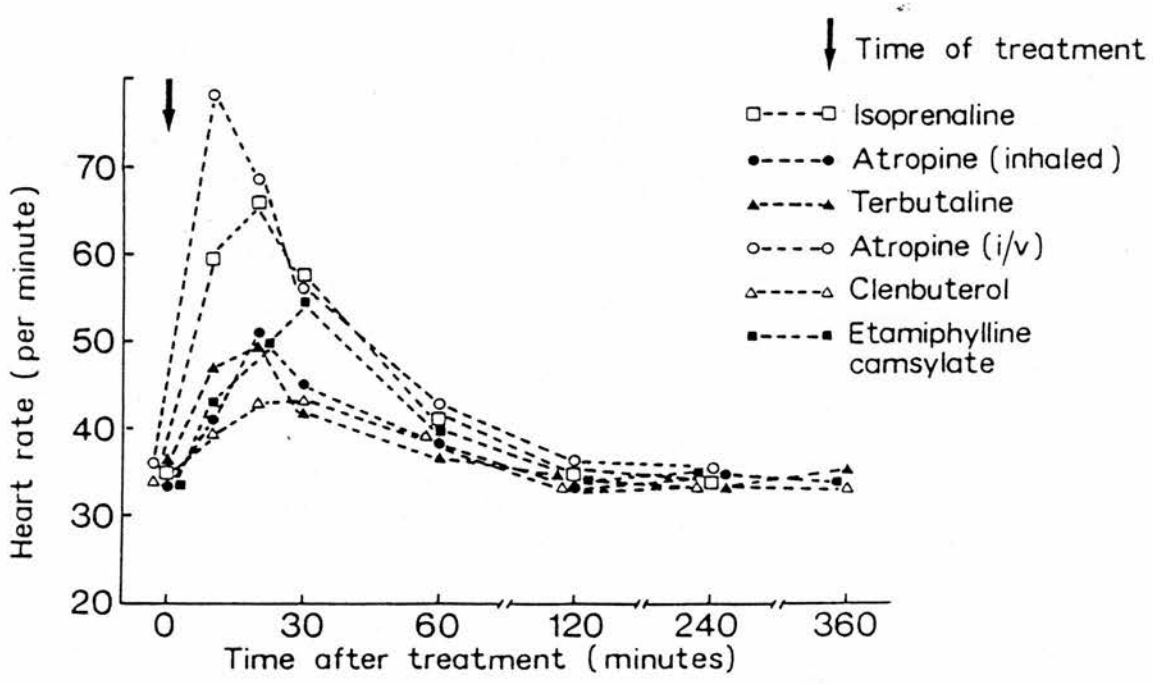


Figure 5.11. Effects of bronchodilator drugs on heart rate in normal horses.

horses before and after treatments as well as statistical analysis of results are presented in Appendices 5.2 to 5.7.

DISCUSSION

The effects of isoprenaline were rapid in onset and marked in effect but of short duration (i.e. ½ - 1 hour) (Figure 5.4). Although the decreases in max. Δ Ppl with terbutaline and clenbuterol were not as great as with isoprenaline, their actions were more prolonged with max. Δ Ppl and respiratory rates significantly decreased for 4 to 6 hours after treatment. Unlike isoprenaline which is rapidly matabolised by catechol-o-methyltransferase (COMT) (Hertting, 1964), terbutaline and clenbuterol are not substrates for this enzyme which accounts for their actions being more prolonged than those of isoprenaline. The half life of clenbuterol in the horse following intramuscular injection is 10 hours (Boehringer, 1980, technical information).

Atropine administration by either route produced a similar degree and duration of max. Δ Ppl response in affected horses (Figures 5.6 and 5.7) but the peak response to inhalation occurred approximately 7 minutes later than after intravenous injection.

In the COPD affected horses, etamiphylline camsylate caused a significant decrease in max. Δ Ppl and respiratory rate for 2 hours after treatment. The

period of efficacy was therefore considerably shorter than those of terbutaline and clenbuterol but may be explained by etamiphylline camsylate having a half life of just 2 hours in the horse following parenteral administration (Bogan, J.A., 1980, personal communication). Although etamiphylline camsylate is recommended by the manufacturers for the treatment of a wide range of pulmonary disorders in the horse there appear to be no reported studies on its efficacy.

Max. Δ Ppl, which was one of the main parameters used to assess pulmonary function in these experiments, was found to decrease greatly after administration of all drugs. While this max. Δ Ppl decrease could most obviously be attributed to a direct airway smooth muscle dilating action, some other factors have also to be considered, i.e. an increase in respiratory rate and/or a decrease in tidal volume could also lead to a decrease in max. Δ Ppl. However, in these studies, the respiratory rate decreased significantly in COPD affected horses as a result of treatment. Although the effects of bronchodilator drugs on tidal volume were not recorded in these experiments neither Muylle and Oyaert (1973) nor Sasse and Hajer (1977) were able to demonstrate any significant change in tidal volume in COPD affected horses after intravenous atropine or clenbuterol administration. It is further possible that atropine could have decreased airway resistance

by decreasing 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 horses to other bronchodilator drugs which do not have a drying effect on secretions, it is unlikely that this phenomenon contributed significantly to the observed decrease in max. Δ Ppl after atropine administration.

After treatment with all drugs, the COPD affected horses showed a marked clinical improvement corresponding with the time that significant decreases in max. Δ Ppl and respiratory rate occurred. It is probable that this rapid improvement in pulmonary function which was associated with a decrease in the work of respiration was, in fact, due to a functional decrease in airway resistance (i.e. airway dilation) induced by the drug administration. In contrast, no such response was observed in normal horses and so these results suggest that airway smooth muscle spasm is involved in the pathogenesis of equine COPD. Although the disease is anatomically a predominantly small airways disease, the site or sites of airway spasm in the tracheobronchial tree is unknown. In bronchial asthma, functional studies have shown that spasm occurs in both the bronchi and bronchioles (Cade et al., 1971; Despas, Le Roux and Macklem, 1972). In addition, there is some suggestion that the response of the various size airways

to antigen provocation may be different. The bronchi tend to increase their resistance quickly when stimulated and are, therefore, predominantly involved in acute asthma, but the effect can be quickly reversed through bronchodilator therapy. The reaction of the bronchioles, however, tends to be slower but more intense and persistent (McFadden, Kiser and de Groot, 1973; McFadden and Lyons, 1969). Until similar studies are performed in horses with COPD, the sites of airway spasm remain unknown.

Despite the marked decrease in max. Δ Ppl in COPD horses after bronchodilator administration their max. Δ Ppl and PaO_2 values still remained significantly different from those of normal horses indicating that their airway obstruction was not fully alleviated. This residual airway obstruction is undoubtedly due to the previously noted anatomical changes in COPD i.e. widespread exudative bronchiolitis (Nicholls, 1978).

The effects of bronchodilator therapy on arterial blood gas tensions in horses affected with COPD do not appear to have been reported previously. The initial drop in PaO_2 levels in both normal and COPD animals occurred, without a simultaneous increase in $PaCO_2$ levels. De Moor (1968) also noted a transitory hypoxaemia in normal horses after intravenous atropine administration.

Reports on the effects of clenbuterol on arterial

blood gas tensions in normal horses differ. Whereas Shapland, Garner and Hatfield (1981) recorded no significant changes in arterial blood gas levels in 4 normal horses following clenbuterol administration, Lieske and Deegan (1980) reported PaO_2 decreases of between 4.6 mm Hg and 29.3 mm Hg in 15 normal horses, 15 minutes after clenbuterol treatment. In the 8 normal horses treated in this study the mean PaO_2 levels decreased by 6 mm Hg for 2 hours following clenbuterol administration.

Hypoxaemia has been found to temporarily (for about 1 hour) intensify in human bronchial asthma patients shortly after treatment with isoprenaline, atropine, the xanthine derivatives and to a lesser extent, the beta 2 sympathomimetic bronchodilators (Knudson and Constantine, 1967; Field, 1967; Ingram et al., 1970; Chick, Nicholson and Johnson, 1973; Pain, 1973). This fall in PaO_2 which occurs irrespective of the route of drug administration (Svedmyr and Simonsson, 1978), is generally thought to be caused by an intensification of pre-existing ventilation - perfusion inequalities resulting from direct pulmonary vasodilation induced by these drugs (Field, 1967; West, 1976).

McFadden (1981) recorded the effects of bronchodilator therapy on PaO_2 in normal and asthmatic humans. He found that the higher the pretreatment PaO_2 levels, the greater the fall in PaO_2 following treatment and

that asthmatics with initial low PaO_2 tensions usually show no further PaO_2 decreases after bronchodilator treatment. This author concluded therefore, that the initial hypoxaemia-inducing effects of bronchodilators are not of clinical importance in human bronchial asthma. The situation in hypoxaemic COPD affected horses appears different however, as they suffered further transient PaO_2 decreases following bronchodilator therapy which were similar in magnitude to those PaO_2 decreases recorded in normal horses. Bronchodilator treatment of severely hypoxaemic horses might therefore be contra-indicated unless oxygen therapy can be given in addition.

The dual action of isoprenaline on both beta 1 and beta 2 receptors is disadvantageous in that bronchodilation is usually accompanied by marked tachycardia, as was observed in both normal and COPD affected horses. Although terbutaline and clenbuterol exhibit a useful degree of selectivity for beta 2 receptors, the increased heart rates noted in these experiments indicate that these agents, even in clinical doses may exert significant beta 1 receptor activity in the horse. Another possible way in which bronchodilator drugs could cause tachycardia is indirectly through causing a fall in peripheral vascular resistance thereby causing a reflex increase in heart rate to maintain blood pressure (Sackner et al., 1975). The decrease in pulmonary artery pressure recorded in normal horses

following clenbuterol administration by Shapland, Garner and Hatfield (1981) and Lieske and Deegan (1980) were ascribed by these authors to a fall in the peripheral vascular resistance. A transient tachycardia was also recorded in these horses. A decrease in pulmonary artery pressure can also result from the relief of hypoxaemic pulmonary vasoconstriction (Dixon, 1979). However, this mechanism was not considered by Shapland, Garner and Hatfield (1981) or Lieske and Deegan (1980) probably because these authors were using normal horses which were, therefore, not hypoxaemic at the outset of the trials and consequently no increase in PaO_2 levels were recorded with the decreases in pulmonary artery pressure.

Tachycardia and muscle tremor are common side effects of the selective beta 2 stimulating drugs in man (Bowman and Raper, 1976). Whereas the cardiac stimulating effect can be prevented by pretreatment with a selective beta 1 blocking drug, skeletal muscle tremor which was observed in horses following terbutaline and clenbuterol is mediated by beta 2 receptors and is therefore an unavoidable side-effect of this group of drugs (Larsson and Svedmyr, 1974). Xanthine derivatives cause tachycardia through their myocardial stimulating effects (Nimmo, 1976). This would explain the transient increase in heart rate recorded in normal and COPD horses after etamiphylline camsylate treatment.

In addition to their airway dilating properties,

the sympathomimetic amines have other actions which could play a role in improving lung function but which have not as yet been widely reported. In man, the beta agonists have been shown to facilitate mucociliary transport (Santa Cruz et al., 1974) and are thought to inhibit mast cell degranulation in sensitised individuals on bronchial provocation tests (McFadden, 1981).

The short duration of action and the previously mentioned side effects of isoprenaline (i.e. cardiac stimulation) and atropine (i.e. cardiac stimulation, increased viscosity of bronchial secretions, reduced bowel motility and mydriasis) preclude their widespread use as therapeutic agents for equine COPD. The use of the longer acting bronchodilator drugs for the treatment of COPD remains subject to many limitations in that they do not control the cause of the disease, they only partially alleviate the physiological changes and clinical signs and the resulting improvement is of short duration. However, terbutaline, clenbuterol and etamiphylline camsylate could be used for the treatment of acute attacks of COPD. Orally administered preparations of the latter two drugs are commercially available and may be found useful when continuing bronchodilator treatment in horses following the initial parenteral dose. Further studies on the orally administered compounds are described in Chapter 6.

CONCLUSIONS

Administration of bronchodilator drugs to horses

affected with COPD brought about a temporary, marked improvement in clinical dyspnoea, accompanied by significant decreases in max. ΔPpl and respiratory rate and significant increases in PaO_2 values. However, bronchodilator treatment failed to restore normal pulmonary function in COPD affected horses. None of the above effects were observed in normal horses. This suggests that airway spasm is involved in the pathogenesis of equine COPD.

Although the use of inhaled or intravenous bronchodilator drugs in the treatment of equine COPD is subject to many limitations, bronchodilator therapy could be of value as a temporary measure in the treatment of acute and severe attacks.

CHAPTER 6.

THE EFFECTS OF ORALLY ADMINISTERED BRONCHODILATOR DRUGS AND ENVIRONMENTAL CONTROL ON COPD AFFECTED HORSES.

INTRODUCTION

Studies described in Chapter 5 and those of Obel and Schmiterlöw (1948), Muylle and Oyaert (1973) and Sasse and Hajer (1977) have shown that a single parenteral treatment or inhalation with a variety of bronchodilator drugs brings about a marked improvement in the clinical appearance and respiratory function of COPD affected horses. These beneficial effects are, however, temporary although some agents, for example, the beta 2 sympathomimetic bronchodilators are longer acting than their predecessors.

Repeated bronchodilator inhalation or injections for COPD treatment would be impractical except as a temporary measure in severely affected animals. However, orally administered compounds are now commercially available and the use of oral clenbuterol (Ventipulmin, Boehringer, Ingleheim, BRD) twice daily for 14 days has been reported to cause an improvement in COPD affected horses (Sasse and Hajer,1977). However, the response was assessed by the owners' observations only. Further investigations including veterinary clinical examinations and pulmonary function testing are therefore required to objectively examine the efficacy of these orally administered bronchodilator drugs for equine COPD treatment.

This chapter aims to examine the effects of two bronchodilating agents, namely clenbuterol and

etamiphylline camsylate on (1) the clinical signs and respiratory function in COPD affected horses when administered without environmental control measures and (2) these same parameters in affected horses when administered in conjunction with environmental control measures.

MATERIALS AND METHODS

HORSES.

16 COPD affected horses were divided into 2 groups; horses Nos. B93 - 100 (Appendix 3.2) were treated with clenbuterol in trials 6.A. and B. Horses Nos. B101-108 (Appendix 3.2) received etamiphylline camsylate in trials 6.C. and D. Each horse acted as its own control. All these COPD affected horses suffered respiratory hypersensitivity to M. faeni and all showed clinical signs of COPD when exposed to the natural challenge environment (p 55).

CLENBUTEROL TRIALS.

The flow diagram (Figure 6.1) indicates the sequence of management changes and drug administration.

6.A. Effects of oral clenbuterol treatment on horses housed in the natural challenge environment.

6.A.(i). Challenge environment, no treatment.

8 COPD affected horses, (Nos. B93 - 100, Appendix 3.2) were exposed to the natural challenge environment for 14 days without any treatment. The horses were given daily clinical examinations and their

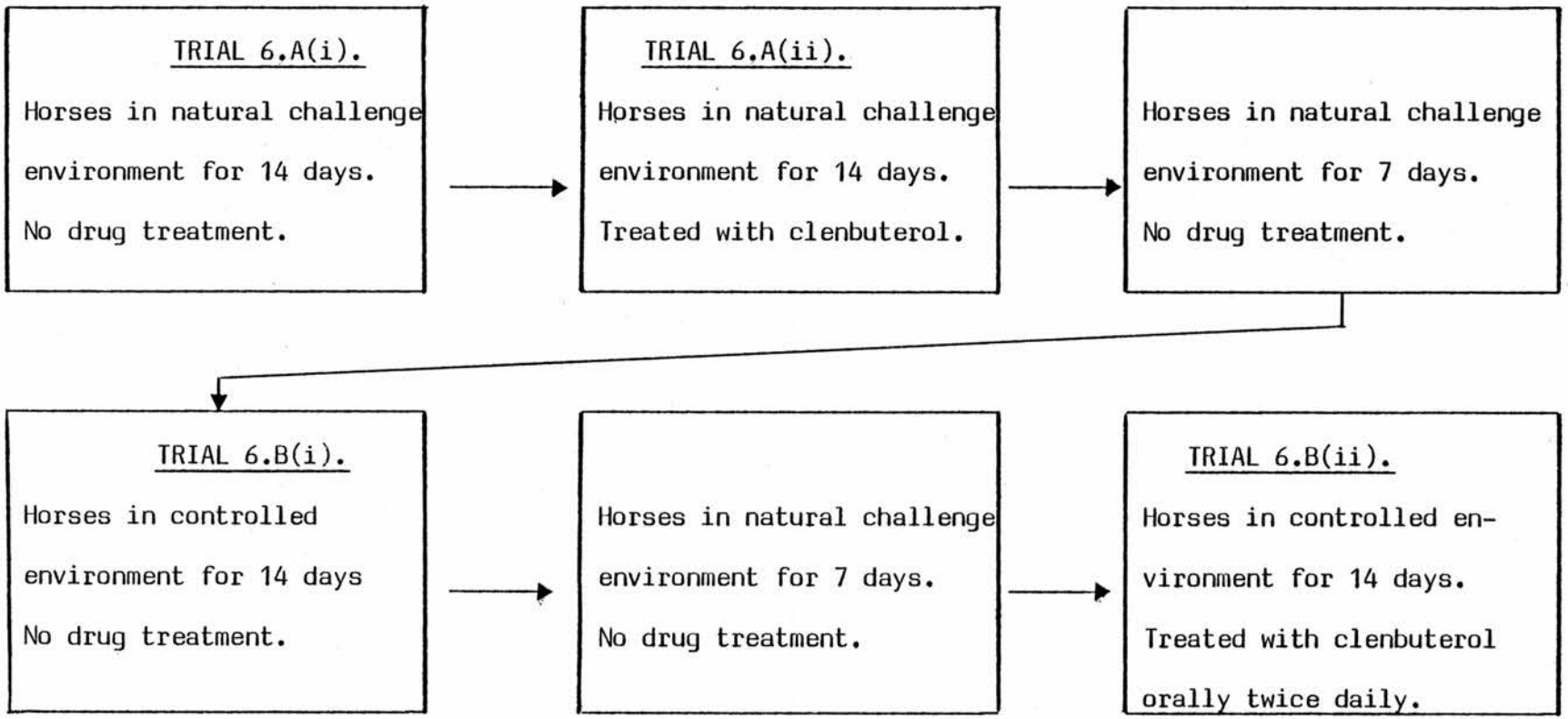


Figure 6.1. Flow diagram of the studies on oral clenbuterol administration in the treatment of equine COPD.

max. ΔPpl, PaO_2, $PaCO_2$ and arterial blood pH values were recorded, using techniques previously described (pp.49,54), on days 0, 1, 2, 4, 7, 9, 11 and 14.

6.A(ii). Challenge environment plus clenbuterol treatment.

For the following 14 days, the horses were kept in a similar environment and were given clenbuterol. Treatment was initiated with a single intravenous injection of clenbuterol (Ventipulmin, Boehringer, Sohn., Ingleheim, BRD) at a dosage of 0.8 µg/kg and continued by twice daily oral administration in their concentrate feed (0.8 µg/kg b.i.d.) for 14 days. In this trial and all ensuing treatment trials, the feed was made into a mash to ensure that the full dose was taken and a deep heavy trough was used to prevent drug spillage. Clinical examinations and respiratory function parameters were recorded as described in trial 6.A(i).

On completion of this trial, horses were maintained untreated in the same environment for 7 days prior to commencement of trial 6.B.

6.B. Effects of oral clenbuterol treatment on COPD affected horses housed in the controlled environment.

6.B(i). Controlled environment, no drug treatment.

The 8 COPD horses described in trial

6.A were examined clinically on day 0 and max. ΔPpl, $PaCO_2$ and arterial pH recorded. Thereafter horses were housed in a controlled environment (p. 57) for 14 days during which time, they received no drug treatment. The horses received daily clinical examinations and, as in the previous trial (6.A), the same respiratory function parameters were recorded on days 1, 2, 4, 7, 9, 11 and 14.

Upon completion of the controlled environment trial the horses were re-exposed to the natural challenge environment for 7 days prior to commencement of trial 6.B(ii).

6.B(ii). Controlled environment plus clenbuterol treatment.

As in the preceding trial, the 8 horses were clinically examined and respiratory function parameters recorded at the start of the trial (day 0). Thereafter, the horses were treated with clenbuterol (Ventipulmin, Boehringer Sohn., Ingleheim, BRD) at a dosage of 0.8 µg/kg intravenously and were moved to the controlled environment where they remained for 14 days. Throughout that period, the horses received oral clenbuterol treatment (0.8 µg/kg) twice daily in the feed. Clinical examinations and respiratory function parameters were recorded during this trial, as described in the previous trial.

ETAMIPHYLLINE CAMSYLATE TRIALS.

The design of the etamiphylline camsylate trials is similar to the clenbuterol trials and is demonstrated in Figure 6.2.

6.C. Effects of oral etamiphylline camsylate treatment on COPD affected horses housed in the natural challenge environment.

This trial followed the same protocol as the first clenbuterol trial (6.A).

6.C(i). Challenge environment, no treatment.

8 COPD affected horses, (Nos. B101 - 108, Appendix 3.2) were housed, examined and their respiratory function monitored, as previously described in trial 6.A(i).

6.C(ii). Challenge environment plus etamiphylline camsylate treatment.

The horses were maintained in the challenge environment and received 3.0 mg/kg etamiphylline camsylate (Millophyline - V, Dales Pharmaceuticals Ltd., Skipton) intravenously on day 0, followed by 2.25 mg/kg orally in the concentrate feed three times daily for 14 days. Clinical examinations and respiratory function parameters were recorded as described under trial 6.A(i).

The affected horses were housed in the natural challenge environment, free of treatment, for 7 days after completion of this trial prior to commencement

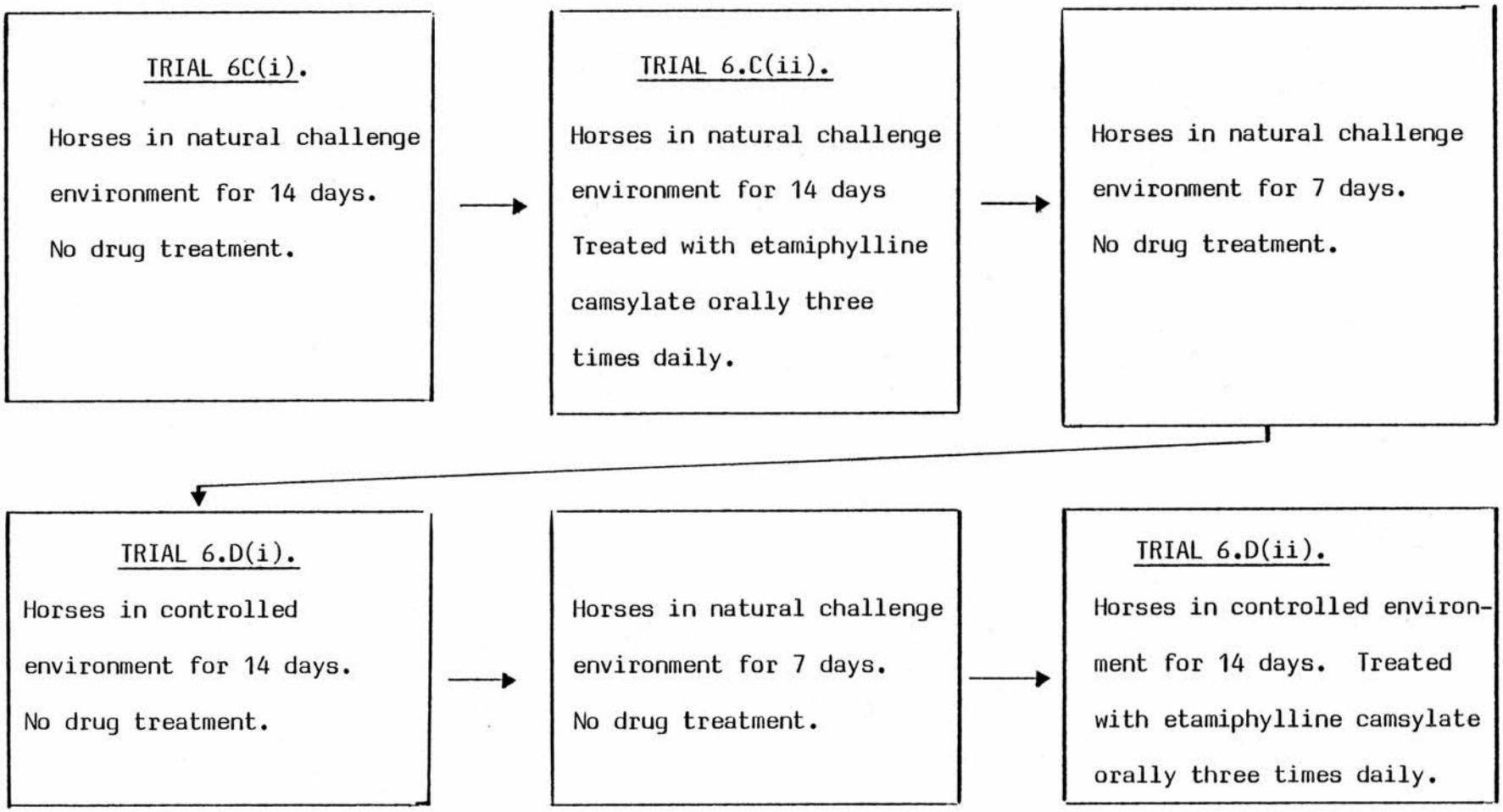


Figure 6.2. Flow diagram of the studies on oral etamiphylline camsylate administration in the treatment of equine COPD.

of trial 6.D (See Figure 6.2).

6.D. Effects of oral etamiphylline camsylate treatment on COPD affected horses housed in the controlled environment.

The protocol for this trial is similar to that described in the corresponding clenbuterol trial (6.B(i), (ii)).

6.D(i). Controlled environment, no drug treatment.

This controlled environment study was carried out as previously described in trial 6.B(i) using COPD affected horses Nos. B101 - 108 (Appendix 3.2).

At the end of this trial, the horses were re-exposed to the natural challenge environment for 7 days prior to commencing trial 6.D(ii).

6.D(ii). Controlled environment plus etamiphylline camsylate treatment.

In this trial, the horses were treated with 3.0 mg/kg etamiphylline camsylate intravenously prior to being moved to the controlled environment. Thereafter, horses received 2.25 mg/kg etamiphylline camsylate orally three times daily for 14 days. Clinical examinations and respiratory function parameters were recorded as described under trial 6.B(ii).

STATISTICAL ANALYSIS OF RESULTS.

In trials 6.A, B, C and D, values for respiratory rate, max.ΔPpl, PaO_2, $PaCO_2$ and arterial blood pH in the untreated horses (in trials 6.A(i), 6.B(i), 6.C(i) and 6.D(i)) on days 0, 2, 4, 7, 9, 11 and 14 were compared to the corresponding values for the treated horses (in trials 6.A(ii), 6.B(ii), 6.C(ii) and 6.D(ii)) by Student's t-test as applied to paired observations.

RESULTS

CLENBUTEROL TRIALS.

6.A. Effects of oral clenbuterol treatment on COPD affected horses housed in the natural challenge environment.

At the start of both the control and treatment trials, all horses were symptomatically affected with COPD. The mean respiratory rate, max.ΔPpl and PaO_2 values are presented in Figures 6.3, 6.4, and 6.5 respectively. Each horse showed many of the clinical signs of COPD although the severity of the disease varied between horses. All horses coughed, had a double expiratory effort with increased and harsh lung sounds. Wheezing audible at the nostril, was present in horses Nos. B96 and B98 at the start of both parts of this trial, and in horse No. B94 at the commencement of trial 6.A(ii) only.

6.A(i). Challenge environment, no treatment.

Throughout this trial, horses continued to be

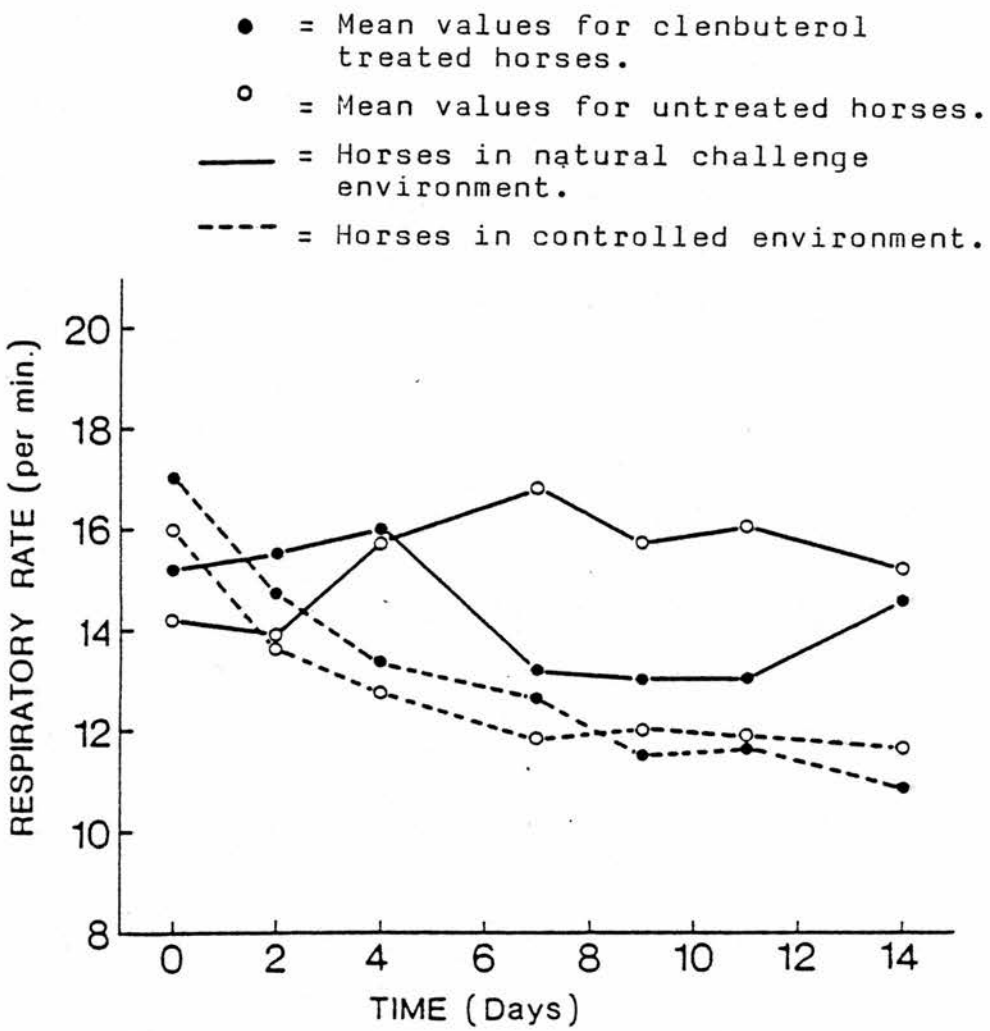


Figure 6.3. Effects of oral clenbuterol treatment on respiratory rate in COPD affected horses, (i) in the natural challenge environment and (ii) in the controlled environment.

● = Mean values for clenbuterol treated horses.

o = Mean values for untreated horses.

____ = Horses in natural challenge environment.

_ _ _ _ = Horses in controlled environment.

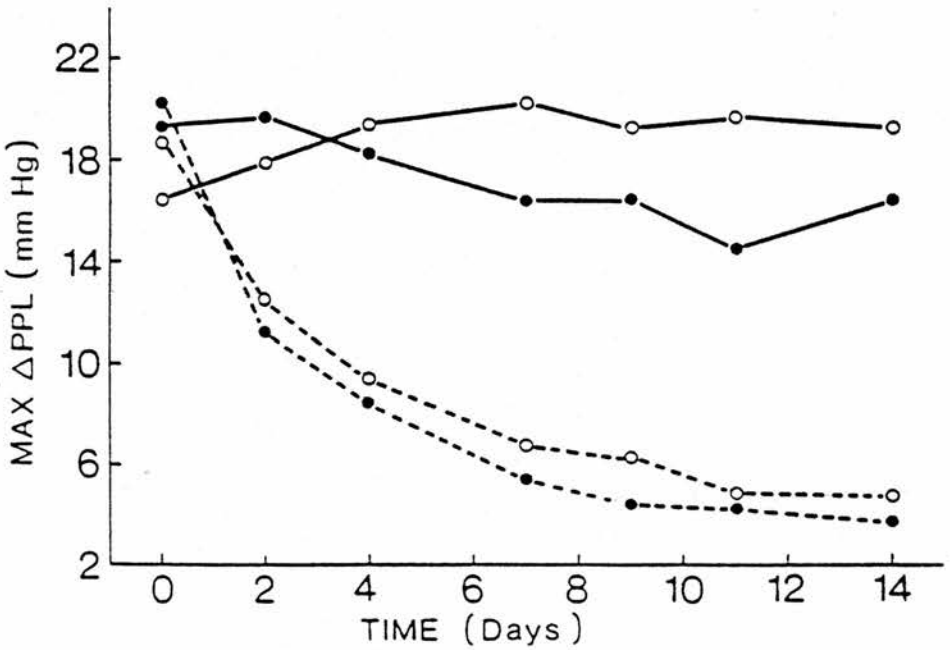


Figure 6.4. Effects of oral clenbuterol treatment on max. ΔPpl in COPD affected horses, (i) in the natural challenge environment and (ii) in the controlled environment.

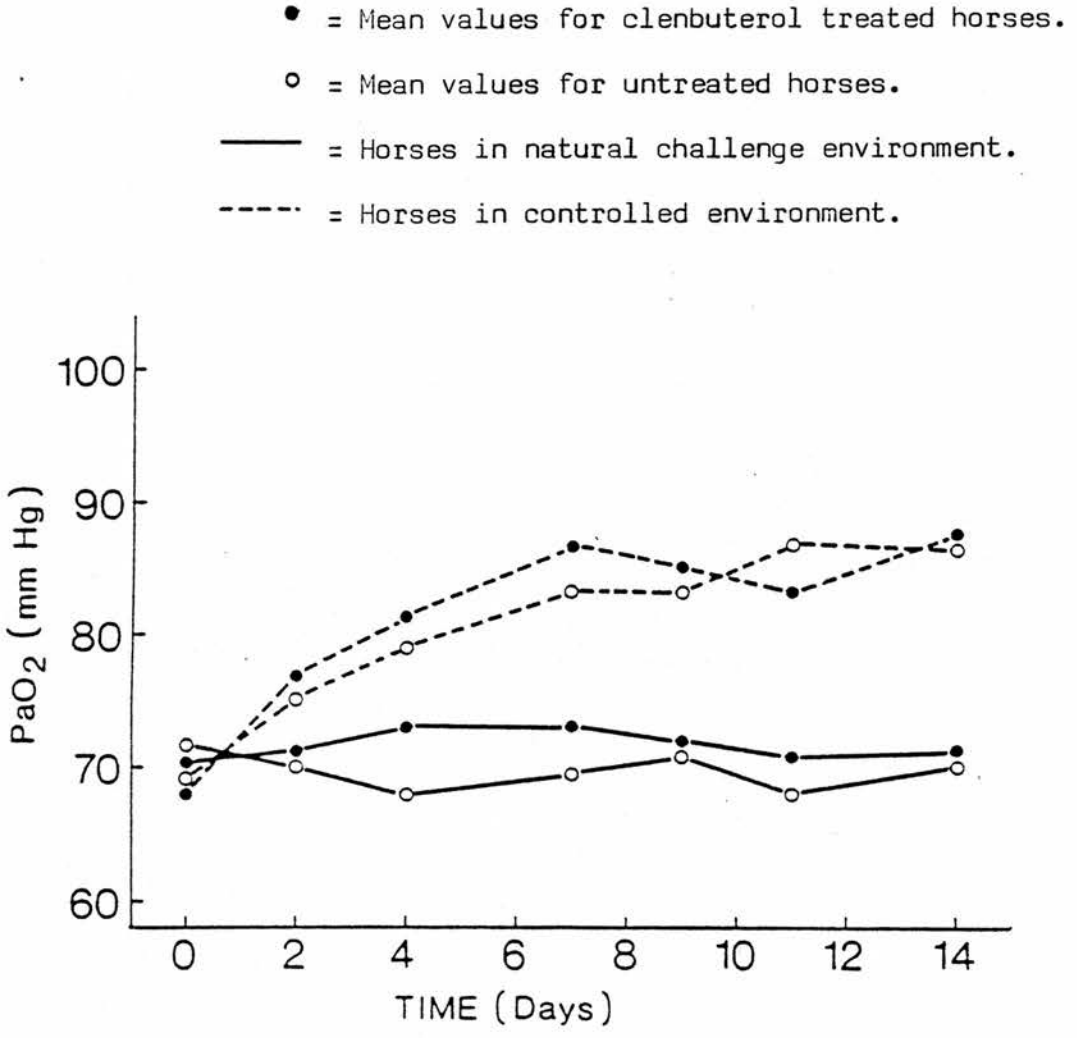


Figure 6.5. Effects of oral clenbuterol treatment on PaO_2 in COPD affected horses, (i) in the natural challenge environment and (ii) in the controlled environment.

symptomatically affected. The mean respiratory rate, PaO_2, $PaCO_2$ and arterial pH did not alter significantly from values recorded on day 0, but the mean max. ΔPpl increased and was significantly higher ($P < 0.05$) than the baseline value on days 7, 11 and 14 (Figure 6.4). Horses Nos. B94, 97 and 98 showed an increasing severity of clinical signs over the 14 days. Their double expiratory efforts became very pronounced, horses coughed more frequently and horse No. B94 began wheezing on day 4 which continued until the end of the trial. The wheezing in horses Nos. B96 and B98 became more pronounced.

6.A(ii). Challenge environment plus clenbuterol treatment.

The effects of clenbuterol treatment on respiratory rate, max. ΔPpl and PaO_2 in the COPD affected horses are shown in Figures 6.3, 6.4 and 6.5 respectively.

During the first 4 days, the respiratory rate did not differ significantly from the values recorded on corresponding days in the challenge environment trial (6.A(ii)), but during the latter 7 days, the mean respiratory rates decreased and were significantly lower ($P < 0.05$) than those for the untreated horses on days 7 and 11. From day 4 onwards, the mean max. ΔPpl values for the treated horses were lower than those for the untreated horses but this decrease was only statistically significant ($P < 0.01$) on day 11.

The only significant difference in PaO_2 values from the challenge environment trial was recorded on day 4 when mean PaO_2 values for the treated horses was 73.4 mm Hg as compared to 68.1 mm Hg for the untreated horses ($P < 0.01$). All horses remained hypoxaemic throughout the trial except for horse No. B95 whose PaO_2 was in the normal range from day 4 to day 9 (Appendix 6.1). There were no significant changes in $PaCO_2$ or arterial pH as a result of treatment.

In general, the horses showed little improvement in their clinical condition whilst on clenbuterol treatment in the challenge environment. Horse No. B95 did, however, improve markedly from day 4 to 10, during which time she ceased coughing and by day 10 only a very slight double expiratory effort was present but, during the last 4 days of the trial, the clinical signs worsened. Horses Nos. B96 and B98 continued wheezing throughout the trial. Horse No. B94 continued to be severely affected throughout the trial with just a slight clinical improvement over the last 7 days.

6.B. Effects of oral clenbuterol treatment on COPD affected horses housed in the controlled environment.

At the outset of both parts of this trial, all horses were symptomatically affected with COPD. The mean values for respiratory rate, max.$\triangle$Ppl and PaO_2

recorded during this trial are shown in Figures 6.3, 6.4 and 6.5 respectively.

6.B(i). Controlled environment, no drug treatment.

When horses were housed in the controlled environment, there was a steady, highly significant ($P < 0.001$) decline in the mean max. ΔPpl and respiratory rate and increase in the mean PaO_2 over the 14 days.

At the start of this trial, the horses' clinical conditions were similar to those described at the start of the challenge environment plus clenbuterol treatment trial (6.A(ii)). As this trial progressed, the horses all showed a marked clinical improvement and became asymptomatic in 6 to 14 days except for horse No. B96 which, although very much improved, was still showing clinical signs of COPD on day 14. The mean ($\pm$ S.D.) time taken for horses to become asymptomatic was 9.25 $\pm$ 4.40 days.

6.B(ii). Controlled environment plus clenbuterol treatment.

At the commencement of this trial, horses were clinically affected to a similar degree as at the beginning of the controlled environment trial (6.B(i)). Once again, there was a marked decrease in the mean max. ΔPpl and respiratory rate and increase in the mean PaO_2 value. Although the max. ΔPpl and PaO_2 changes

occurred slightly faster in this trial (when horses were receiving clenbuterol treatment) than in the preceding trial, there were no significant differences in the values for the above-named parameters recorded on corresponding days, between the two trials. Neither were there any significant differences in $PaCO_2$ or arterial pH levels between treated and untreated horses.

The horses became asymptomatic between days 4 to 12 (mean ($\pm$ S.D.) 7.25 $\pm$ 2.60 days) which is less, but not significantly so ($P > 0.05$), than the time taken for the untreated horses to become asymptomatic.

Horses Nos. B93 and B99 showed sweating, muscle tremor and tachycardia (heart rate 45-55 beats per minute) for 2 to 3 hours after clenbuterol administration on several occasions during both clenbuterol treatment trials (6.A(ii) and 6.B(ii)).

ETAMIPHYLLINE CAMSYLATE TRIALS.

6.C. Effects of oral etamiphylline camsylate treatment on COPD affected horses housed in the natural challenge environment.

At the beginning of both parts of this trial, all horses were symptomatically affected with COPD. The horses coughed, showed a double expiratory effort

and increased harsh lung sounds. Wheezing, audible at the nostril was present in horse No. B107. Mean values for respiratory rate, max. ΔPpl and PaO_2 are presented in Figures 6.6, 6.7 and 6.8 respectively.

6.C(i). Challenge environment, no treatment.

The horses continued to be symptomatically affected throughout this trial. Values for respiratory rate, max. ΔPpl, PaO_2, $PaCO_2$ and arterial pH recorded during the trial did not differ significantly from those recorded on day 0. The horses' clinical signs showed little change during the 14 days of this trial except for horse No. B105 which became much more severely affected from day 6 to day 13, with pronounced dyspnoea and wheezing audible at the nostril during this time.

6.C(ii). Challenge environment plus etamiphylline camsylate treatment.

Similarly to the previous trial (6.C(i)), all horses remained symptomatically affected with COPD when retained in the challenge environment despite treatment with etamiphylline camsylate. The mean values for respiratory rate, max. ΔPpl and PaO_2 during this trial, which are shown in Figures 6.6, 6.7 and 6.8 respectively, did not differ significantly from the corresponding values recorded in the untreated horses

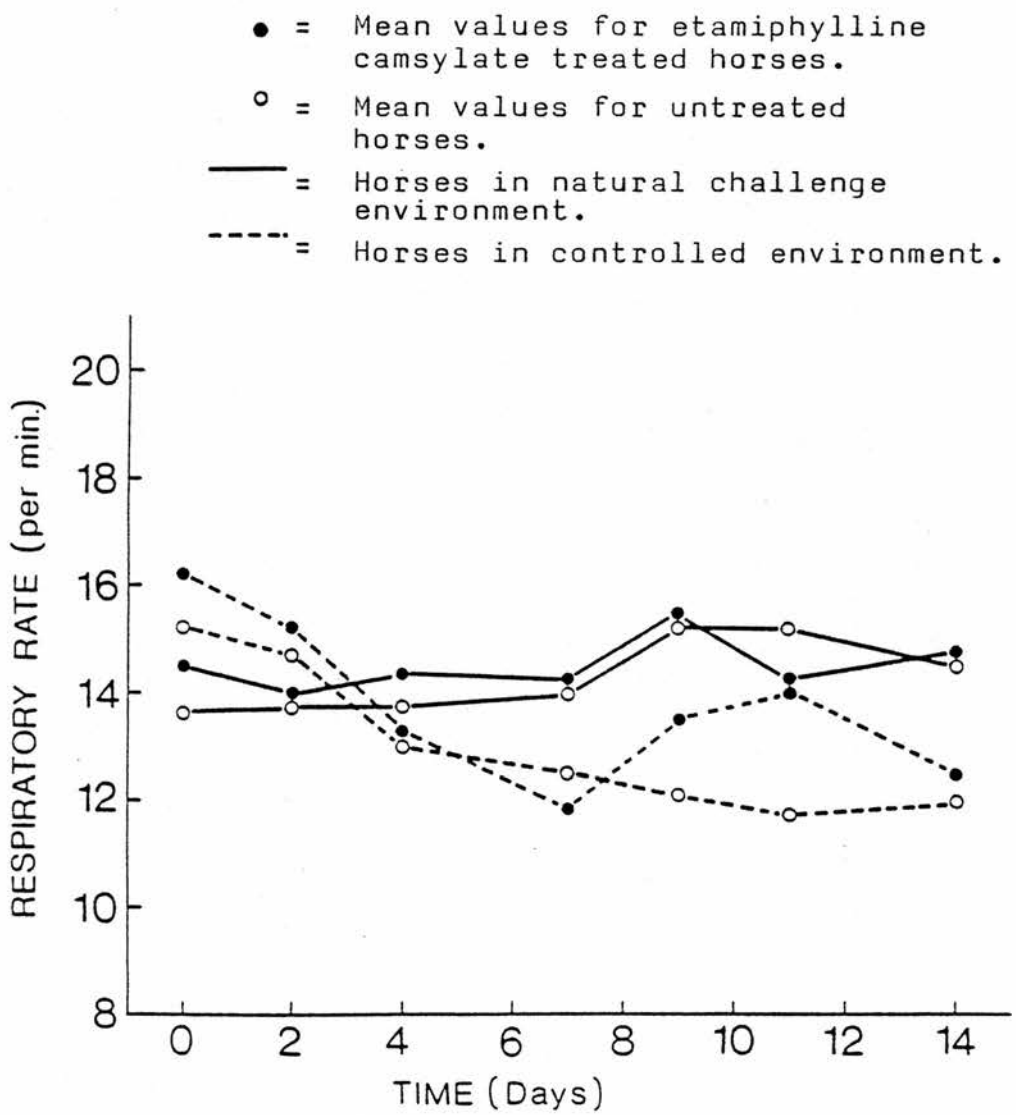


Figure 6.6. Effects of oral etamiphylline camsylate treatment on respiratory rate in COPD affected horses, (i) in the natural challenge environment and (ii) in the controlled environment.

● = Mean values for etamiphylline camsylate treated horses.

○ = Mean values for untreated horses.

——— = Horses in natural challenge environment.

- - - - = Horses in controlled environment.

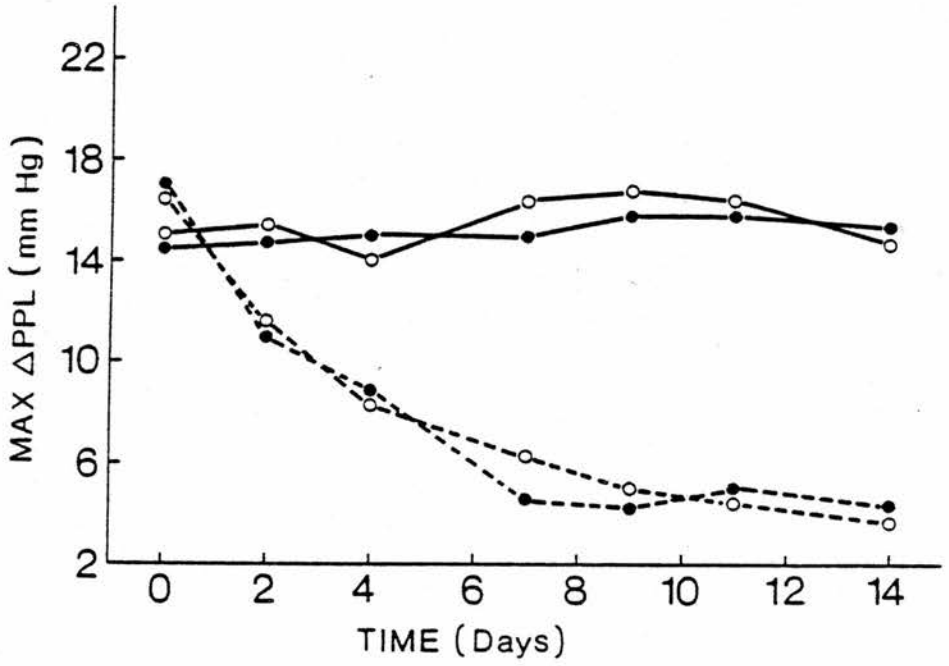


Figure 6.7. Effects of oral etamiphylline camsylate treatment on max. Δ Ppl in COPD affected horses, (i) in the natural challenge environment and (ii) in the controlled environment.

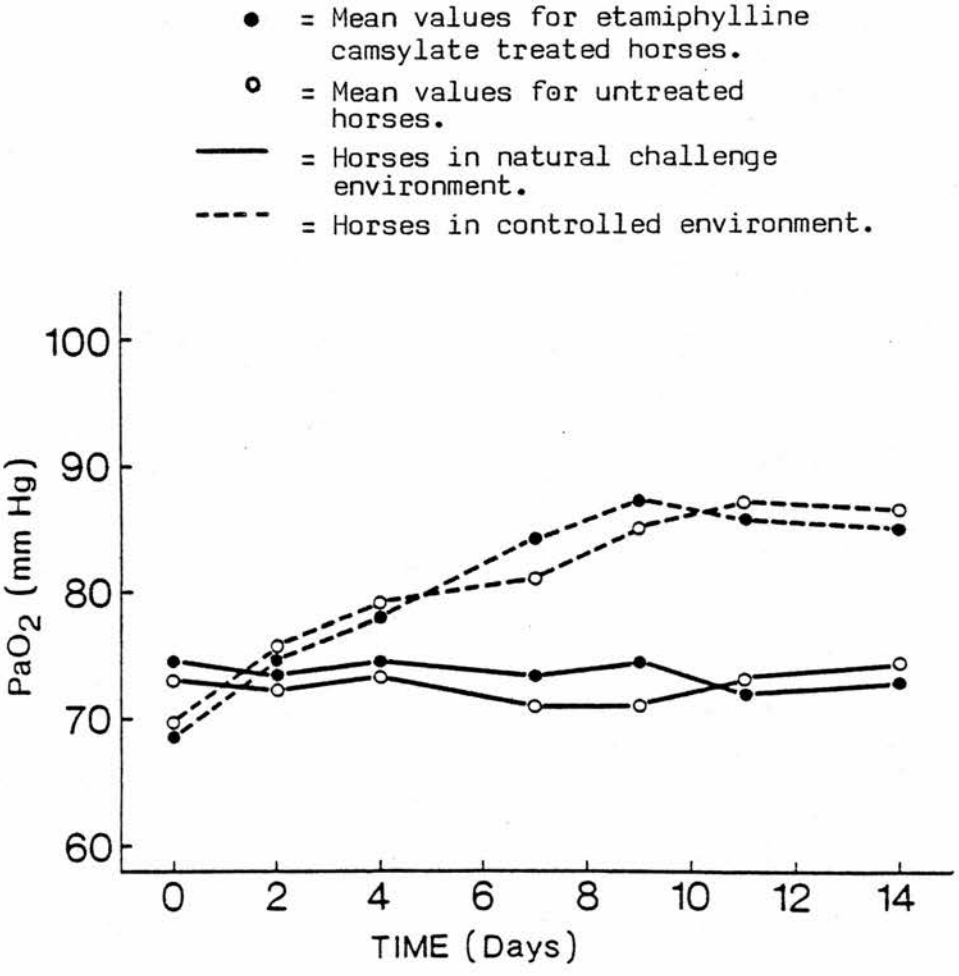


Figure 6.8. Effects of oral etamiphylline camsylate treatment on PaO_2 in COPD affected horses, (i) in the natural challenge environment and (ii) in the controlled environment.

(trial 6.C(i)). Neither were there any significant differences in the horses' $PaCO_2$ or arterial pH values between the two trials.

The clinical signs of COPD in horse No. B101 became less pronounced from the 6th day onwards. No other horses showed any clinical improvement as a result of etamiphylline camsylate treatment whilst retained in the challenge environment.

6.D. Effects of oral etamiphylline camsylate treatment on COPD affected horses housed in the controlled environment.

All horses were symptomatically affected with COPD at the beginning of both parts of this trial. The mean values for respiratory rate, max. Δ Ppl and PaO_2 recorded during the trials are shown in Figures 6.6, 6.7 and 6.8 respectively.

6.D(i). Controlled environment, no drug treatment.

When horses were moved from the natural challenge environment and housed in the controlled environment, there was a gradual, highly significant ($P < 0.001$) decrease in the mean max. Δ Ppl and respiratory rate and increase in the mean PaO_2 over the course of the trial. Most horses showed more advanced clinical signs at the beginning of this trial than at that of the previous trial (6.C), but they improved markedly as this trial progressed and became

asymptomatic in 4 to 14 days (mean ($\pm$ S.D.) 8.13 $\pm$ 3.23 days).

6.D(ii). Controlled environment plus etamiphylline camsylate treatment.

At the commencement of this trial, the horses were affected to a similar degree as at the beginning of the previous trial (6.D(i)). When moved to the controlled environment and given etamiphylline camsylate, changes in max.ΔPpl, PaO_2 and respiratory rate occurred, similar to those for the untreated horses (in trial 6.D(i)) and there were no significant differences between these corresponding values or those of $PaCO_2$ and arterial pH in the two trials.

Horses became asymptomatic in 4 to 10 days (mean ($\pm$ S.D) 6.50 $\pm$ 1.93 days) which is not significantly less ($P > 0.05$) than the time taken for horses to become asymptomatic in the controlled environment trial (6.D(i)).

No side effects were observed as a result of oral etamiphylline camsylate treatment. There were no problems associated with drug palatability, all horses ingested both clenbuterol and etamiphylline camsylate quite readily.

The individual values for respiratory rate, max.ΔPpl, PaO_2, $PaCO_2$ and arterial pH recorded during trials 6.A, B, C and D as well as the results of the

statistical analyses are presented in Appendices 6.1, 6.2, 6.3 and 6.4 respectively.

DISCUSSION

Despite clenbuterol and etamiphylline camsylate having different pharmacological modes of action, these drugs were shown to have similar effects on the respiratory parameters examined in COPD affected horses. The results of the trials will, therefore, be discussed together.

When COPD affected horses, housed in the natural challenge environment, were treated with clenbuterol (trial 6.A(ii)) and etamiphylline camsylate (trial 6.C(ii)), they continued to be clinically affected with COPD and there were very few significant changes in their respiratory function values from those for horses untreated and housed in similar conditions. Despite the decrease in mean max. ΔPpl and increase in mean PaO_2 values when horses were on clenbuterol treatment, these values did not approach normal levels, i.e.; max. ΔPpl < 6 mm Hg and PaO_2 > 82 mm Hg (McPherson et al., 1978). Horse No. B95 alone had values within these ranges on days 4, 7 and 9 of trial 6.A(ii) (Appendix 6.1).

When symptomatically affected horses were housed in the controlled environment, neither drug caused significant changes in max. ΔPpl or PaO_2 from the corresponding values in the preceding trials, when the

horses were housed in this same environment but received no drug treatment. Neither was the remission of clinical signs significantly hastened through treatment with these agents in conjunction with the controlled environment.

The apparent lack of response in COPD affected horses to these orally administered bronchodilator drugs is difficult to explain in view of the marked improvements recorded after their intravenous injection, reported in chapter 5. In keeping with the drug manufacturers' recommendations, the same clenbuterol dose was used for intravenous and oral administration (i.e. 0.8 µg/kg) and a lower dose of etamiphylline camsylate was used for the oral administration trials (i.e. 2.25 mg/kg) than for intravenous administration (i.e. 3.0 mg/kg). Although both drugs are well absorbed from the equine gastrointestinal tract, it is possible that higher doses of orally administered compounds are required to achieve a similar response to that which has been demonstrated with intravenous administration. Detailed dose response studies would be required to elucidate this point. The half lives of clenbuterol and etamiphylline camsylate after oral administration in the horse vary considerably; clenbuterol having a half life of 20 hours (Boehringer, 1980, technical information) whilst that of etamiphylline camsylate is 6 hours (Bogan, J.A., 1981, personal communication). Following intravenous

administration of clenbuterol and etamiphylline camsylate to COPD affected horses, significant pulmonary function improvements were present up to 4 hours and 2 hours respectively (chapter 5). The monitoring time in experiments described in this chapter was constant, i.e. 3 to 3½ hours after the morning treatment. Whilst it is possible that the response occurred prior to monitoring and that the effects were diminishing by 3 to 3½ hours after treatment, this seems unlikely as the response would be more delayed after oral administration than after intravenous injection. In addition, the monitoring time was well within the half life of both drugs.

When the affected horses were treated with clenbuterol or etamiphylline camsylate whilst housed in the challenge environment, it is possible that high levels of aetiological antigens in the atmosphere counteracted the effects of these bronchodilator drugs. However, in the trials where antigen exposure was minimised through use of a controlled environment (trials 6.B(ii) and 6.D(ii)), bronchodilator therapy appeared equally ineffective.

The poor response to oral bronchodilator therapy in clenbuterol and etamiphylline camsylate treatment trials when horses were housed in the challenge environment (6.A(ii) and 6.C(ii)) might have been attributed to the horses having permanent structural pulmonary changes. However, the fact that all horses became

asymptomatic in the controlled environment shortly thereafter indicates that the pulmonary changes were primarily functional (i.e. reversible) rather than structural (i.e. irreversible).

The side effects of oral clenbuterol administration (sweating, muscle tremor and tachycardia) noted in two horses were not recorded by Sasse and Hajer (1977) during their oral clenbuterol trials. However, the response to treatment was assessed by the owners and no details were provided regarding the times of observation after treatment. It is possible, therefore, that the owners failed to notice any transient side effects through infrequent observation. Nausea and gastro-intestinal discomfort commonly occur in man after administration of oral etamiphylline preparations (Flenley, 1981), however, no such side effects were seen in the horse after oral etamiphylline camsylate treatment.

CONCLUSIONS.

In these trials, neither oral clenbuterol nor etamiphylline camsylate were effective in treating symptomatic COPD affected horses exposed to the natural challenge environment. In addition, neither drug significantly hastened the remission of COPD which normally occurred when symptomatic horses were housed in the controlled environment.

CHAPTER 7.

THE EFFECTS OF SODIUM CHROMOGLYCATE ON ANTIGEN INHALATION CHALLENGE IN TWO COPD AFFECTED HORSES.

INTRODUCTION

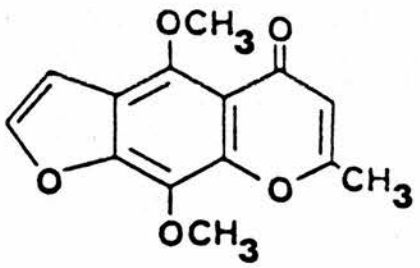
Ammi visnaga is an umbelliferous plant (Figure 7.1) which grows wild in eastern Mediterranean regions. Since ancient times, extracts from the seeds of this plant have been used medicinally by the local population mainly as an antispasmodic in the treatment of renal colic and ureteral spasm (Anrep et al., 1947). Chemical analysis of extracts of this plant showed the active principle to be a chromone named khellin (di-methoxy-methyl-furano-chromone) (Figure 7.2). In vitro studies showed that khellin causes marked relaxation of visceral smooth muscle, including bronchial muscle (Samaan, 1932). Later, khellin was reported to be an efficient bronchodilator in certain forms of human asthma (Kennedy and Stock, 1952).

Sodium cromoglycate was discovered in 1965 during research to find a more effective agent for the treatment of human bronchial asthma. In an attempt to improve the smooth muscle relaxant properties of khellin while eliminating its systemic vasodilatory side effects, a series of synthetic chromones were produced. One of these compounds, a dimeric chromonecarboxylic acid (Figure 7.2) known as sodium cromoglycate became the prototype of a new class anti-allergic drugs (Cairns et al., 1972).

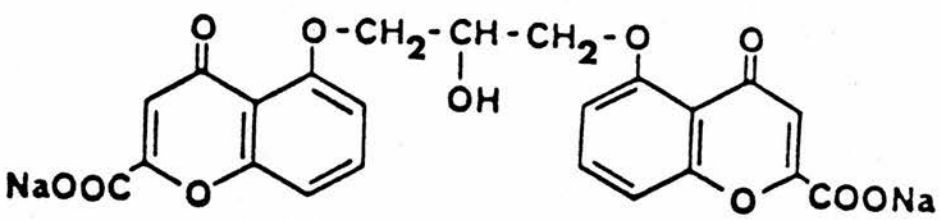
In preliminary studies in man, prophylactic

Figure 7.1. *Ammi visnaga*, an Eastern Mediterannean plant from which khellin is obtained.


Ammi visnaga



KHELLIN.



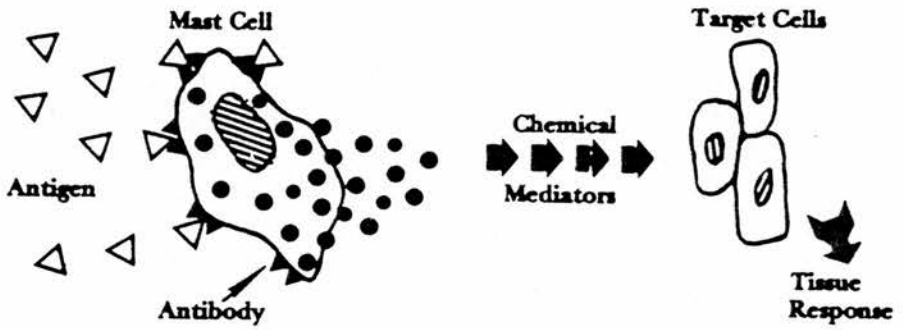
SODIUM CROMOGLYCATE

Figure 7.2. Structural formulae of khellin and sodium cromoglycate.

inhalation treatment with sodium cromoglycate was found to inhibit the bronchospasm which normally occurred in sensitised bronchial asthma affected subjects, following inhalation of the appropriate antigen, although it possessed no direct bronchodilator properties (Altounyan, 1967). This finding led to the widespread use of sodium cromoglycate in the treatment of bronchial asthma and several other allergic diseases including hay fever, gastro-intestinal allergies, ulcerative colitis, chronic proctitis and vernal kerato-conjunctivitis.

The mechanism of action of sodium cromoglycate in bronchial asthma is not completely understood but it is believed to stabilise sensitised pulmonary mast cell membranes. This prevents degranulation of these mast cells after antigen exposure, thus inhibiting the release of pharmacologically active substances (Cox, 1976) (Figure 7.3). These substances including histamine, slow releasing substance of anaphylaxis (SRS-A), serotonin and 5-hydroxytryptamine (5-HT) cause constriction of the airway smooth muscle, inflammation, mucosal secretion and oedema of the airways. The process by which sodium cromoglycate prevents mediator release from the mast cells is incompletely understood. Studies in animals, particularly the rat, have shown that sodium cromoglycate does not appear to affect either fixation of reagenic antibodies to mast cells or the interaction between antigen and

ANTIGEN CHALLENGE WITHOUT PRIOR SODIUM CROMOGLYCATE TREATMENT.



ANTIGEN CHALLENGE FOLLOWING SODIUM CROMOGLYCATE TREATMENT.

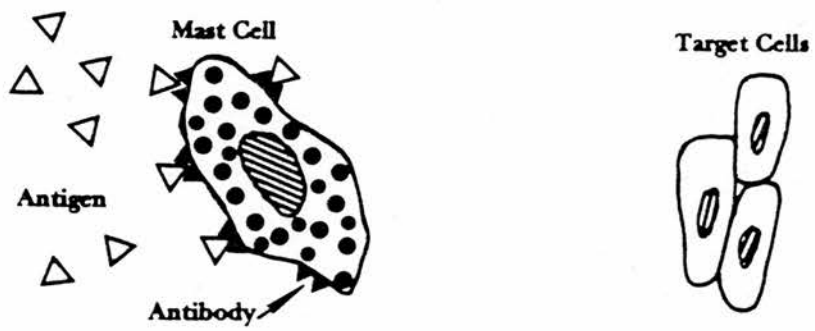


Figure 7.3. Schematic representation of a possible mode of action for sodium cromoglycate (on pulmonary mast cells).

cell-bound antibody (Cox, 1970). However, the biochemical events which occur subsequent to the combination of antigen with cell-bound antibody can be inhibited by sodium cromoglycate. These include a decrease in membrane associated cyclic 3',5'- AMP. This cyclic 3',5'-AMP decrease causes increased mast cell membrane permeability, an influx of calcium into the cell, a decrease in the mast cell cyclic 3',5'-AMP levels followed by mediator release from the mast cell (Church, 1978). Sodium cromoglycate is thought to act by preventing the influx of calcium into the mast cell and thereby halting the biochemical chain of events leading to mediator release (Garland and Mongar, 1974, 1976). Another possible mechanism of action of sodium cromoglycate is that it may cause blockade of airway irritant receptors and this has been demonstrated experimentally in dogs following sodium cromoglycate inhalation (Jackson and Richards, 1977). It has been postulated that these receptors might be involved in the pathogenesis of exercise-induced asthma where mast cell degranulation is not thought to be primarily involved. Sodium cromoglycate is, therefore, not a bronchodilator, a smooth muscle relaxant or anti-inflammatory agent, nor does it antagonise any specific pharmacologically active substance, e.g. histamine.

In the treatment of bronchial asthma, sodium cromoglycate is predominantly used to prevent Type I

(reagin-mediated) allergic lung reactions, however, clinical studies have shown that sodium cromoglycate also protects against the late allergic pulmonary reactions (some possibly of Type III, Arthus reactions) which develop 4 to 6 hours after antigen exposure (Pepys et al., 1968; Laitinen et al., 1978; Booij-Noord, Orie and De Vries, 1971). These late allergic reactions are seen typically in farmer's lung and bird fancier's lung diseases in which extrinsic allergic alveolitis is the important pathological feature. However, late bronchial reactions in man (which occur without many of the features of alveolitis) caused by inhalation of house dust, are also inhibited by sodium cromoglycate (Booij-Noord et al., 1972).

Horses affected with COPD frequently show respiratory hypersensitivity to mycological antigens in stable dust and forage or to pollens. COPD signs may be exacerbated as early as 30 minutes after antigen exposure (Eyre, 1972) or more typically as a delayed response, 4 to 8 hours after challenge (McPherson et al., 1979b). The pathogenesis of equine COPD is not completely understood but airway spasm is known to be involved as well as a widespread exudative bronchiolitis, as was shown in chapter 5. The role of mast cells in this equine disease has not been established, but pulmonary mast cell hyperplasia has been noted in COPD affected horses (Nicholls, 1978). If mast cell

degranulation is involved in the pathogenesis of COPD, it is possible that prophylactic treatment with sodium cromoglycate prior to antigen challenge could prevent the onset or exacerbation of the disease.

This chapter describes some preliminary studies on the effects of prophylactic sodium cromoglycate administration to COPD affected horses.

MATERIALS AND METHODS.

HORSES.

Two horses (horses Nos. B111 and B112, Appendix 3.2) were diagnosed as being affected with COPD according to the criteria of McPherson *et al.*, (1978). Horse No. B111 showed respiratory hypersensitivity to *M. faeni* and *A. fumigatus*, whilst horse No. B112 was hypersensitive to *M. faeni* only. The horses were housed in the controlled environment (p. 57) during the trials.

SODIUM CROMOGLYCATE TREATMENT.

Sodium cromoglycate B.P. 1% w/v solution (Intal, Fisons Ltd., Loughborough) was aerosolised using a Wright's nebuliser and administered via a mask which covered the horse's mouth and nose (p. 57). 80 mg sodium cromoglycate was administered as a single dose.

ANTIGEN INHALATION CHALLENGE.

Prior to antigen challenge, the horses' max. Δ Ppl, PaO_2 and clinical state were determined as previously

described (pp.49,54). M. faeni and A. fumigatus antigens were prepared and administered as described on p. 57. Clinical observations were made hourly thereafter and the max. Δ Ppl and PaO_2 were re-measured five hours after antigen inhalation. The following max. Δ Ppl values were taken as indicating a positive response; where the pre-exposure max. Δ Ppl value was less than 6 mm Hg and increased after antigen challenge to greater than or equal to 6 mm Hg (the value delineating affected animals by McPherson et al., 1978) or where the pre-exposure max. ΔPpl was already greater than 6 mm Hg, an increase of 15% in this figure was considered to indicate a positive reaction (McPherson et al., 1979b).

TRIALS.

7.A. Antigen challenge, no drug treatment.

To establish the mean response of each horse to antigen inhalation, both horses were challenged with M. faeni on three occasions. Horse No. B111 was also challenged with A. fumigatus on three occasions.

7.B. A single M. faeni challenge following sodium cromoglycate treatment.

80 mg sodium cromoglycate was administered 20 to 30 minutes prior to the M. faeni challenge of both horses on three occasions and the response monitored at 5 hours after challenge as previously described.

7.C. Repeated M. faeni challenge following sodium cromoglycate treatment.

The duration of protection provided by a single sodium cromoglycate treatment in the affected horses against repeated M. faeni challenge was tested as follows:

(i). The horses were treated with sodium cromoglycate and challenged as in trial 7.B and thereafter were challenged daily with M. faeni until a positive response was recorded.

(ii). Horses received sodium cromoglycate treatment on day 1 and were challenged with M. faeni on days 2 and 4.

(iii). Antigen challenge was applied only at the end of the protective period as established in (i), on day 4 for horse No. B111 and days 4 and 5 for horse No. B112.

7.D. Multiple antigen challenge following sodium cromoglycate treatment.

The effects of multiple antigen challenge on the duration of protection provided by a single sodium cromoglycate treatment was tested in horse No. B111, using M. faeni and A. fumigatus challenge on alternate days.

(i). Sodium cromoglycate 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.

(ii). The trial took the same form as (i) except that A. fumigatus was administered on days 1 and 3 and M. faeni on day 2.

RESULTS

7.A. Antigen challenge, no drug treatment.

The effects of M. faeni and A. fumigatus inhalation challenge (on max. ΔPpl and PaO_2) on horses Nos. B111 and B112 are shown in Table 7.1. The horses were not always in an identical disease state at the time of antigen challenge and this accounts for the wide range in max. ΔPpl values for horse B111 before and after A. fumigatus challenge. There was a mean max. ΔPpl increase of 7 mm Hg in horse No. B111 five hours after M. faeni challenge, 8 mm Hg in the same animal after A. fumigatus challenge and 8 mm Hg in horse No. B112 after M. faeni challenge. These increases all constituted positive responses, being well in excess of the required 15% increase. Examples of max. ΔPpl recordings from horse No. B111 before and after M. faeni inhalation challenge are shown in Figure 7.4.

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

TABLE 7.1.

Max. Δ Ppl and PaO_2 values (mean values $\pm$ S.D.) recorded before and 5 hours after *M. faeni* and *A. fumigatus* inhalation challenge in 2 COPD affected horses.

Horse No.		B111	B111	B112
Antigen		*M. faeni*	*A. fumigatus*	*M. faeni*
No. of exposures		3	3	3
Rest	Max. Δ Ppl (mm Hg)	6.83 $\pm$ 2.84	8.00 $\pm$ 4.50	4.67 $\pm$ 1.16
	PaO_2 (mm Hg)	82.93 $\pm$ 2.15	77.70 $\pm$ 4.29	84.87 $\pm$ 5.26
5 hours	Max. Δ Ppl (mm Hg)	13.67 $\pm$ 2.30	16.00 $\pm$ 4.27	12.50 $\pm$ 2.00
	PaO_2 (mm Hg)	76.80 $\pm$ 4.25	74.47 $\pm$ 9.17	80.23 $\pm$ 2.15
$\bar{d}$	Max. Δ Ppl (mm Hg)	7.17 $\pm$ 1.15	7.67 $\pm$ 0.29	7.83 $\pm$ 0.76
	PaO_2 (mm Hg)	-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.

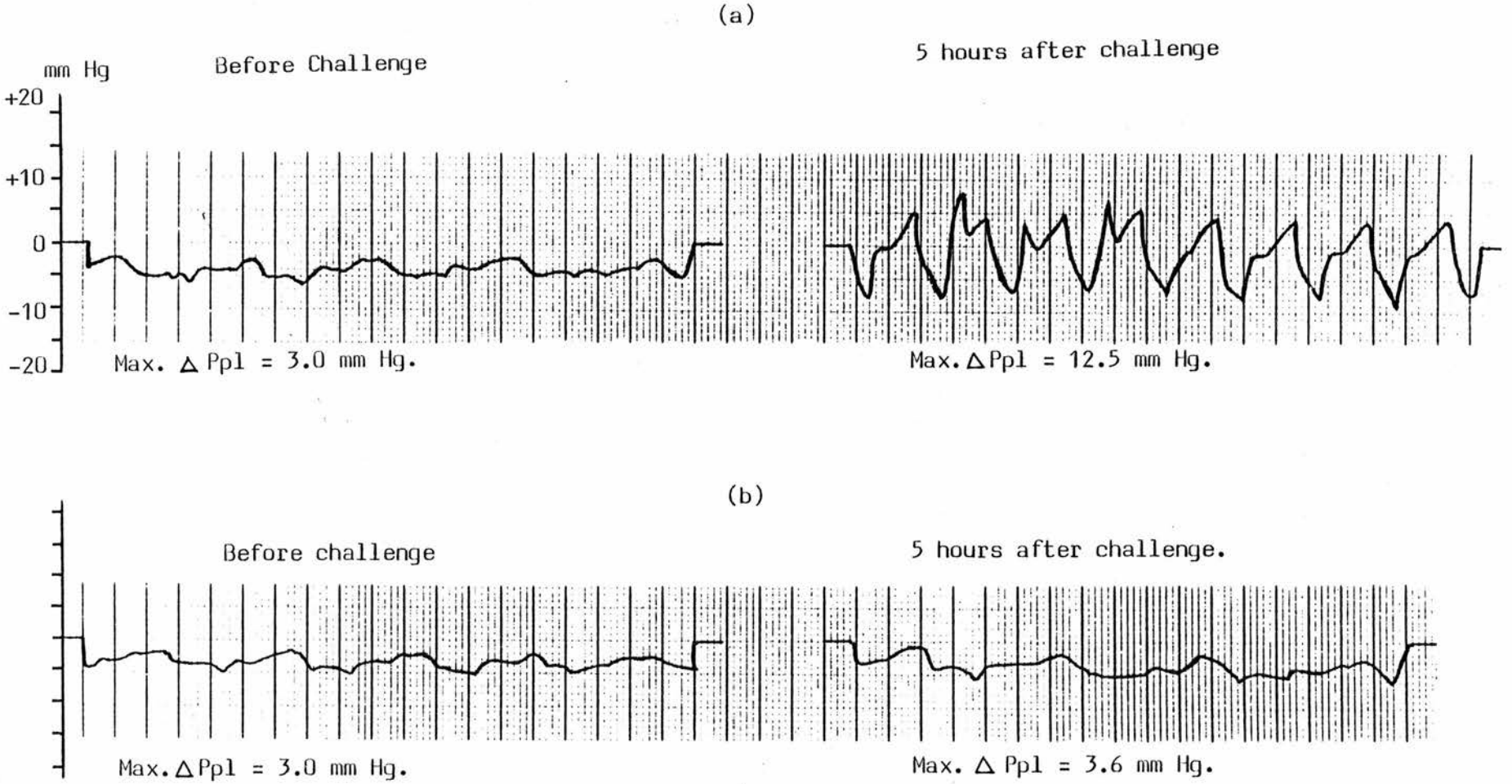


Figure 7.4. Max. ΔPpl recordings from a COPD affected horses (B111) before and 5 hours after M. faeni inhalation challenge. (a) no prophylactic sodium cromoglycate treatment. (b) with prophylactic sodium cromoglycate treatment.

7.B. A single M. faeni challenge folowing sodium cromoglycate treatment.

Sodium cromoglycate inhalation did not induce any clinical, max. ΔPpl or PaO_2 changes in either animal with 30 minutes of treatment. Table 7.2 shows the mean max. ΔPpl and PaO_2 values at rest (after sodium cromoglycate 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 7.1, little change was recorded in max. ΔPpl values in either horse, with the mean values at 5 hours after challenge being less than 6 mm Hg. 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. Examples of max. ΔPpl recordings from horse No. B111 which had been prophylactically treated with sodium cromoglycate before M. faeni inhalation challenge are shown in Figure 7.4.

When symptomatic at the commencement of the trial, horse No. B111 showed no further increase in max. ΔPpl after antigen challenge and a PaO_2 decrease of 4.2 mm Hg, whilst in horse No. B112 max. ΔPpl increased from 6 mm Hg to 7 mm Hg with a PaO_2 increase of 3.2 mm Hg.

7.C. Repeated M. faeni challenge following sodium cromoglycate treatment.

(i). Horses Nos. B111 and B112, challenged daily

TABLE 7.2.

Max.ΔPpl and PaO_2 values (mean values $\pm$ S.D.) recorded before and 5 hours after *M. faeni* inhalation challenge in 2 COPD affected horses prophylactically treated with sodium cromoglycate.

Horse No.		B111	B112
No. of exposures		3	3
Rest	Max. Δ Ppl (mm Hg)	4.33 $\pm$ 1.89	4.67 $\pm$ 1.61
	PaO_2 (mm Hg)	81.97 $\pm$ 3.61	85.43 $\pm$ 2.64
5 hours post challenge	Max. Δ Ppl (mm Hg)	4.66 $\pm$ 2.08	4.50 $\pm$ 1.50
	PaO_2 (mm Hg)	85.30 $\pm$ 4.85	86.90 $\pm$ 4.35
$\bar{d}$	Max. Δ Ppl (mm Hg)	0.33 $\pm$ 0.29	-0.17 $\pm$ 0.58
	PaO_2 (mm Hg)	3.33 $\pm$ 5.12	1.47 $\pm$ 3.92

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

with M. faeni, did not show positive responses to antigen challenge until days 4 and 5 respectively after sodium cromoglycate treatment. Table 7.3 shows the daily pre- and post-challenge max. Δ Ppl and PaO_2 values. In horse No. B111 on day 5, the pre-exposure max. ΔPpl value was elevated, PaO_2 depressed and the horse was showing clinical signs of COPD. This was possibly as a result of the positive response to challenge on day 4. This reaction was intensified by antigen challenge on day 5.

(ii). When the frequency of M. faeni inhalation challenge was reduced to days 2 and 4 after sodium cromoglycate treatment, both horses showed positive responses to challenge on day 4.

(iii). A single antigen challenge on day 4 after sodium cromoglycate treatment induced a positive response in horses No. B111 but not in horse No. B112. A second challenge on day 5 produced a positive response in horse No. B112.

7.D. Multiple antigen challenge following sodium cromoglycate treatment.

The duration of protection after sodium cromoglycate treatment in horse No. B111 was shorter in the face of multiple antigen challenge. In parts (i) and (ii) this animal showed an increase in max. ΔPpl of 2 and 2.5 mm Hg respectively on day 2. However, the post-challenge value on this day was less than 6 mm Hg. On day 3, max. ΔPpl

TABLE 7.3.

Max.ΔPpl and PaO_2 values in 2 COPD affected horses initially treated with sodium cromoglycate followed by daily M. faeni inhalation challenge. Mean values (± S.D.) recorded before and 5 hours after M. faeni challenge on days 1 to 5.

Horse No.			B111	B112
No. of exposures			5	5
Rest	Max. Δ Ppl (mm Hg)	Days 1-3	3.67 ± 1.55	3.50 ± 0.5
		Day 4	3.5	3.5
		Day 5	9.0	4.0
	PaO_2 (mm Hg)	Days 1-3	85.50 ± 1.55	87.37 ± 1.07
		Day 4	86.7	88.9
		Day 5	72.4	84.9
5 hours post-challenge	Max. Δ Ppl (mm Hg)	Days 1-3	3.83 ± 0.58	3.16 ± 0.29
		Day 4	10.0	4.5
		Day 5	14.5	9.5
	PaO_2 (mm Hg)	Days 1-3	85.77 ± 3.93	89.27 ± 3.89
		Day 4	80.4	90.4
		Day 5	70.6	80.6

increased by 5.5 and 7.5 mm Hg respectively and the horse 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 sodium cromoglycate. This protection was most efficient when horses were asymptomatic, but when horses were symptomatic sodium cromoglycate prevented the normally observed intensification of the disease after challenge. A single sodium cromoglycate inhalation in symptomatically affected animals did not produce any apparent clinical improvement, this being similar to the findings in bronchial asthma in man where sodium cromoglycate is of no value in the symptomatic treatment of the disease (Cox, 1970).

The studies showed that the duration of protection of COPD affected horses after a single sodium cromoglycate dose was much longer than that for human bronchial asthma patients where sodium cromoglycate inhibits antigen-induced bronchoconstriction for less than 24 hours (Kolotkin, Lee and Townley, 1973). The reason for the prolonged protection in horse may be due to differences in the pathogenesis of these two diseases or to interspecies differences in, for instance, the metabolism and excretion of the drug or mast cell

longivity, between the horse and man. The half life of sodium cromoglycate in the horse has not been established, but in man, it varies from 45 to 90 minutes (Cox, 1970).

The duration of sodium cromoglycate 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 therefore similar to that in bronchial asthma patients where protection occurs for up to 24 hours when the same antigen is used sequentially but not when a dissimilar antigen is introduced 5 hours after sodium cromoglycate treatment (Kolotkin, Lee and Townley, 1973).

Although these trials were performed on only two horses, they suggest that sodium cromoglycate is effective in controlling COPD in horses. Consequently, a clinical trial to evaluate the efficacy of sodium cromoglycate on a larger number of affected horses is described in the following chapter (chapter 8).

CONCLUSIONS.

These trials showed that prophylactic treatment of COPD affected horses with sodium cromoglycate prevents the exacerbation of respiratory disease, normally observed at 4 to 8 hours after inhalation challenge with an appropriate antigen. The duration of

protection against antigen challenge after a single sodium cromoglycate treatment is approximately 4 to 5 days and protection does not appear to be prolonged by reducing the frequency of antigen challenge. However, multiple antigen challenge appears to shorten the protective period.

CHAPTER 8.

THE PROPHYLACTIC EFFECTS OF REPEATED SODIUM CROMOGLYCATE TREATMENT ON COPD AFFECTED HORSES.

INTRODUCTION.

In the preliminary studies (chapter 7) on 2 COPD affected horses with respiratory hypersensitivity to M. faeni, sodium cromoglycate inhalation 20-30 minutes prior to artificial antigen inhalation challenge, prevented the exacerbation of respiratory disease normally observed 4 to 8 hours after challenge. Following a single sodium cromoglycate treatment, this protection lasted for 4 to 5 days. However, the horses, being in a controlled environment, were only exposed to daily artificial challenge with a single antigen and not continuously exposed to a range of aetiological allergens as they could be if housed in the natural challenge environment.

This chapter describes a clinical trial to evaluate the prophylactic effects of sodium cromoglycate treatment on a greater number of COPD affected horses continuously stabled in the natural challenge environment. The effects of repeated sodium cromoglycate administration on the duration of the protective period in COPD affected horses was also examined.

MATERIALS AND METHODS.

HORSES.

56 confirmed COPD cases (horses Nos. B109-164, Appendix 3.2), were divided into 4 groups, each group receiving a different number of sodium cromoglycate

treatments. Their breed/type, age and duration of illness prior to the trial are presented in Table 8.1.

TRIAL PROTOCOL.

8.A. Natural challenge environment, no drug treatment.

Initially, the COPD affected horses were housed in the natural challenge environment (p. 55) for 4 days to establish that this environment induced an exacerbation of COPD signs. Respiratory function parameters including respiratory rate, max. ΔPpl, PaO_2 and $PaCO_2$ were measured before and after 24 hours in this environment and horses received daily clinical examinations during this phase.

8.B. Controlled environment, no drug treatment.

The horses were then housed in the controlled environment (p, 57) until clinical signs abated and their max. ΔPpl and PaO_2 levels were within normal ranges.

8.C. Sodium cromoglycate treatment, (in controlled environment).

As soon as the horse became asymptomatic in the controlled environment, they were given 80 mg sodium cromoglycate B.P. 1% W/V solution (Cromovet, Fisons Ltd., Loughborough) nebulised into a face mask. Two types of nebulisation apparatus were used, namely, the Wright's nebuliser and face mask previously described for antigen inhalation challenge (p. 57) or a Pulmo-Aide, series 561 Portable Compressor (Devilbiss,

TABLE 8.1.

The breed/type, age and duration of illness of COPD affected horses treated with sodium cromoglycate.

Group Number	Number of days treat-ment	Number and Identification of horses	BREED/TYPE			Age (mean ± S.D. in years)	Duration of illness (mean ± S.D. in months)
			Thoroughbred	Hunter	Pony		
1	1	9 (Horses* B109 - 117)	4	4	1	9.33 ± 3.20	22.11 ± 17.57
2	2	23 (Horses* B118 - 140)	10	7	6	10.65 ± 5.27	22.17 ± 25.56
3	3	16 (Horses * B141 - 156)	7	6	3	8.56 ± 2.96	14.50 ± 12.03
4	4	8 (Horses * B157 - 164)	1	6	1	13.25 ± 4.40	40.50 ± 18.07

* Appendix 3.2.

Pennsylvania) along with a Cromovet nebuliser and face mask (Fisons Ltd., Loughborough) (p. 59). Both systems are alleged to produce a droplet size of less than 8 microns. Sodium cromoglycate treatment of COPD affected horses using both systems had been shown by previous pilot experiments, to be equally effective.

Sodium cromoglycate was administered daily for 1, 2, 3, or 4 successive days to 9, 23, 16 and 8 horses respectively (Groups 1, 2, 3 and 4; Table 8.1).

8.D. Natural challenge environment after sodium cromoglycate treatment.

On completion of the sodium cromoglycate administration, horses were immediately returned to the natural challenge environment and were subjected to daily clinical examinations with particular attention paid to the presence and degree of double expiratory effort, coughing, respiratory rate and chest sounds. Max. Δ Ppl, PaO_2 and $PaCO_2$ levels were monitored as previously described (pp.49, 54) at 2 and 3 day intervals.

8.E. End of the trial.

The end of the protective period (induced by sodium cromoglycate treatment) was established by both clinical assessment and when values for max. ΔPpl and PaO_2 became abnormal i.e. max. ΔPpl > 6 mm Hg and PaO_2 82 < mm Hg.

STATISTICAL ANALYSIS OF RESULTS.

Within each group of horses, values for respiratory rate, max. Δ Ppl, PaO_2 and $PaCO_2$ recorded during the 4 phases of this trial (i.e. 8.A. Challenge environment, no drug treatment, 8.B. Controlled environment, no drug treatment, 8.D. Challenge environment after sodium cromoglycate treatment, 8.E. End of the trial) were individually compared using the paired Student's t-test.

The correlation between the number of sodium cromoglycate treatments and the duration of the protective period after treatment was calculated by regression analysis (Snedecor and Cochran, 1967). The significance of the correlation between the duration of the protective period after treatment and age, bodyweight, duration of illness, max. ΔPpl, PaO_2 and $PaCO_2$ values for untreated symptomatic horses, was tested by regression analysis. These correlations were tested within each group and on the massed data from the four groups.

RESULTS.

8.A. Natural challenge environment, no drug treatment.

Exposure to the natural challenge environment (without sodium cromoglycate treatment) induced clinical signs of COPD in all horses after 24 hours. Mean

values for max. Δ Ppl and blood gases also showed that the horses became symptomatic (Table 8.2). Horses remained symptomatic throughout the 4 days that they remained in this environment.

8.B. Controlled environment, no drug treatment.

When placed in the controlled environment, COPD affected horses became asymptomatic in 4 to 32 days (mean ($\pm$ S.D.) 9.1 $\pm$ 4.9 days). Mean values for respiratory rate, max. ΔPpl and blood gases in asymptomatic horses are shown in Table 8.2.

Compared to the previous phase (8.A), the decreases in respiratory rate and max. ΔPpl and increase in PaO_2 were highly significant ($P < 0.001$) in all groups. A significant decrease ($P < 0.05$) in $PaCO_2$ was recorded in all groups.

8.D. Natural challenge environment after sodium cromoglycate treatment.

Sodium cromoglycate treatment prior to natural antigen challenge temporarily prevented an exacerbation of COPD in the affected horses. The duration of this protective period ranged from a mean of 3.6 days after a single day's treatment to a mean of 24.3 days after treatment on 4 successive days (Figure 8.1). The correlation between the number of sodium cromoglycate treatments and the duration of the protective period was highly significant; correlation coefficient r (54) = +0.709 ($P < 0.001$). The line of best fit was linear (Figure 8.1).

TABLE 8.2.

Respiratory rate, max. Δ Ppl and arterial blood gas values in COPD affected horses before and after 1 to 4 days inhaled sodium cromoglycate treatment.

Number of days treatment (Group Number)	Number of horses	Challenge environment no drug treatment.				Controlled environment no drug treatment.				Challenge environment after sodium cromoglycate treatment.				End of trial.			
		Respiratory Rate (per min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	Respiratory Rate (per min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	Respiratory Rate (per min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	Respiratory Rate (per min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)
1	9	16.1 ± 2.6	10.4 ± 3.1	72.8 ± 6.0	39.9 ± 1.9	10.3 ± 1.8	3.6 ± 0.4	89.0 ± 2.7	36.5 ± 2.2	11.2 ± 1.2	3.9 ± 0.4	85.4 ± 2.3	37.8 ± 1.3	13.2 ± 1.7	6.8 ± 1.1	79.0 ± 3.7	39.1 ± 1.9
2	23	15.6 ± 2.6	12.3 ± 6.3	69.9 ± 8.1	39.0 ± 3.8	9.8 ± 1.6	3.5 ± 0.5	88.7 ± 2.5	36.0 ± 1.8	10.2 ± 0.9	3.9 ± 0.4	88.0 ± 2.7	36.5 ± 1.2	12.6 ± 1.0	7.7 ±1.8	76.2 ± 5.9	38.6 ± 2.1
3	16	15.4 ± 2.7	12.9 ± 6.4	70.1 ± 7.6	40.0 ± 4.5	10.7 ± 1.4	3.7 ± 0.6	88.7 ± 3.3	37.1 ± 2.8	10.5 ± 0.9	3.8 ± 0.4	87.0 ± 2.2	37.2 ± 1.1	13.1 ± 1.8	7.3 ± 1.4	75.9 ± 7.5	38.0 ± 1.7
4	8	16.4 ± 3.8	15.0 ± 5.8	69.9 ± 4.9	38.5 ± 1.4	11.8 ± 0.7	3.9 ± 0.7	89.5 ± 2.4	35.7 ± 1.8	11.8 ± 1.2	4.0 ± 0.6	84.8 ± 1.9	36.2 ± 1.7	12.5 ± 3.2	8.3 ± 2.0	79.6 ± 3.9	36.3 ± 3.2

⬆

Challenge environment, no drug treatment - Mean values recorded after 24 hours in challenge environment.

Controlled environment, no drug treatment - Mean values recorded when horses were clinically asymptomatic.

Challenge environment after sodium cromoglycate treatment - Mean values recorded on a composite of days, at 2 and 3 day intervals throughout each horse's period of protection.

End of trial - Mean values recorded at the end of the sodium cromoglycate protective period.

⬆ = Sodium cromoglycate inhalation.

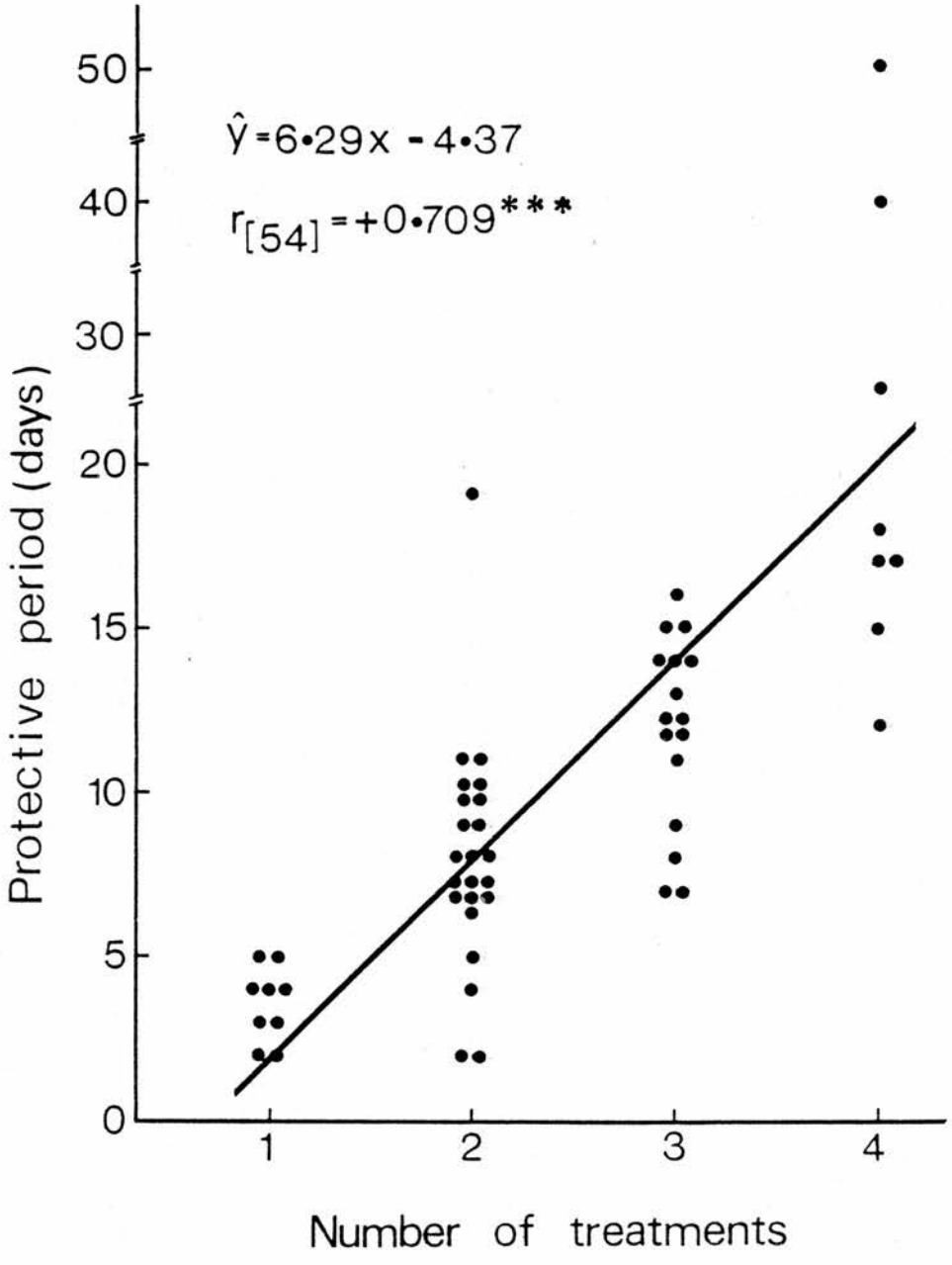


Figure 8.1. The relationship between the number of sodium cromoglycate treatments and the protective period in COPD affected horses housed in the natural challenge environment. (● = values recorded for individual horses, *** = $P < 0.001$).

The mean values for respiratory rate, max. ΔPpl and blood gases recorded during the protective period are shown in Table 8.2. an average of 3 pulmonary function examinations were made from each horse in Group 1, 5 per horse in Group 2, 7 per horse in Group 3 and 12 per horse in Group 4. The values expressed are a mean of these recordings for all horses.

The differences in the horses' respiratory rate, max. Δ Ppl and PaO_2 values between when they were untreated and symptomatically affected (8.A) and during the protective period were highly significant ($P < 0.001$) whereas the decrease in $PaCO_2$ was less significant ($P < 0.05$) (Table 8.2).

Comparison of the values recorded for these parameters when the horses were asymptomatic in the controlled environment (8.B) and during the protective period showed no significant differences ($P > 0.05$) in max. ΔPpl, respiratory rate or $PaCO_2$ between the two phases. In groups 2 and 3, there were no significant PaO_2 differences ($P > 0.05$) between these same two phases but PaO_2 values in Groups 1 and 4 were significantly lower ($P < 0.01$) during the protective period as compared to the asymptomatic period in the controlled environment (8.B).

There were no significant correlations between the duration of the protective period after sodium cromoglycate treatment and the horses' age, bodyweight, duration of illness, symptomatic max. ΔPpl, PaO_2 and

$PaCO_2$ values or the time required to become asymptomatic in the controlled environment, either within each group or on the massed data from the 4 groups. The correlation coefficients from the massed data are shown in Table 8.3.

8.E. End of the trial.

Towards the end of the protective period, faint, harsh respiratory sounds were auscultated and these became louder over the following 1 to 3 days. This was accompanied by a slight double expiratory effort which became increasingly more pronounced and, in some cases, an increase in respiratory rate and coughing. Mean values for respiratory rate, max. ΔPpl and blood gases when symptoms became re-established at the end of the protective period are shown in Table 8.2. The increase in max. ΔPpl and decrease in PaO_2 were significant ($P < 0.001$ and $P < 0.01$ respectively) compared to values recorded during the protective period (8.D) but there were no significant changes in respiratory rate or $PaCO_2$ values.

No untoward side effects were observed in any of the horses following sodium cromoglycate treatment.

The individual values for respiratory rate, max. Δ Ppl, PaO_2 and $PaCO_2$ recorded from the COPD affected horses during the trial as well as duration of the protective period are presented in Appendices 8.1 to 8.4.

TABLE 8.3.

Statistical correlation of the duration of protection in COPD affected horses after sodium cromoglycate treatment, with their age, bodyweight, symptomatic max. ΔPpl and PaO_2 values, duration of illness and remission time (time taken to become asymptomatic in the controlled environment).

FACTOR	CORRELATION COEFFICIENT Ø	SIGNIFICANCE
Age	r(54)=0.226	$P > 0.05^{NS}$
Bodyweight	r(54)=0.004	$P > 0.05^{NS}$
Symptomatic max. ΔPpl	r(54)=-0.101	$P > 0.05^{NS}$
Symptomatic PaO_2	r(54)=0.220	$P > 0.05^{NS}$
Duration of illness	r(54)=0.010	$P > 0.05^{NS}$
Remission time	r(54)=-0.183	$P > 0.05^{NS}$

Ø The correlation coefficients are from the massed data, from Groups 1, 2, 3 and 4.

NS = not significant.

DISCUSSION

From this study, it is apparent that sodium cromoglycate administration to asymptomatic COPD affected horses is effective as a short-term prophylactic measure against exposure to the natural challenge environment. Each horse acted as its own control by virtue of its previous positive response to the natural antigen challenge.

The protective period afforded to COPD affected horses by sodium cromoglycate depended most on the number of successive days treatment with this drug prior to antigen challenge. The protective period of 3.6 $\pm$ 1.1 days (mean $\pm$ S.D.) after a single sodium cromoglycate inhalation is similar to the protective period recorded in chapter 7 where 2 horses failed to respond to M. faeni inhalation challenge for 4 and 5 days after a single sodium cromoglycate treatment. With increasing daily sodium cromoglycate inhalations, the protective period increased linearly. The protective period was 8.0 $\pm$ 3.4 days (mean $\pm$ S.D.) after 2 days treatment, 11.9 $\pm$ 2.9 days after 3 days treatment and 24.3 $\pm$ 13.4 days after 4 days treatment.

There was a wide range in the duration of the protective period from all 4 treatment schedules, particularly after the 2 days treatment and 4 days treatment when the respective protective periods ranged from 2 to 19 days and 12 to 50 days (Figure 8.1, Appendices 8.2 and 8.4). Statistical analysis showed

no significant correlations between the duration of the protective period and the parameters shown in Table 8.3 i.e.: age, duration of illness, symptomatic max. Δ Ppl and PaO_2. As the challenge conditions were similar for all horses, these differences in the duration of the protective period are difficult to explain.

During the protective period (8.D), values for the horses' respiratory function parameters did not differ significantly from those when the horses were asymptomatic in the controlled environment (8.B) except for PaO_2 in Groups 1 and 4. In these groups several horses became hypoxaemic 1 to 2 days before clinical signs of COPD and max. Δ Ppl increases occurred. These reduced PaO_2 values account for the lower mean PaO_2 values during the protective period in Groups 1 and 4 (Appendices 8.1 and 8.4).

The prolonged periods of protection recorded in many horses after sodium cromoglycate treatment (e.g. 24 days after 4 days treatment) is in contrast to the usual situation in human bronchial asthma patients where sodium cromoglycate inhalation is required 4 times daily in order to maintain adequate protection. However, some workers have demonstrated prolonged prophylactic effects of sodium cromoglycate in a small percentage of bronchial asthma patients. Bernstein et al., (1972), Kennedy (1969) and Silver (1971) all

recorded remission of bronchial asthma in some patients for up to 4 weeks following cessation of treatment. In addition, it has been clinically noted that prolonged sodium cromoglycate therapy in bronchial asthma patients is often associated with periods of almost total remission requiring no therapy or only occasional therapy (Pain, 1973). However, the reason for this prolonged efficiency has not been elucidated. In this respect, Bernstein et al., (1972) suggested that possible non-immunological effects of the drug may be involved as they considered that its effects on immunological reactions mediated by reaginic or Arthus-type antibodies could hardly account for such long-term remission from clinical disease. Altounyan, Cox and Orr (1971) postulated that desensitisation to specific antigens under the protective cover of sodium cromoglycate may also account for the long-term effects of the drug, whilst Kerr, Govindaraj and Patel (1970) suggested that this effect might arise from sodium cromoglycate exerting a specific protective effect upon the bronchial smooth muscle membrane. As yet, there is no experimental evidence to support any of the above hypotheses.

A feature of sodium cromoglycate treatment in bronchial asthma patients is the variability in response to treatment, in both the proportion of patients that respond to treatment and the quality of the observed response. In a review of numerous therapeutic trials,

Brogden, Speight and Avery (1974) calculated that the proportion of asthma patients that responded to long-term sodium cromoglycate treatment varied from 52 to 100%. . These authors suggested that, in general, young patients, extrinsic asthmatics and exercise-induced asthmatics are most likely to respond. A possible explanation for the disparity in response to sodium cromoglycate in bronchial asthma patients is given by Bryant, Burns and Lazarus (1973) who reported that sodium cromoglycate is effective in the majority of patients with IgE mediated reactions whereas it was ineffective in patients with IgG mediated reactions. As it is not yet possible to measure IgE levels in the horse, the relationship between IgE mediated reactions in equine COPD patients and their duration of protection following sodium cromoglycate treatment has not been tested.

COPD affected horses are usually capable of working normally as long as they remain asymptomatic and this can best be achieved by implementing environmental control measures as previously outlined (p. 57). In some situations, this regime is impractical, for instance, during transportation or when horses are moved temporarily away from the home environment, e.g. for competitive events. Even limited antigen exposure may provoke clinical signs of COPD and render the horse incapable of performing to its full potential. In such cases, prophylactic sodium cromoglycate administration may prove beneficial to COPD affected horses

during periods of unavoidable challenge. This drug cannot, however, be used in race horses in Great Britain and Ireland under the current rules of racing.

Ensuring strict environmental control for an individual horse frequently proves difficult in large stables, particularly when horses are kept at livery. Such cases could benefit from long term prophylactic treatment which would avoid having to make special provision for the management of these horses (personal observations). It was postulated that additional courses of sodium cromoglycate treatment administered to COPD affected horses prior to the end of their sodium cromoglycate protective period, might prevent the onset of clinical signs over a more prolonged period than after a single course of treatment. Studies to investigate this are described in the following chapter.

CONCLUSIONS.

Prophylactic treatment of asymptomatic COPD affected horses with inhaled sodium cromoglycate is an effective method of controlling the onset of COPD. A linear response exists between the number of successive days treatment with this drug and the duration of remission of COPD while horses are exposed to natural antigen challenge.

CHAPTER 9.

THE PROPHYLACTIC EFFECTS OF INTERMITTENT SODIUM CROMOGLYCATE TREATMENT ON COPD AFFECTED HORSES.

INTRODUCTION.

In the previous two chapters (chapters 7 and 8), prophylactic sodium cromoglycate administration to asymptomatic COPD affected horses was shown to temporarily prevent the onset of COPD after exposure of susceptible horses to the aetiological antigens.

This chapter describes studies to assess whether the protective period afforded to COPD affected horses by sodium cromoglycate can be prolonged by repeating treatment whilst horses are housed in the challenge environment, prior to the anticipated reappearance of COPD signs.

MATERIALS AND METHODS.

HORSES.

Prior to the commencement of the trial, 8 COPD affected horses (horses Nos. B117, B121, B126, B130, B140, B154, B162 and B163, Appendix 3.2) were housed in the controlled environment (p. 57) for 14 days and by the end of this period all horses were asymptomatic.

TRIAL PROTOCOL.

The sequence of the ensuing intermittent sodium cromoglycate treatment trial is shown in Figure 9.1.

9.A. Challenge environment, no drug treatment.

Initially, the COPD affected horses were housed in the natural challenge environment (p. 55) for 28 days without receiving any drug treatment. The

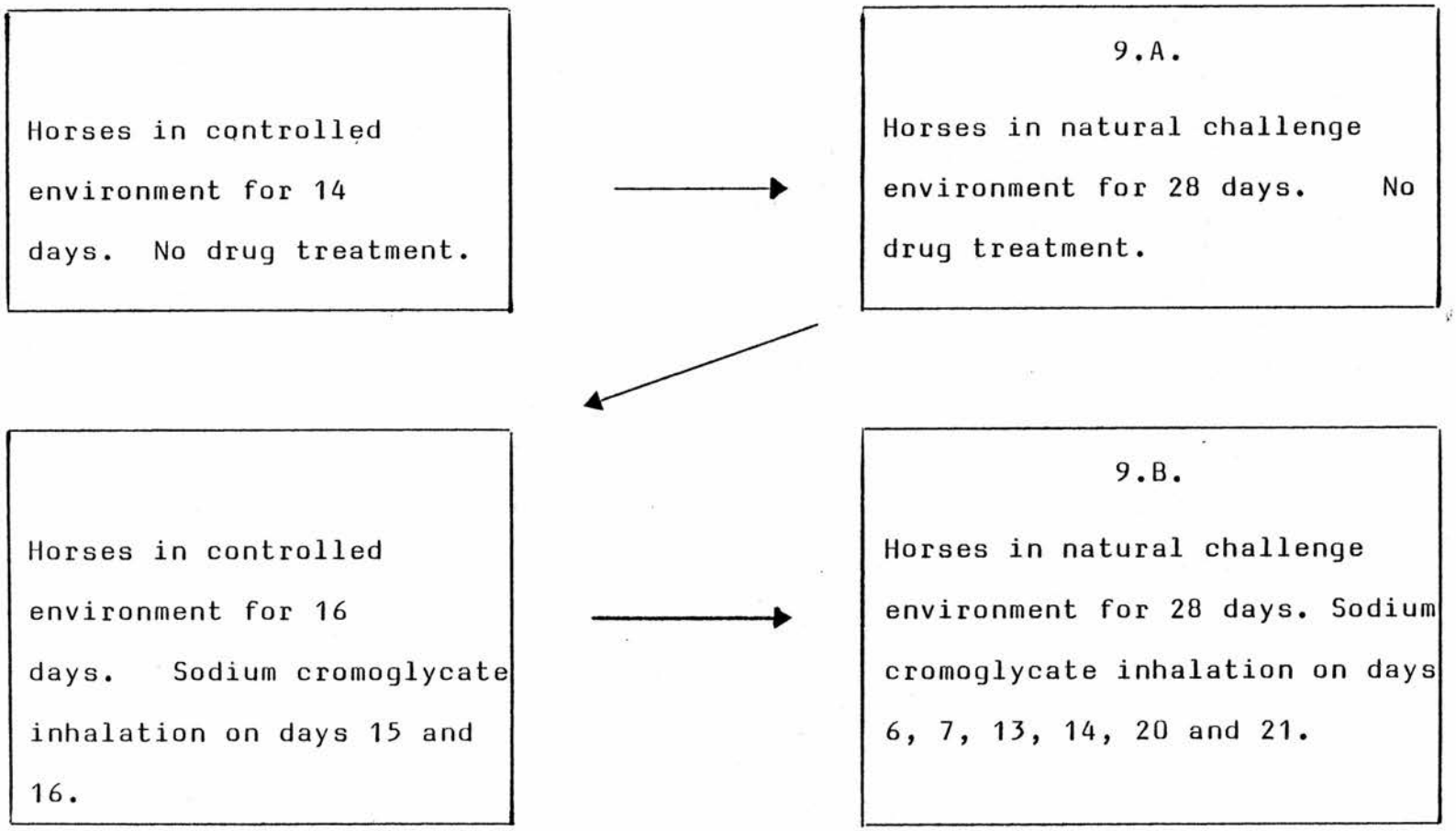


Figure 9.1. Flow diagram of the studies on intermittent sodium cromoglycate inhalation for the therapy of equine COPD.

horses received daily clinical examinations and values for max. Δ Ppl and arterial blood gases were recorded as previously described (pp 49,54) three times weekly throughout this phase of the trial.

On day 28, the horses were returned to the controlled environment for 14 days and by the end of this period they were all asymptomatic. The horses received no drug treatment during this time. On the following 2 days (days 15 and 16) horses were treated with 80 mg sodium cromoglycate by inhalation using the Cromovet inhalation system, as previously described (p. 59). Following treatment on day 16, the horses' max. ΔPpl and arterial blood gas values were monitored and thereafter, horses were returned to the challenge environment.

9.B. Challenge environment and intermittent sodium cromoglycate treatment.

For the ensuing 28 days, the horses were maintained in the challenge environment. 80 mg sodium cromoglycate was administered using the Cromovet inhalation system on days 6, 7, 13, 14, 20 and 21. As in 9.A, the horses received daily clinical examinations and their max. Δ Ppl and arterial blood gas values were recorded three times weekly throughout the 28 days.

STATISTICAL ANALYSIS OF RESULTS.

The Student's t-test as applied to paired observations was used to compare:-

(a) Values for respiratory rate, max. ΔPpl, PaO_2, $PaCO_2$ and arterial pH recorded immediately prior to challenge (on day 0), with values recorded during exposure to the challenge environment in trials 9.A and 9.B.

(b) Values for respiratory rate, max. ΔPpl, PaO_2, $PaCO_2$ and arterial pH recorded during 9.A when horses were untreated, with the corresponding values recorded during 9.B when horses were treated with sodium cromoglycate.

RESULTS.

9.A. Challenge environment, no drug treatment.

All horses were asymptomatic at the start of this phase. The mean ($\pm$ S.D.) values for max. ΔPpl and PaO_2 were 3.74 $\pm$ 0.53 mm Hg and 88.51 $\pm$ 2.98 mm Hg respectively. After being housed in the challenge environment for 1 day, all horses became symptomatically affected with COPD. The increase in mean max. ΔPpl and the decrease in mean PaO_2 values were both highly significant ($P < 0.001$).

The mean max. ΔPpl continued to increase steadily up to 19.20 $\pm$ 5.52 mm Hg (mean $\pm$ S.D.) on day 16 and

remained between 18.01 ± 6.44 mm Hg and 19.6 ± 6.22 mm Hg until the end of the 28 day period (Figure 9.2). The mean respiratory rate was very significantly elevated ($P < 0.001$) above the baseline value on day 2 and remained so throughout the 28 days (Figure 9.3). The mean PaO_2 decreased steadily to 68.40 ± 5.30 mm Hg (mean ± S.D.) on day 16 and remained between 67.89 ± 5.00 mm Hg and 70.54 ± 5.26 mm Hg during the remainder of this phase (Figure 9.4). $PaCO_2$ levels were increased ($P < 0.05$) from day 1 onwards (Figure 9.5) but there was no significant alteration in the pH values.

Marked clinical signs of COPD were present in all horses throughout this period.

9.B. Challenge environment and intermittent sodium cromoglycate treatment.

The horses were once again asymptomatic at the start of this phase and mean values for max. ΔPpl, respiratory rate, PaO_2, $PaCO_2$ and arterial pH were similar to those recorded at the beginning of 9.A. Upon exposure to the challenge environment, most horses showed little clinical alteration in their respiratory signs. Mean (± S.D.) values for max. Δ Ppl, respiratory rate, PaO_2 and $PaCO_2$ recorded during the trial are shown in Figures 9.2, 9.3, 9.4 and 9.5 respectively.

During this period (9.B), no significant differences ($P > 0.05$) were recorded in respiratory rate, $PaCO_2$ or arterial pH from the immediate pre-challenge

= Sodium cromoglycate inhalations.

= Exposure to challenge environment commences.

= Mean ± S.D. values for sodium cromoglycate treated horses.

= Mean ± S.D. values for untreated horses.

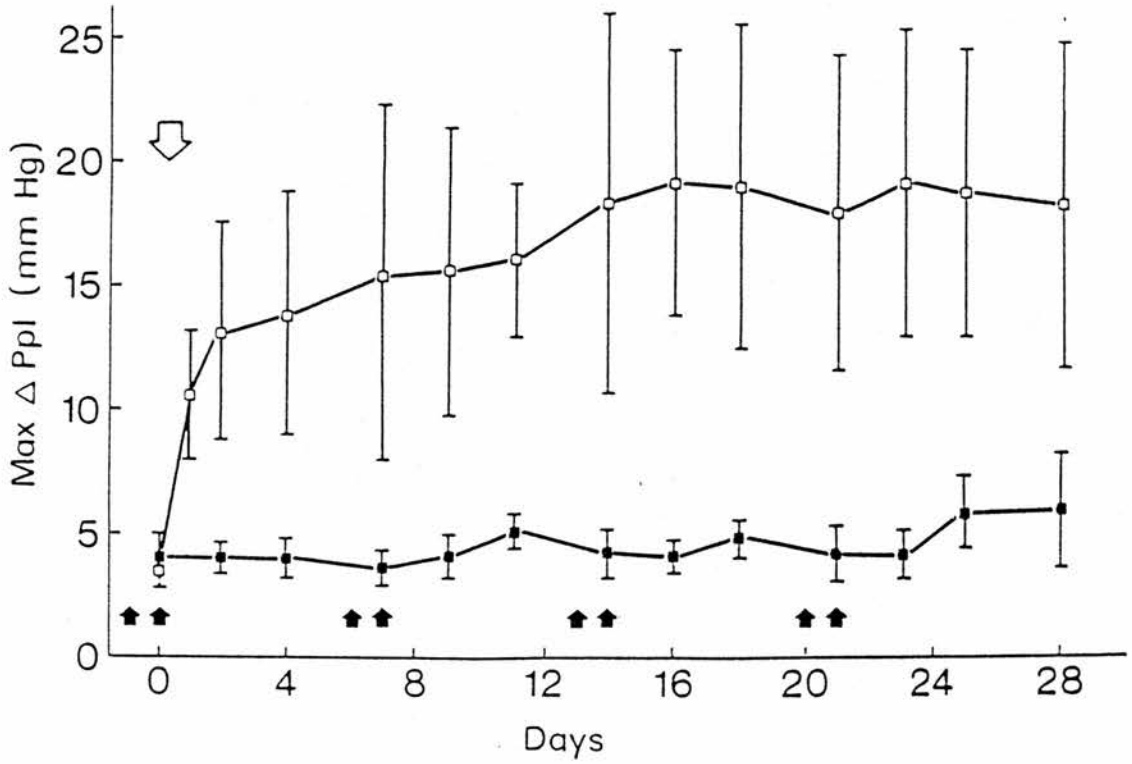


Figure 9.2. Effects of intermittent sodium cromoglycate treatment on max. Δ Ppl in COPD affected horses.

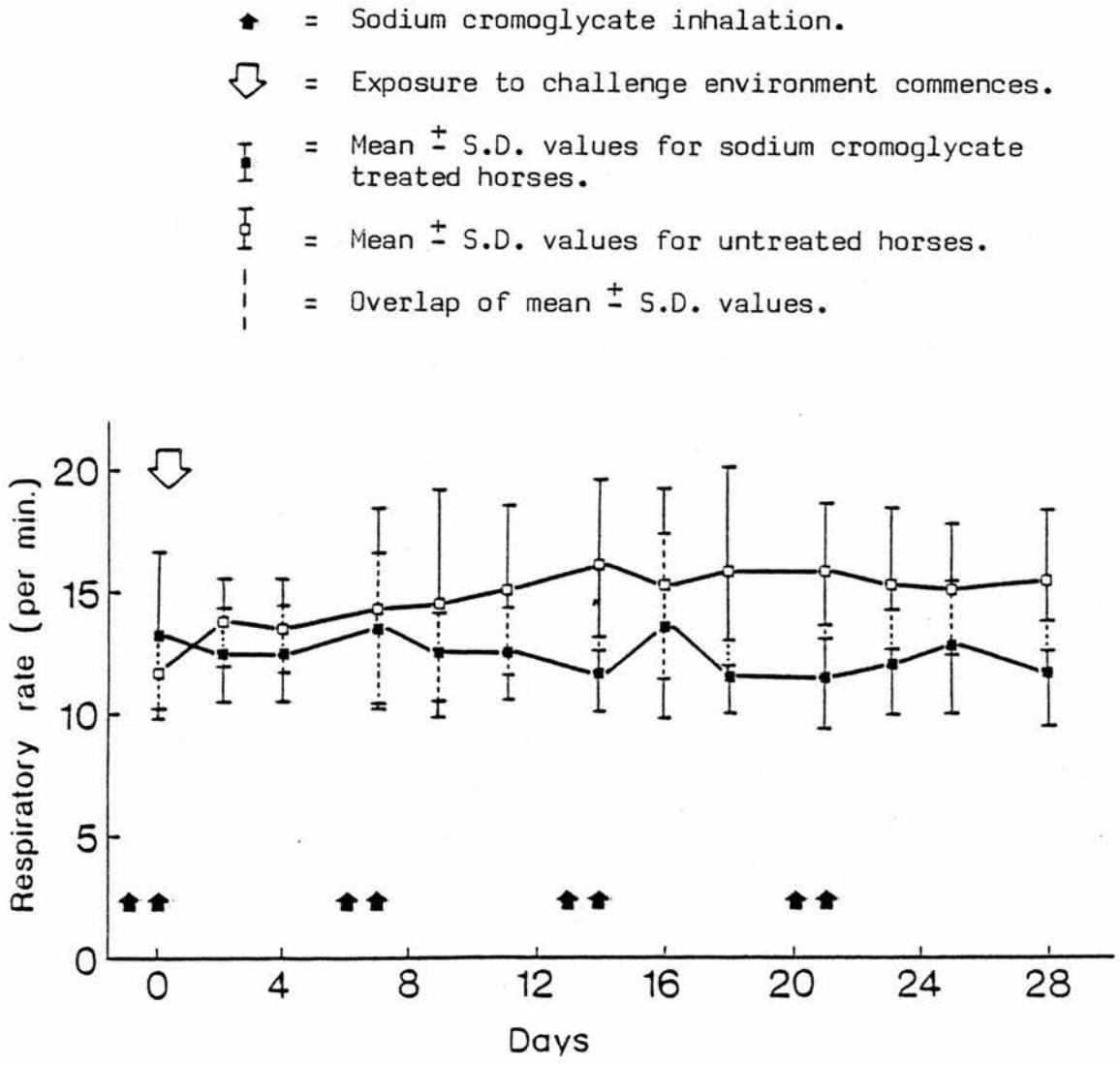


Figure 9.3. Effects of intermittent sodium cromoglycate treatment on respiratory rate in COPD affected horses.

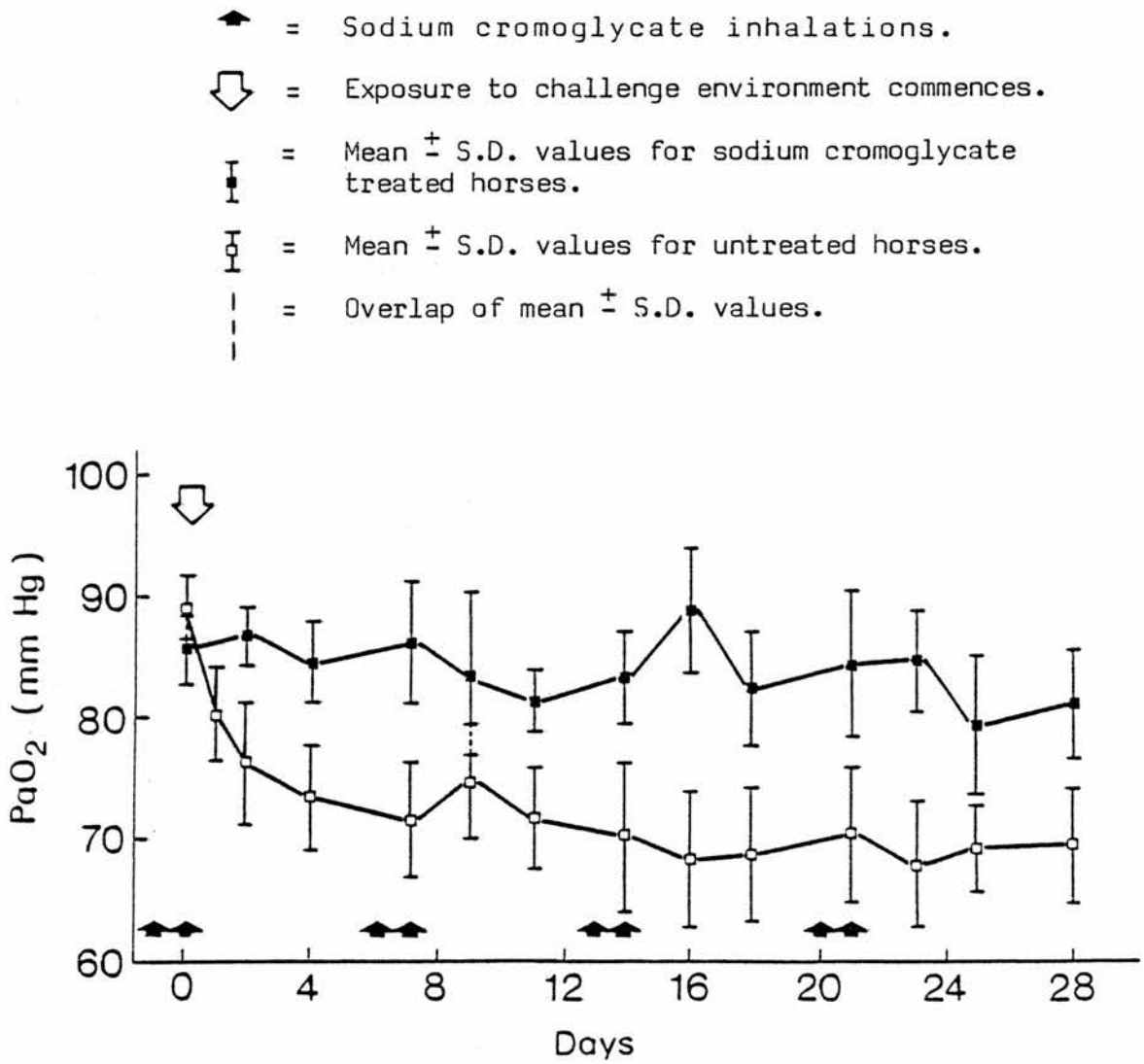


Figure 9.4. Effects of intermittent sodium cromoglycate treatment on PaO_2 in COPD affected horses.

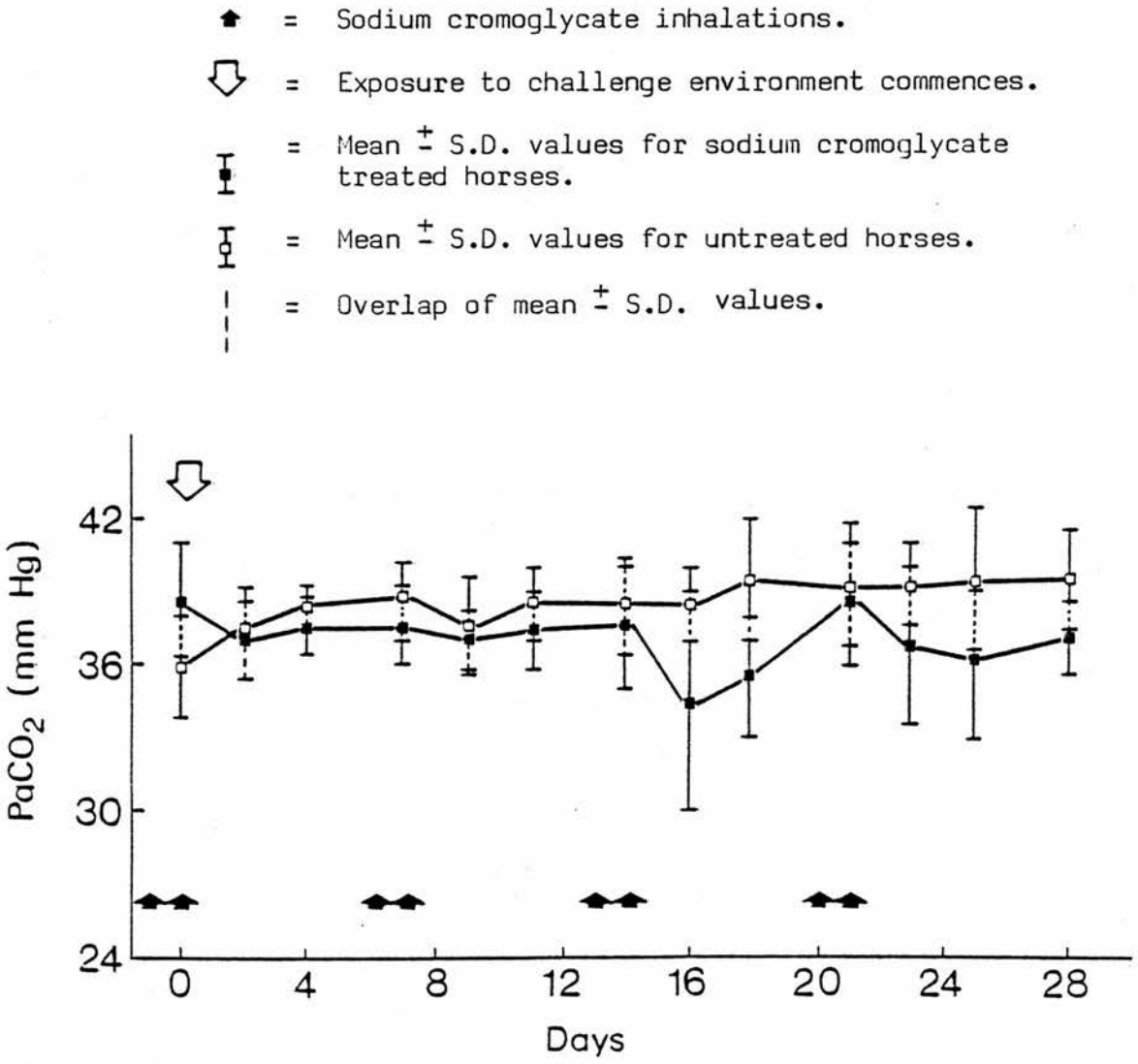


Figure 9.5. Effects of intermittent sodium cromoglycate treatment on $PaCO_2$ in COPD affected horses.

values (recorded on day 0). Max. Δ Ppl values were, however, significantly elevated ($P < 0.05$) above the pre-challenge values on days 11, 25 and 28 while PaO_2 values were significantly decreased ($P < 0.05$) on days 11, 18, 25 and 28 (Appendices 9.1 and 9.2). Despite significant max.ΔPpl increases on certain days, mean max.ΔPpl values did not exceed 6 mm Hg (Figure 9.2).

Throughout this trial, max.ΔPpl and PaO_2 values were significantly ($P < 0.001$) lower and higher respectively than the corresponding values for these horses when untreated (in 9.A). The mean respiratory rate and $PaCO_2$ levels were lower when horses were treated as compared to when they were untreated. The decreases in respiratory rate were significant ($P < 0.05$) in 7 out of 13 pulmonary function examinations. Similarly, decreases in $PaCO_2$ were significant ($P < 0.05$) in 5 out of 13 recordings (Appendices 9.1 and 9.2).

Horses Nos. B117, B126, B130, B154 and B163 remained asymptomatic throughout the 28 days while horse No. B121 was asymptomatic up to day 27. Horses Nos. B140 and B162 showed varying degrees of COPD signs at intervals during this phase. Horse No. B162 had a slight double expiratory effort, slightly increased and harsh inspiratory lung sounds and coughed occasionally on days 14 to 20 and 25 to 28 but was asymptomatic at other times. Horse No. B140 was asymptomatic up to day 21 apart from an occasional cough and slightly increased and harsh

inspiratory lung sounds on isolated days. However, from day 21 to 28, this horse showed a marked double expiratory effort, frequent coughing and increased, harsh inspiratory sounds were present on auscultation.

Individual values for respiratory rate, max. ΔPpl, PaO_2 and $PaCO_2$ recorded from horses during 9.A. and 9.B as well as the results of statistical analyses are presented in Appendices 9.1 and 9.2.

DISCUSSION.

The results of this short-term trial indicate that in the majority of cases, intermittent sodium cromoglycate is effective in preventing the onset of COPD in affected horses continuously exposed to the challenge environment.

In chapter 8, the mean ($\pm$ S.D.) protective period after 2 consecutive days sodium cromoglycate treatment was 8.0 $\pm$ 3.4 days. In this trial, the 2 consecutive days sodium cromoglycate treatment were repeated at 5 day intervals to fall within the previously recorded mean protective period following the 2 days sodium cromoglycate treatment. Increases in max. ΔPpl and decreases in PaO_2 values tended to occur towards the end of the interval between sodium cromoglycate inhalations, i.e. days 11, 18, 25 and 28. This might indicate that

COPD affected horses housed in the natural challenge environment and intermittently treated with sodium cromoglycate may require treatment at shorter intervals than might be expected from the results of chapter 8, i.e. the mean protective period recorded in chapter 8 following 4 consecutive days sodium cromoglycate treatment was 24.3 days. Intermittent 4 day sodium cromoglycate treatment should therefore be administered at shorter time intervals, e.g. 20 or 21 days.

The results of studies into the efficacy of long-term sodium cromoglycate therapy for the control of human bronchial asthma are inconsistent. Some authors have found the efficacy of sodium cromoglycate to decline with prolonged use (Stewart, 1971; Vialatte and Delayeun, 1971) although other reports do not support this (Medical Research Council Collaborative Trial, 1972; Silverman et al., 1972; Hermance and Brown, 1976). In the COPD affected horses, the overall response to sodium cromoglycate treatment did not decline with successive inhalations over the 28 days. However, a more prolonged trial lasting several months would be required to more accurately assess the efficacy of long-term sodium cromoglycate treatment in COPD affected horses.

The individual response to treatment was consistently good in 6 horses but unsatisfactory at times in the remaining 2 (horses Nos. B140 and 162). Horse

No. B140 was a 23 year old mare which showed a very marked response to the challenge environment when untreated (in 9.A), with max. ΔPpl varying between 30 to 35 mm Hg and PaO_2 as low as 58.5 mm Hg (Appendix 9.1). It appears, therefore, that she was extremely sensitive to the natural challenge environment and the protracted exposure to this environment in 9.B might have accounted for the unsatisfactory response to sodium cromoglycate treatment during the last week of the trial. During the treatment of horse No. B162, problems arose with the Cromovet nebuliser unit on days 13, 14 and 21. The nebuliser unit became partially blocked with dust particles on several occasions which necessitated repeated cleaning and re-assembly of the unit. It is possible that there was a slight loss of sodium cromoglycate during this process which resulted in the horse having a lower dose and consequently incomplete protection. Alternatively, nebulisation of the drug whilst the nebuliser unit was partially blocked could have resulted in increased droplet size, reduced drug distribution into the smaller airways and hence lowered the efficacy of the treatment.

Intermittent sodium cromoglycate treatment may be found useful for the management of COPD affected horses in general practice. 4-day courses of sodium cromoglycate treatment would result in a more prolonged period of protection and hence reduce the frequency of

treatment. However, this form of treatment is by its nature very flexible and individual treatment schedules could be designed according to each horse's response to treatment and the owner's requirements.

CONCLUSIONS.

In a 28 day trial, twice weekly sodium cromoglycate inhalation was effective in preventing the onset of COPD in 6 out of 8 affected horses which were continuously housed in the natural challenge environment.

CHAPTER 10.

GENERAL DISCUSSION AND CONCLUSIONS.

The studies described in this thesis provide more information both on the therapy of equine COPD and on the pathophysiology of the disease itself.

Minimising exposure to the aetiological antigens through use of a controlled environment allowed COPD affected horses to become asymptomatic within 4 to 32 days (mean $\pm$ S.D. = 9.0 $\pm$ 4.8 days). The time taken to become asymptomatic correlated significantly with the severity and the duration of the disease.

When the horses were asymptomatic, their pulmonary function values (i.e.: respiratory rate, max. ΔPpl, tidal volume, inspiratory and expiratory flow rates, PaO_2, and $PaCO_2$ and arterial pH) did not differ significantly from those of normal horses. It is, therefore, apparent that the pathophysiological changes occurring in equine COPD are largely reversible and that most affected horses are capable of regaining normal pulmonary function when contact with the aetiological antigens is minimised. These findings are in agreement with the observations of several authors who reported clinical improvement in COPD affected horses kept permanently outdoors or housed in minimal dust environments (Thurlbeck and Lowell, 1964; Eyre, 1972; Cook, 1976) and are also in accordance with the findings of Meister, Gerber and Tschudi (1976) and Dixon (1979) who additionally recorded reversal of hypoxaemia in COPD affected horses when housed in

controlled environments.

Two avenues of chemotherapy were investigated i.e. bronchodilator therapy of symptomatic COPD affected horses and prophylactic sodium cromoglycate treatment of asymptomatic horses.

Intravenous administration of atropine, clenbuterol or etamiphylline camsylate and inhalation of atropine, isoprenaline or terbutaline to symptomatic COPD affected horses brought about a temporary, marked improvement in clinical signs, accompanied by significant decreases in max. ΔPpl (by 60 to 70% of the pre-treatment value) and respiratory rate and significant increases in PaO_2 values. These findings indicate that airway spasm does play a role in the pathogenesis of equine COPD. Despite the marked improvement at the peak response to treatment, the horses failed to regain normal pulmonary function. This is undoubtedly due to the widespread bronchiolitis which occurs in symptomatically affected horses which would not be compensated for by bronchodilator treatment.

Whereas the beneficial effects of isoprenaline, atropine and etamiphylline camsylate did not last more than 2 hours in affected horses, the actions of terbutaline and clenbuterol were more prolonged with significant improvement in pulmonary function for 4 to 6 hours after treatment. Although terbutaline,

clenbuterol and etamiphylline camsylate could be useful for the treatment of acute attacks of COPD, parenteral bronchodilator treatment remains subject to many limitations in that it only partially alleviates the clinical signs and the resulting improvement is of short duration. In addition, side effects are frequently observed following parenteral bronchodilator administration although these are minimal with the use of the more specific beta 2 sympathomimetic bronchodilators (i.e. terbutaline and clenbuterol) and with etamiphylline camsylate.

The clinical improvement in COPD affected horses recorded in these trials following atropine, isoprenaline and clenbuterol treatment is similar to that previously reported by other authors (Obel and Schmiterlöw, 1948; Muylle and Oyaert, 1973; Sasse and Hajer, 1977). The effects of terbutaline and etamiphylline camsylate on respiratory function in COPD affected horses do not appear to have been described previously.

Studies into the efficacy of orally administered bronchodilator drugs in the treatment of equine COPD proved disappointing. When horses were housed in the challenge environment and treated with oral clenbuterol or etamiphylline camsylate, they continued to be symptomatically affected with COPD. Apart from the significant decreases in respiratory rate and max. Δ Ppl

on days 7 and 11 of the clenbuterol trial, there were no significant changes in their respiratory function values from those recorded when horses were untreated and housed in similar conditions. In addition, neither drug significantly hastened the remission of clinical signs of COPD which normally occurred when symptomatic horses were housed in the controlled environment.

The reason for this apparent lack of response of the COPD affected horses to oral bronchodilator treatment is difficult to explain, especially after the marked response shown by affected horses to these same agents administered intravenously. In keeping with the drug manufacturer's recommendations, the same clenbuterol dose was used for intravenous and oral administration (i.e. 0.8 μg/kg) and a lower dose of etamiphylline camsylate was used for oral administration (i.e. 2.25 mg/kg) than for intravenous administration (i.e. 3.0 mg/kg). Although both drugs are well absorbed from the equine gastrointestinal tract, it is possible that higher doses of orally administered compounds are required to achieve a similar response to that which has been demonstrated with intravenous administration. Detailed dose-response studies on orally administered bronchodilator drugs in COPD affected horses would be required to elucidate this point.

To date, there appears to be only one published report on the oral administration of bronchodilator drugs

to COPD affected horses (Sasse and Hajer, 1977). In contrast to the findings in this thesis, Sasse and Hajer (1977) reported a good response in horses treated with clenbuterol at a dosage rate of 0.8 µg/kg for 14 days. However, as the response to treatment was only subjectively assessed by the owners' observations it is impossible to critically compare the results of the two studies.

Should higher doses of the orally administered bronchodilator drugs prove more successful in treating COPD, it still seems unlikely that this form of therapy would be useful as a long-term measure as it does not control the cause of this disease. Unless contact with the aetiological antigens is minimised, COPD signs would recur on cessation of treatment. Although it is alleged that long-term clenbuterol administration also aids in the treatment of bronchiolitis in COPD through its mucolytic effect which prevents "pooling" of the mucus in the airways (Boehringer, technical information, 1980), there have been no published studies to support this claim.

In contrast to the results of bronchodilator treatment, studies on the prophylactic treatment of asymptomatic COPD affected horses with sodium cromoglycate proved hopeful. In the initial studies (chapter 7), prophylactic sodium cromoglycate inhalation in 2 affected horses prevented the exacerbation of

respiratory disease, normally observed in COPD affected horses 4 to 8 hours after M. faeni inhalation challenge. These studies were followed by a clinical trial using 56 horses (chapter 8) in which it was shown that a linear response exists between the number of successive days treatment with this drug and the duration of the protective period while horses are exposed to the natural challenge environment. The protective period was 3.6 $\pm$ 1.1 days (mean $\pm$ S.D.) after a single sodium cromoglycate treatment, 8.0 $\pm$ 3.4 days after 2 days treatment, 11.9 $\pm$ 2.9 days after 3 days treatment and 24.3 $\pm$ 13.4 days after 4 days treatment. In a 28 day trial (chapter 9), two successive days sodium cromoglycate inhalation administered at weekly intervals was effective in preventing the onset of COPD in 6 out of 8 affected horses which were continuously housed in the natural challenge environment.

These experiments show that prophylactic inhalation treatment of asymptomatic COPD affected horses with sodium cromoglycate is an effective method of controlling the disease in the short term. As sodium cromoglycate is believed to act by stabilising the mast cell membranes, these results suggest that pulmonary mast cell degranulation is involved in the pathogenesis of equine COPD.

Whilst environmental control is a preferable form of therapy for COPD, sodium cromoglycate treatment may be found useful when unavoidable exposure to the

aetiological antigens is anticipated. This could occur, for instance, during transportation or when horses are moved temporarily away from the home environment. 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.

There is, however, one main disadvantage to sodium cromoglycate treatment of COPD affected horses, i.e. the method of drug inhalation is labour intensive (20 to 30 minutes per dose) and can be prolonged by faulty equipment, which may, in addition, reduce the efficacy of treatment. It would, therefore, be preferable if an orally administered compound with similar activity to inhaled sodium cromoglycate could be developed for equine COPD treatment.

Since the introduction of sodium cromoglycate, numerous related compounds have been synthesised as potential anti-allergic drugs. These include benzopyrans, xanthones, quinolines, azapurinones and oxanilic acid (Church, 1978). Many of these compounds can be administered either by inhalation or orally and in experimental animals, have been found to possess even greater anti-allergic activity than sodium cromoglycate. However, clinical tests in bronchial asthma patients have shown that none of these cromoglycate analogues are more effective than sodium cromoglycate.

The orally administered cromoglycate-like compounds are still undergoing investigation for the treatment of asthma. If successful, the use of these orally administered drugs for the treatment of equine COPD merits investigation.

There are a number of other drugs with anti-allergic properties which might be beneficial for the treatment of equine COPD but which have not as yet been investigated for this purpose. These include the non-steroidal anti-inflammatory drugs (NSAIDS), diethycarbamazine, ketotifen and oxatomide (Eyre and Hanna, 1980; Hawking, 1979; Daniel and Schromm, 1980).

In addition to investigation into the efficacy of newer anti-allergic drugs in COPD affected horses, more fundamental research into the disease is required in order to gain a better understanding of the immunological and biochemical processes occurring in the pathogenesis of equine COPD.

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APPENDICES

APPENDIX 3.1.

Normal Horses: Case details, resting respiratory function measurements, arterial blood gas and pH values.

* Respiratory
X Inspiratory
Ø Expiratory

Horse No.	Breed Type	Age	Sex	(kg) Weight	*Resp. Rate/ Minute	Max. ΔPpl (mm Hg)	Tidal Volume (litres)	Minute Volume litres/min	Max. XInsp. Flow Rate litres/min	Max. ØExp. Flow Rate litres/min	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	Arterial pH
A 1	Hunter	9	Mn	481.4	10	3.0	6.5	65.0	240.1	216.7	88.4	36.1	7.446
A 2	H.Hunter	13	Mn	584.5	9	3.5	8.2	73.0	180.6	165.5	92.1	39.5	7.420
A 3	Hunter	9	F	434.5	12	3.6	4.2	50.8	236.0	210.4	86.3	37.5	7.422
A 4	Hunter	4	Mn	495.9	12	3.5	5.8	68.6	200.8	186.5	90.1	34.3	7.440
A 5	TB	5	F	539.1	11	3.1	6.0	66.6	180.0	156.0	87.3	37.0	7.427
A 6	Hunter	8	Mn	553.6	8	3.0	7.8	60.9	230.8	164.1	88.1	37.0	7.460
A 7	TB	4	Mn	485.5	12	2.5	6.5	78.8	246.1	200.9	94.5	39.0	7.450
A 8	Hunter	9	Mn	470.9	16	4.7	5.1	84.6	214.0	192.6	85.2	39.6	7.460
A 9	Hunter	20	Mn	614.6	8	3.5	8.5	68.5	318.7	275.1	84.2	44.8	7.437
A 10	Hunter	9	Mn	543.6	10	3.8	5.4	54.0	182.8	199.1	89.0	37.7	7.458
A 11	TB	7	Mn	586.4	10	3.5	7.6	76.0	230.8	275.6	89.9	36.1	7.456
A 12	Hunter	10	F	493.2	12	3.9	5.8	68.6	208.3	272.4	92.6	32.1	7.447
A 13	Hunter	3	Mn	445.5	13	4.2	4.9	65.7	195.8	182.7	85.5	37.9	7.425
A 14	TB	2	Mn	365.5	16	2.5	3.9	60.4	210.5	182.3	89.1	39.5	7.435
A 15	Hunter	10	F	528.2	16	3.0	6.5	108.6	257.2	229.3	85.1	37.6	7.448
A 16	H.Hunter	8	Mn	631.8	12	5.0	9.2	109.4	216.4	187.9	90.6	39.5	7.420
A 17	Norwegan Fjoord	10	M	407.3	16	4.8	4.3	62.8	215.4	210.3	84.5	44.3	7.382
A 18	Hunter	12	F	489.1	12	3.7	5.7	69.6	192.3	187.5	89.8	40.4	7.436
A 19	TB	6	Mn	540.0	11	3.9	8.4	93.1	319.4	296.1	83.2	36.3	7.429
A 20	TB	10	Mn	459.0	10	3.8	6.0	60.0	208.9	167.3	87.2	43.0	7.446
A 21	Welsh Pony	4	Mn	318.2	12	4.0	-	-	-	-	87.2	37.6	7.420
A 22	Pony	4	Mn	340.9	9	3.5	-	-	-	-	94.5	31.2	7.462
A 23	Pony	5	F	326.3	12	3.6	-	-	-	-	93.0	32.7	7.432
A 24	Hunter	22	Mn	463.6	13	3.0	-	-	-	-	88.1	34.0	7.430
A 25	Hunter	17	Mn	510.9	9	3.0	-	-	-	-	91.2	32.1	7.426
A 26	Hunter	9	Mn	522.7	10	3.4	-	-	-	-	92.1	34.6	7.439
A 27	TB	12	Mn	536.8	10	3.7	-	-	-	-	92.1	35.4	7.442
A 28	TB	5	F	488.2	12	2.8	-	-	-	-	89.0	35.0	7.421
A 29	Pony	6	F	372.7	24	3.0	-	-	-	-	94.1	36.0	7.424
A 30	Pony	7	F	380.4	18	3.0	-	-	-	-	85.5	37.2	7.446
A 31	TB	3	Mn	571.4	9	3.3	-	-	-	-	87.0	36.3	7.432
A 32	Hunter	16	Mn	500.8	9	3.1	-	-	-	-	95.7	36.0	7.422
A 33	TB	8	Mn	586.4	8	4.5	-	-	-	-	91.3	37.0	7.415
A 34	Hunter	7	Mn	523.6	10	2.7	-	-	-	-	90.2	35.4	7.446
A 35	TB	10	F	550.0	10	3.9	-	-	-	-	92.5	34.6	7.420
A 36	Arab	5	Mn	434.5	14	3.5	-	-	-	-	96.0	32.1	7.431
A 37	Pony	6	Mn	336.4	9	3.6	-	-	-	-	84.0	33.8	7.428
A 38	Pony	4	F	309.1	12	4.5	-	-	-	-	90.6	34.5	7.445
A 39	Welsh	7	F	354.5	18	3.0	-	-	-	-	89.7	33.5	7.430
A 40	Fell X	13	Mn	405.5	8	3.2	-	-	-	-	92.0	33.4	7.428
A 41	TB	4	Mn	573.2	8	3.6	-	-	-	-	91.3	33.0	7.421
A 42	Hunter	6	Mn	538.6	8	3.4	-	-	-	-	96.0	32.1	7.447
A 43	TB	12	Mn	535.0	14	4.0	-	-	-	-	97.9	30.2	7.432
A 44	Trotter	4	F	420.6	10	3.8	-	-	-	-	94.4	39.3	7.450
A 45	Arab	7	Mn	415.8	13	4.4	-	-	-	-	92.1	34.6	7.418
A 46	Pony	7	Mn	343.6	16	2.5	-	-	-	-	96.9	36.2	7.432
A 47	Hunter	9	Mn	502.9	10	4.5	-	-	-	-	88.3	37.5	7.415
A 48	TB	10	F	496.0	13	3.0	-	-	-	-	98.4	34.1	7.440
A 49	TB	5	F	492.6	16	3.0	-	-	-	-	93.4	37.6	7.409
A 50	Pony	3	Mn	338.6	12	5.4	-	-	-	-	88.4	37.6	7.423
A 51	TB	8	Mn	578.3	14	3.7	-	-	-	-	89.5	40.6	7.420
A 52	Hunter	24	Mn	461.2	24	3.0	-	-	-	-	89.8	35.4	7.459
A 53	Pony	6	F	392.1	14	3.1	-	-	-	-	84.9	36.5	7.432
A 54	Pony	10	Mn	369.4	16	4.3	-	-	-	-	86.5	38.1	7.425
A 55	TB	4	Mn	427.0	9	3.0	-	-	-	-	92.1	35.3	7.460
A 56	Hunter	8	Mn	536.4	12	3.0	-	-	-	-	90.5	38.9	7.429
A 57	TB	6	Mn	500.2	10	4.5	-	-	-	-	83.9	40.1	7.369
A 58	Pony	20	F	388.8	14	3.7	-	-	-	-	96.2	31.3	7.442
A 59	TB	4	F	429.5	16	2.8	-	-	-	-	90.2	37.7	7.374
A 60	TB	6	Mn	551.3	11	3.7	-	-	-	-	84.0	36.4	7.408
A 61	Pony	6	F	362.4	10	3.9	-	-	-	-	87.3	32.1	7.452
A 62	TB	4	Mn	540.1	12	5.2	-	-	-	-	91.7	34.3	7.429
A 63	TB	8	Mn	566.3	8	3.0	-	-	-	-	85.0	36.1	7.440
A 64	TB	3	Mn	506.4	12	4.0	-	-	-	-	85.9	36.4	7.422
A 65	TB	10	Mn	580.6	11	3.5	-	-	-	-	87.2	43.0	7.446
A 66	Pony	10	F	395.0	14	2.8	-	-	-	-	92.3	38.6	7.439
A 67	TB	6	Mn	529.4	12	4.4	-	-	-	-	88.3	34.1	7.448
A 68	TB	5	F	457.8	10	4.1	-	-	-	-	86.8	32.4	7.452
n		68		68	68	68	20	20	20	20	68	68	68
x̄		8.13		473.7	12.07	3.59	6.32	72.25	224.25	207.92	89.82	36.34	7.433
S.D.		4.60		82.85	3.34	0.66	1.53	15.93	39.00	41.40	3.77	3.10	0.018

APPENDIX 3.2.

Symptomatic COPD affected horses: Case details, resting respiratory function measurements, arterial blood gas and pH values.

* Respiratory
X Inspiratory
Ø Expiratory

Horse No.	Breed Type	Age	Sex	(kg) Weight	*Resp. Rate/ Minute	Max. ΔPpl (mm Hg)	Tidal Volume (litres)	Minute Volume litres/ min.	Max. XInsp. Flow Rate litres/ min.	Max. ØExp. Flow Rate litres/ min.	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	Art- erial pH	Duration of illness (months)
B 1	TB	10	Mn	480.0	14	9.0	6.4	89.0	297.0	240.2	67.7	42.1	7.453	12
B 2	Hunter	8	Mn	452.3	21	9.6	4.4	96.4	280.6	220.1	72.0	40.2	7.457	8
B 3	Hunter	15	F	511.8	14	19.7	6.0	84.8	360.7	200.5	78.3	35.4	7.457	48
B 4	Pony	6	Mn	383.6	16	12.3	4.0	69.9	294.5	208.3	85.7	35.8	7.445	5
B 5	Hunter	10	Mn	584.1	26	7.0	6.3	169.8	260.8	241.0	65.1	37.0	7.460	9
B 6	Hunter	12	Mn	509.1	13	12.4	8.2	72.8	233.5	189.0	70.2	44.0	7.433	10
B 7	Hunter	12	Mn	519.1	26	19.5	5.8	140.8	346.1	229.2	69.1	41.0	7.428	30
B 8	Hunter	23	F	458.2	10	33.0	8.4	84.0	340.9	262.0	72.6	34.5	7.436	52
B 9	H.Hunter	13	Mn	636.7	9	8.2	9.9	89.4	296.3	235.3	76.0	34.7	7.430	36
B 10	H.Hunter	10	Mn	659.1	20	10.1	9.2	186.6	237.2	168.6	75.9	38.5	7.438	12
B 11	Hunter	16	Mn	409.1	16	24.5	4.4	73.4	195.5	192.3	65.7	35.7	7.430	48
B 12	Hunter	10	Mn	521.8	8	17.5	8.5	67.0	173.1	162.0	72.1	38.9	7.449	60
B 13	TB	11	M	525.5	12	15.5	7.6	94.2	227.1	204.6	73.9	37.6	7.437	26
B 14	TB	7	Mn	469.3	14	12.4	6.3	87.2	219.4	165.3	69.9	42.4	7.416	5
B 15	Hunter	5	F	492.6	18	8.5	5.2	95.3	337.9	231.4	75.5	33.6	7.448	6
B 16	TB	14	Mn	515.7	11	14.3	6.9	75.0	194.0	162.3	74.6	39.8	7.433	14
B 17	TB	12	Mn	529.0	12	19.6	6.0	74.1	302.4	189.3	67.8	44.5	7.396	12
B 18	TB	8	F	488.2	12	10.5	6.6	78.0	237.1	210.9	76.4	37.5	7.428	16
B 19	Hunter	7	F	526.4	9	12.0	7.2	64.5	177.4	150.3	75.2	39.0	7.446	6
B 20	Hunter	10	Mn	559.7	12	13.2	5.8	70.6	215.2	175.4	76.6	40.8	7.430	9
B 21	Pony	5	F	354.5	18	10.5	-	-	-	-	80.1	40.1	7.429	6
B 22	TB	9	Mn	509.1	20	23.2	-	-	-	-	79.1	36.0	7.416	12
B 23	Hunter	7	Mn	518.2	18	14.5	-	-	-	-	73.0	38.0	7.442	9
B 24	Hunter	9	Mn	467.7	16	17.0	-	-	-	-	72.0	41.2	7.439	18
B 25	Draught	14	Mn	636.4	18	13.5	-	-	-	-	67.2	36.1	7.447	24
B 26	TB	8	Mn	499.1	9	16.5	-	-	-	-	69.4	37.3	7.425	8
B 27	TB	9	Mn	515.9	9	18.0	-	-	-	-	72.0	39.2	7.446	10
B 28	TB	6	F	462.7	12	26.4	-	-	-	-	64.7	36.5	7.400	32
B 29	TB	9	Mn	500.8	12	15.6	-	-	-	-	63.9	44.3	7.382	13
B 30	Hunter	12	F	486.4	20	13.5	-	-	-	-	76.0	40.5	7.416	40
B 31	H.Hunter	12	F	520.6	33	33.2	-	-	-	-	62.8	43.2	7.388	72
B 32	H.Hunter	7	Mn	608.1	20	26.0	-	-	-	-	58.5	38.2	7.436	15
B 33	TB	11	F	457.3	28	18.4	-	-	-	-	72.9	37.2	7.428	7
B 34	Hunter	7	Mn	534.0	18	20.1	-	-	-	-	65.6	41.2	7.392	62
B 35	TB	8	F	451.9	12	23.5	-	-	-	-	66.7	43.9	7.386	48
B 36	TB	3	F	459.1	24	14.0	-	-	-	-	85.1	37.1	7.426	4
B 37	Pony	6	F	400.0	20	12.5	-	-	-	-	66.4	38.3	7.409	6
B 38	TB	9	Mn	525.9	19	15.5	-	-	-	-	71.5	39.0	7.422	12
B 39	TB	7	Mn	520.5	28	21.5	-	-	-	-	68.9	40.1	7.446	10
B 40	Hunter	9	Mn	512.2	20	19.0	-	-	-	-	70.4	39.9	7.412	11
B 41	Pony	8	Mn	376.9	12	13.5	-	-	-	-	72.6	37.0	7.429	30
B 42	Pony	10	Mn	350.9	14	15.0	-	-	-	-	68.5	39.3	7.436	16
B 43	Hunter	12	Mn	449.8	12	17.4	-	-	-	-	70.0	40.2	7.406	20
B 44	Hunter	10	Mn	422.6	16	14.5	-	-	-	-	71.0	37.3	7.449	24
B 45	TB	12	Mn	534.0	12	19.5	-	-	-	-	66.1	38.5	7.426	50
B 46	Hunter	7	Mn	515.9	14	12.6	-	-	-	-	73.0	38.0	7.433	12
B 47	Hunter	9	F	495.5	16	14.5	-	-	-	-	71.0	39.6	7.429	9
B 48	TB	7	Mn	528.2	18	18.0	-	-	-	-	66.0	42.9	7.388	11
B 49	TB	12	Mn	565.6	12	14.5	-	-	-	-	66.9	42.9	7.400	14
B 50	TB	7	F	522.7	10	26.1	-	-	-	-	65.8	44.4	7.427	48
B 51	Hunter	13	F	459.1	24	30.0	-	-	-	-	63.2	38.9	7.459	66
B 52	Hunter	8	Mn	527.3	23	27.0	-	-	-	-	54.8	42.9	7.361	54
B 53	TB	9	F	555.5	24	20.3	-	-	-	-	66.4	32.6	7.419	12
B 54	Draught	7	Mn	620.5	16	12.7	-	-	-	-	80.4	34.1	7.445	24
B 55	Draught	8	Mn	650.0	23	10.5	-	-	-	-	76.4	40.6	7.424	18
B 56	Pony	8	F	396.2	18	12.0	-	-	-	-	82.4	44.8	7.430	6
B 57	Hunter	14	Mn	372.7	21	19.5	-	-	-	-	69.5	38.5	7.419	36
B 58	TB	7	Mn	617.2	21	12.2	-	-	-	-	72.5	36.1	7.433	24
B 59	TB	9	Mn	518.2	17	18.4	-	-	-	-	63.0	39.1	7.396	12
B 60	Hunter	7	Mn	536.8	18	14.5	-	-	-	-	76.1	37.1	7.442	14
B 61	Hunter	9	Mn	545.0	20	22.0	-	-	-	-	68.2	37.9	7.430	40
B 62	Hunter	9	F	482.1	18	15.3	-	-	-	-	73.8	41.2	7.416	9
B 63	Anglo Arab	6	M	494.0	24	13.0	-	-	-	-	77.5	40.1	7.405	9
B 64	TB	8	F	532.6	19	26.6	-	-	-	-	61.0	42.8	7.396	42
B 65	TB	7	Mn	534.5	30	22.2	-	-	-	-	59.9	42.7	7.381	12
B 66	H.Hunter	8	Mn	604.9	20	19.0	-	-	-	-	63.0	42.0	7.400	16
B 67	Hunter	11	Mn	472.3	18	23.6	-	-	-	-	79.1	36.0	7.410	15
B 68	H.Hunter	13	Mn	592.8	24	12.5	-	-	-	-	66.1	36.1	7.412	20
B 69	Hunter	15	F	531.4	16	15.1	-	-	-	-	70.8	39.9	7.402	66
B 70	Hunter	22	F	408.9	16	28.0	-	-	-	-	70.5	43.3	7.400	60
B 71	Pony	5	F	368.2	16	11.4	-	-	-	-	73.4	39.8	7.431	12
B 72	Hunter	9	Mn	480.4	14	18.3	-	-	-	-	65.4	39.6	7.410	6
B 73	TB	7	Mn	580.5	13	8.5	-	-	-	-	78.3	42.9	7.420	18
B 74	TB	7	Mn	485.5	12	12.0	-	-	-	-	64.4	41.8	7.417	7
B 75	HeavyHunter	10	Mn	592.7	22	23.1	-	-	-	-	71.0	43.3	7.326	5
B 76	Hunter	10	Mn	529.1	16	16.6	-	-	-	-	72.9	38.7	7.425	6
B 77	TB	7	Mn	541.8	14	14.5	-	-	-	-	75.7	37.9	7.421	9
B 78	TB	10	F	420.5	13	13.7	-	-	-	-	70.6	38.9	7.417	12
B 79	Hunter	15	Mn	592.7	18	21.0	-	-	-	-	64.9	43.7	7.372	24
B 80	Hunter	10	Mn	556.3	26	32.7	-	-	-	-	60.3	43.9	7.396	18

APPENDIX 3.2.

(Continued).

Horse No.	Breed Type	Age	Sex	(kg) Weight	Resp. Rate/ Minute	Max. Δ Ppl (mm Hg)	Tidal Volume (litres)	Minute Volume litres/ min.	Max. Insp. Flow Rate litres/ min.	Max. Exp. Flow Rate litres/ min.	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	Art- erial pH	Duration of illness (months)
B 81	Pony	14	Mn	409.3	16	12.2	-	-	-	-	72.9	34.5	7.442	48
B 82	Pony	8	F	397.6	14	15.0	-	-	-	-	69.3	36.2	7.418	10
B 83	TB	9	F	495.0	12	9.5	-	-	-	-	74.6	40.7	7.439	7
B 84	Hunter	12	F	545.2	10	10.1	-	-	-	-	77.8	33.0	7.432	30
B 85	TB	5	Mn	563.4	13	17.6	-	-	-	-	67.2	39.2	7.401	7
B 86	TB	7	Mn	550.1	12	13.8	-	-	-	-	73.0	35.1	7.456	16
B 87	Arab	6	F	446.4	18	9.0	-	-	-	-	76.1	35.9	7.437	12
B 88	Hunter	11	Mn	586.3	14	23.6	-	-	-	-	65.5	37.2	7.415	10
B 89	TB	6	F	522.1	16	16.0	-	-	-	-	70.9	36.0	7.460	10
B 90	Fell Pony	8	F	468.5	11	10.0	-	-	-	-	70.2	39.9	7.440	5
B 91	TB	13	Mn	500.9	20	12.9	-	-	-	-	72.5	42.1	7.433	18
B 92	TB	5	F	536.5	16	18.3	-	-	-	-	68.0	34.3	7.400	6
B 93	HavyHunter	14	Mn	615.0	14	15.0	-	-	-	-	68.5	39.7	7.408	42
B 94	Hunter	11	Mn	502.9	22	29.7	-	-	-	-	65.7	42.4	7.385	48
B 95	Hunter	16	F	445.9	18	14.5	-	-	-	-	73.9	38.4	7.413	62
B 96	Hunter	24	F	463.0	22	28.9	-	-	-	-	64.0	44.8	7.376	84
B 97	TB	9	F	496.2	13	17.5	-	-	-	-	73.9	39.5	7.416	12
B 98	H.Hunter	13	Mn	645.3	16	22.4	-	-	-	-	62.1	43.9	7.382	42
B 99	H.Hunter	10	Mn	609.7	13	14.7	-	-	-	-	71.6	36.2	7.420	24
B100	TB	11	M	510.8	10	12.0	-	-	-	-	80.7	33.2	7.428	10
B101	TB	7	Mn	538.6	16	18.1	-	-	-	-	69.7	39.9	7.408	24
B102	TB	7	Mn	578.3	10	11.5	-	-	-	-	72.5	40.9	7.416	20
B103	Pony	6	F	388.6	16	15.7	-	-	-	-	69.3	37.4	7.409	18
B104	Hunter	10	Mn	539.1	12	14.0	-	-	-	-	76.9	36.9	7.430	36
B105	Hunter	14	Mn	512.5	20	25.1	-	-	-	-	63.0	43.9	7.400	48
B106	Hunter	16	F	475.3	18	17.3	-	-	-	-	71.2	38.4	7.412	60
B107	Hunter	15	Mn	587.6	18	20.6	-	-	-	-	59.7	43.4	7.402	54
B108	TB	10	F	551.9	12	10.0	-	-	-	-	76.3	39.6	7.415	11
B109	H.Hunter	12	Mn	610.9	18	14.5	-	-	-	-	63.4	40.2	7.426	48
B110	TB	7	F	444.5	18	8.1	-	-	-	-	76.1	37.6	7.421	7
B111	Hunter	9	Mn	488.2	16	13.0	-	-	-	-	72.4	39.0	7.405	36
B112	Hunter	14	F	533.4	20	10.4	-	-	-	-	75.9	38.2	7.412	12
B113	TB	12	F	520.9	14	9.2	-	-	-	-	75.2	38.5	7.422	10
B114	TB	7	Mn	543.6	14	6.0	-	-	-	-	82.9	39.9	7.418	7
B115	WelshPony	6	F	338.2	18	6.9	-	-	-	-	74.1	41.0	7.444	7
B116	TB	7	Mn	489.5	12	12.2	-	-	-	-	64.4	42.0	7.447	24
B117	H.Hunter	11	Mn	625.0	15	13.5	-	-	-	-	71.0	43.5	7.376	48
B118	TB	9	Mn	497.3	15	10.5	-	-	-	-	73.0	38.3	7.416	4
B119	Pony	6	F	352.1	16	8.0	-	-	-	-	78.6	44.7	7.405	6
B120	TB	8	Mn	548.7	15	13.2	-	-	-	-	60.9	39.2	7.416	9
B121	Hunter	14	Mn	405.1	18	15.0	-	-	-	-	68.5	39.1	7.412	36
B122	Hunter	9	Mn	497.6	13	7.6	-	-	-	-	78.1	38.5	7.424	3
B123	TB	8	Mn	493.2	16	8.3	-	-	-	-	82.3	39.0	7.426	6
B124	TB	7	Mn	526.3	12	6.1	-	-	-	-	62.5	40.5	7.416	3
B125	AngloArab	9	M	483.0	20	7.0	-	-	-	-	61.9	40.0	7.401	6
B126	TB	12	Mn	532.0	18	20.3	-	-	-	-	65.6	41.2	7.412	60
B127	TB	3	Mn	515.4	16	8.5	-	-	-	-	59.7	44.5	7.402	6
B128	TB	7	Mn	469.8	19	22.9	-	-	-	-	61.0	45.9	7.41	12
B129	TB	11	F	450.2	12	8.0	-	-	-	-	74.6	39.2	7.429	12
B130	Hunter	15	F	486.6	18	22.2	-	-	-	-	63.8	36.4	7.413	48
B131	Fell Pony	13	Mn	426.9	10	8.5	-	-	-	-	72.3	37.6	7.424	12
B132	Pony	25	Mn	398.4	18	11.8	-	-	-	-	67.5	42.6	7.417	96
B133	Fell Pony	12	Mn	414.9	18	6.0	-	-	-	-	83.6	30.0	7.435	14
B134	Hunter	8	Mn	537.0	14	9.2	-	-	-	-	71.9	36.1	7.415	3
B135	Pony	12	F	389.2	14	27.3	-	-	-	-	58.0	35.0	7.419	24
B136	Hunter	6	Mn	572.9	12	10.1	-	-	-	-	75.0	34.1	7.420	10
B137	HavyHunter	13	Mn	642.5	18	22.0	-	-	-	-	59.4	44.6	7.406	48
B138	TB	12	Mn	488.3	16	12.5	-	-	-	-	72.6	37.4	7.419	12
B139	TB	7	Mn	519.0	15	6.8	-	-	-	-	83.0	34.9	7.421	8
B140	Hunter	23	F	431.0	15	12.9	-	-	-	-	73.4	38.0	7.415	72
B141	Pony	5	F	406.5	20	9.5	-	-	-	-	69.5	43.2	7.416	6
B142	TB	7	F	509.2	18	34.0	-	-	-	-	64.4	47.0	7.415	9
B143	TB	7	Mn	572.0	14	18.0	-	-	-	-	63.9	44.3	7.415	12
B144	Pony	10	F	388.8	18	11.5	-	-	-	-	69.4	38.2	7.415	6
B145	Arab	4	Mn	463.5	16	7.0	-	-	-	-	62.0	41.9	7.420	5
B146	Hunter	7	Mn	536.4	15	8.5	-	-	-	-	72.0	36.3	7.420	24
B147	TB	8	Mn	559.2	12	15.5	-	-	-	-	58.4	42.1	7.409	16
B148	Hunter	9	Mn	563.1	15	12.2	-	-	-	-	66.1	34.8	7.420	14
B149	TB	7	F	546.5	15	14.0	-	-	-	-	81.9	36.1	7.423	12
B150	Knap strap	7	Mn	586.0	18	12.7	-	-	-	-	74.1	38.2	7.420	12
B151	Hunter	12	F	510.5	16	13.0	-	-	-	-	63.1	46.0	7.408	4
B152	TB	12	Mn	497.5	14	13.4	-	-	-	-	62.5	44.6	7.419	6
B153	TB	6	Mn	526.4	12	8.3	-	-	-	-	82.3	29.7	7.430	12
B154	TB	12	F	540.2	11	10.0	-	-	-	-	76.5	37.8	7.416	10
B155	Hunter	16	F	492.0	20	10.5	-	-	-	-	76.2	39.0	7.419	48
B156	Hunter	9	Mn	542.9	14	8.2	-	-	-	-	79.4	38.3	7.415	36
B157	Hunter	11	Mn	566.3	15	9.5	-	-	-	-	70.6	39.0	7.408	36
B158	Hunter	14	F	500.0	18	14.2	-	-	-	-	68.1	39.2	7.418	48
B159	Hunter	15	F	482.6	16	10.5	-	-	-	-	73.9	38.4	7.412	48
B160	Hunter	24	F	508.5	24	25.0	-	-	-	-	64.4	39.2	7.401	72
B161	Hunter	16	F	496.7	16	12.5	-	-	-	-	74.3	37.5	7.429	36
B162	HavyHunter	13	Mn	627.4	12	15.5	-	-	-	-	68.2	36.1	7.410	12
B163	TB	13	M	542.4	12	10.1	-	-	-	-	77.0	38.2	7.430	24
B164	Pony	15	Mn	400.7	18	22.5	-	-	-	-	63.0	40.7	7.402	48
n		164		164	164	164	20	20	20	20	164	164	164	164
$\bar{x}$		9.99		506.42	16.35	15.44	6.65	93.14	261.34	201.90	70.55	39.28	7.419	23.10
S.D.		4.02		67.94	4.40	6.23	1.61	33.56	58.76	31.78	6.25	3.21	0.020	19.66

APPENDIX 4.1.

Asymptomatic COPD affected Horses: Resting respiratory function measurements, arterial blood gas and pH values and time taken to become asymptomatic in the controlled environment.

* Respiratory
X Inspiratory
Ø Expiratory

Horse No.	*Resp. Rate/ Minute	Max. ΔPpl (mm Hg)	Tidal Volume (litres)	Minute Volume litres/min.	Max. XInsp. Flow Rate litres/min.	Max. ØExp. Flow Rate litres/min.	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	Arterial pH	Time (days) to become Asymptomatic
B 1	12	3.5	4.8	58.6	155.6	155.0	89.0	36.5	7.434	5
B 2	14	3.8	5.9	80.6	296.2	216.5	84.9	40.1	7.443	7
B 3	14	3.5	6.1	85.9	185.3	213.1	89.6	35.4	7.442	7
B 4	16	3.0	4.5	76.3	256.5	200.9	85.0	37.2	7.434	6
B 5	14	3.6	5.8	93.8	280.3	256.9	83.7	38.4	7.432	8
B 6	9	3.0	8.5	74.5	230.8	217.9	89.8	44.6	7.452	5
B 7	13	5.2	6.0	78.0	227.3	194.5	84.7	36.3	7.415	10
B 8	12	4.5	8.0	98.6	210.7	186.5	87.0	34.9	7.431	24
B 9	10	3.7	9.6	96.0	275.0	246.5	86.7	37.7	7.420	4
B 10	12	4.2	9.4	108.2	206.2	200.1	88.2	38.4	7.439	7
B 11	11	5.1	5.0	56.0	188.3	180.0	82.1	42.7	7.416	14
B 12	10	3.9	8.1	81.0	217.9	186.4	86.3	39.4	7.424	16
B 13	9	3.2	8.5	74.5	203.5	171.2	94.7	39.6	7.447	6
B 14	12	4.1	5.9	70.6	209.1	172.6	88.3	36.4	7.439	8
B 15	13	2.9	5.4	72.7	185.3	159.5	90.7	33.1	7.446	4
B 16	12	3.5	7.3	86.4	231.7	200.6	88.0	36.5	7.451	9
B 17	10	5.2	6.5	65.0	218.4	190.7	90.7	39.7	7.430	10
B 18	9	3.1	6.4	59.0	177.0	170.6	87.5	42.1	7.441	5
B 19	14	3.5	7.8	85.0	192.5	210.7	83.9	44.0	7.427	6
B 20	12	3.0	6.0	72.9	249.6	230.1	90.6	38.7	7.439	6
B 93	10	4.0	-	-	-	-	87.1	37.9	7.427	9
B 94	12	5.1	-	-	-	-	84.7	37.6	7.420	7
B 95	14	3.5	-	-	-	-	84.0	38.9	7.420	6
B 96	16	5.0	-	-	-	-	83.4	39.0	7.402	18
B 97	13	3.1	-	-	-	-	88.5	35.4	7.434	7
B 98	12	4.5	-	-	-	-	86.1	39.9	7.422	14
B 99	11	3.4	-	-	-	-	87.9	38.4	7.419	7
B100	16	3.0	-	-	-	-	87.0	34.0	7.444	6
B101	10	4.2	-	-	-	-	84.5	37.5	7.441	7
B102	9	3.5	-	-	-	-	84.5	37.6	7.420	4
B103	12	4.3	-	-	-	-	84.0	38.5	7.426	8
B104	12	3.3	-	-	-	-	87.0	38.7	7.418	7
B105	12	5.0	-	-	-	-	84.6	38.5	7.428	11
B106	14	4.2	-	-	-	-	84.5	34.3	7.440	9
B107	12	4.6	-	-	-	-	86.0	38.9	7.406	14
B108	12	3.5	-	-	-	-	86.3	36.5	7.436	5
B109	12	4.1	-	-	-	-	87.3	38.0	7.420	7
B110	12	4.0	-	-	-	-	86.4	36.0	7.420	6
B111	10	3.1	-	-	-	-	91.6	35.7	7.420	7
B112	12	3.4	-	-	-	-	88.0	37.0	7.426	15
B113	9	3.7	-	-	-	-	93.4	35.3	7.412	7
B114	7	3.2	-	-	-	-	86.2	39.0	7.430	7
B115	12	3.4	-	-	-	-	88.9	37.5	7.450	7
B116	15	4.1	-	-	-	-	87.2	38.5	7.420	12
B117	12	3.0	-	-	-	-	92.0	31.6	7.436	11
B118	12	3.0	-	-	-	-	89.7	36.5	7.428	7
B119	10	3.0	-	-	-	-	89.7	37.5	7.415	7
B120	8	4.3	-	-	-	-	88.1	36.4	7.425	7
B121	12	4.1	-	-	-	-	89.5	36.4	7.429	14
B122	16	3.1	-	-	-	-	88.9	36.5	7.429	7
B123	14	3.5	-	-	-	-	92.4	37.0	7.430	7
B124	8	3.0	-	-	-	-	91.2	34.0	7.432	8
B125	12	3.0	-	-	-	-	84.5	38.7	7.426	7
B126	12	4.5	-	-	-	-	87.4	37.9	7.420	12
B127	10	4.1	-	-	-	-	88.4	35.3	7.431	10
B128	12	3.4	-	-	-	-	82.3	39.6	7.420	16
B129	7	3.0	-	-	-	-	90.0	33.1	7.436	7
B130	12	3.5	-	-	-	-	89.2	34.0	7.428	7
B131	9	3.6	-	-	-	-	89.0	32.5	7.436	7
B132	10	3.5	-	-	-	-	87.0	36.1	7.424	10
B133	8	3.3	-	-	-	-	91.6	37.6	7.432	7
B134	8	3.5	-	-	-	-	92.5	33.4	7.420	7
B135	12	4.3	-	-	-	-	87.0	36.5	7.417	28
B136	15	3.0	-	-	-	-	90.5	35.2	7.426	7
B137	12	3.6	-	-	-	-	86.4	37.9	7.415	12
B138	9	3.1	-	-	-	-	86.3	36.4	7.426	7
B139	12	3.3	-	-	-	-	90.5	34.0	7.430	5
B140	10	3.0	-	-	-	-	87.6	35.9	7.420	7
B141	12	4.1	-	-	-	-	84.4	41.0	7.420	7
B142	9	4.6	-	-	-	-	88.7	37.6	7.424	12
B143	12	4.0	-	-	-	-	87.0	38.5	7.423	14
B144	10	3.4	-	-	-	-	87.6	34.0	7.423	16
B145	12	4.9	-	-	-	-	87.3	37.9	7.425	4
B146	9	3.0	-	-	-	-	88.2	37.9	7.425	6
B147	12	4.2	-	-	-	-	89.9	38.6	7.420	15
B148	10	3.0	-	-	-	-	93.5	36.5	7.426	7
B149	9	3.2	-	-	-	-	94.6	36.5	7.436	7
B150	10	3.5	-	-	-	-	88.4	37.4	7.434	7
B151	14	4.0	-	-	-	-	87.9	37.3	7.418	7
B152	16	3.8	-	-	-	-	89.5	37.5	7.421	7
B153	12	3.3	-	-	-	-	94.6	31.7	7.432	4
B154	8	3.0	-	-	-	-	88.5	33.9	7.428	7
B155	12	3.5	-	-	-	-	90.5	37.1	7.425	7
B156	10	3.4	-	-	-	-	84.9	34.9	7.424	7
B157	10	3.7	-	-	-	-	88.1	35.0	7.427	10
B158	12	5.0	-	-	-	-	80.9	37.6	7.420	7
B159	12	3.2	-	-	-	-	92.1	34.6	7.436	7
B160	12	4.0	-	-	-	-	89.9	35.6	7.423	32
B161	12	4.4	-	-	-	-	85.6	32.1	7.420	9
B162	12	3.1	-	-	-	-	90.6	37.2	7.409	10
B163	12	3.0	-	-	-	-	92.5	37.1	7.440	7
B164	12	4.5	-	-	-	-	87.3	37.4	7.428	14
n	92	92	20	20	20	20	92	92	92	92
$\bar{x}$	11.49	3.71	6.78	78.68	219.86	198.02	87.99	37.01	7.428	9.00
S.D.	2.07	0.63	1.52	13.81	36.87	27.24	2.91	2.40	0.010	4.75

APPENDIX 5.1.

Pilot in vitro hypersensitivity studies using pulmonary tissues from normal and COPD affected horses.

INTRODUCTION.

As these *in vitro* studies do not follow the general pattern of this thesis (i.e. the therapy of equine COPD), they are presented as an appendix.

In vitro studies on isolated equine pulmonary tissues have been performed by several workers. Chand and Eyre (1978) showed that strips of equine tracheal and bronchial muscle contracted *in vitro* in the presence of histamine, 5-HT, prostaglandin $F_{2\alpha}$, SRS-A, bradykinin and carbachol and relaxed in the presence of isoprenaline, prostaglandin E_1 and E_2. They also demonstrated that mepyramine selectively antagonised contractions to histamine. Earlier, Eyre (1972) had examined the responses of pulmonary venous strips from 3 COPD affected horses which had positive intradermal reactions to *A. fumigatus in vivo*. Tissues from 2 of these horses contracted *in vitro* when challenged with *A. fumigatus* indicating pulmonary vascular hypersensitivity to this antigen in these animals (a Schultz-Dale reaction) (Schultz, 1910; Dale, 1913). Burka *et al.*, (1976) sensitised 4 normal ponies to bovine plasma by an initial intravenous injection of whole bovine plasma followed by a second injection of bovine plasma in Freund's complete adjuvant, given

subcutaneously. Subsequently, pulmonary venous strips from 3 of these same animals contracted *in vitro* to bovine plasma, thus these authors also demonstrated Schultz-Dale reactions in horses.

The aim of the following pilot studies was to determine whether a similar phenomenon could be demonstrated in the pulmonary tissues of COPD affected horses upon antigen challenge. Such information could lead to a better understanding of the pathogenesis of equine COPD.

MATERIALS AND METHODS.

HORSES.

Pulmonary tissues of 10 confirmed COPD affected horses were used in these studies (Table 1). All horses responded positively to *M. faeni* inhalation challenge *in vivo* and were symptomatically affected with COPD at the time of death.

11 horses which (except for horse No. E8) had no recent history of respiratory illness, which had no clinical abnormality of the respiratory system and which were normal on pulmonary function testing, were used as controls (Table 2). Horse No. E8 had an ethmoid haematoma, horses Nos. E1, 5, 7, 9, 10 and 11 suffered various chronic, mainly musculo-skeletal conditions and animals Nos. E2, 3, 4 and 6 were normal experimental ponies.

PREPARATION OF TISSUES.

Each horse was killed by an overdose of pentobarbitone

TABLE 1.

The case details of the COPD affected horses used in the *in vitro* studies.

Horse No.	Breed/Type	Age	Sex	Weight (Kg)
D1	Hunter	23	F	422
D2	Pony	3	F	330
D3	Heavy Hunter	12	Mn	586
D4	T.B.	12	F	475
D5	T.B.	13	F	524
D6	Hunter	16	Mn	530
D7	T.B.	5	Mn	506
D8	Heavy Hunter	11	F	618
D9	Pony	9	Mn	392
D10	T.B.	8	Mn	490

TABLE 2.

The case details of the control horses used in the *in vitro* studies.

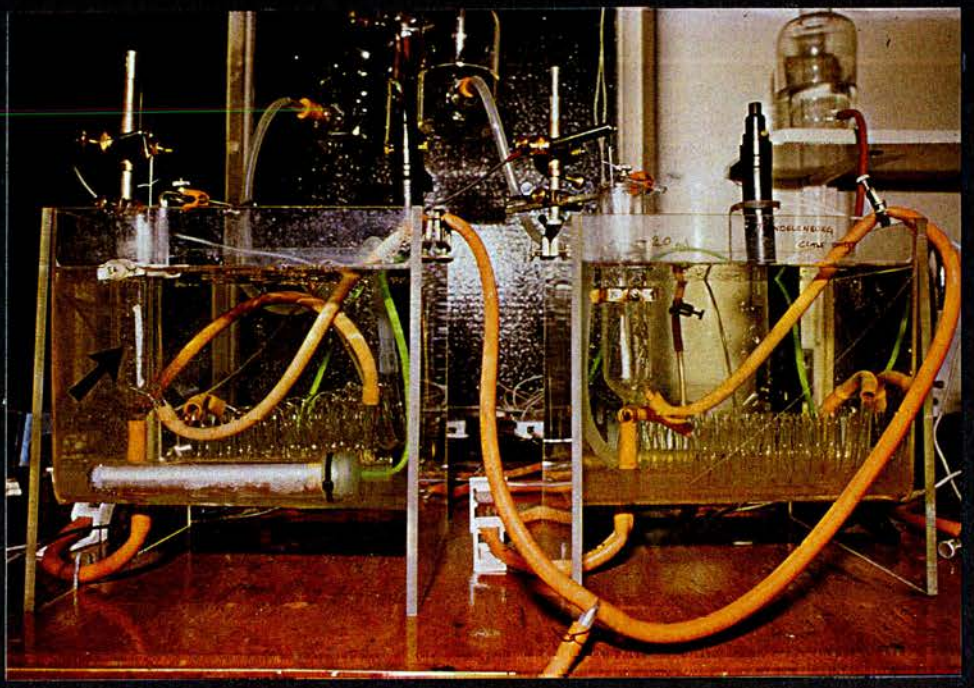
Horse No.	Breed/Type	Age	Sex	Weight (Kg)	Reason for Euthenasia
E1	Hunter	22	Mn	480	Advanced ringbone
E2	Pony	4	Mn	365	Experimental pony
E3	Pony	5	F	348	Experimental pony
E4	Pony	8	Mn	360	Experimental pony
E5	T.B.	12	Mn	465	Navicular disease
E6	Pony	7	F	320	Experimental pony
E7	T.B.	4	M	472	Chronic hind limb lameness
E8	T.B.	10	Mn	510	Ethmoid haematoma and chronic epistaxis
E9	T.B.	9	Mn	456	Navicular disease
E10	Pony	9	Mn	386	Intermittent colic, 1 year duration. Fibrous abdominal adhesions on p.m.
E11	T.B.	12	Mn	465	Navicular disease

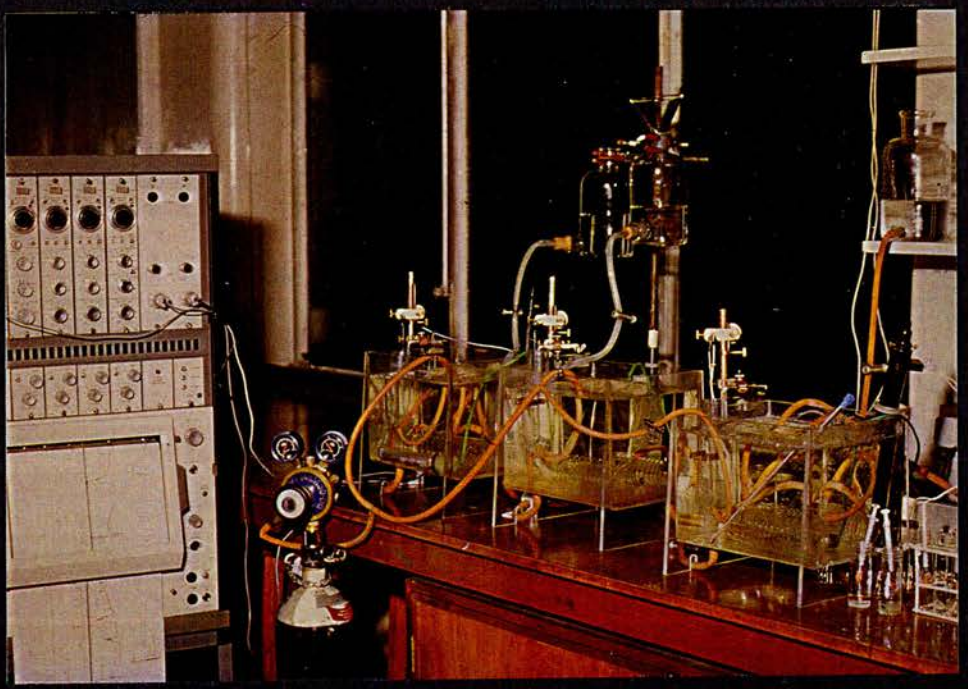
sodium given intravenously. The animals were exanguinated and the lungs were removed within 5 to 10 minutes of death (except for COPD affected horse No. D5, where there was a delay of 20 to 30 minutes between death and the excision of pulmonary tissues). Blocks of pulmonary tissue were rapidly exised and immediately placed in ice-cold Krebs-Henseleit solution. Segments of fourth generation bronchi, pulmonary veins and pulmonary arteries were then cut into spiral strips approximately 30 x 3 mm. Lung parenchymal strips of approximately 30 x 3 x 3 mm were taken from the ventral border of the diaphragmatic lobe using the technique of Lulich, Mitchell and Sparrow (1976). Each preparation was mounted in a 50 ml organ bath (Figure A) and aerated with a mixture of 95% oxygen and 5% carbon dioxide. The organ baths were maintained at 37°C. The strips were allowed to equilibrate under a tension of 3 grams for at least 1 hour. During this time the Krebs-Henseleit solution in the organ baths was changed at 15 minute intervals.

Changes in active tone of the tissues were measured using isometric force transducers (serial no. 4151, Ormed Engineering Ltd., Welwyn Garden City) and recorded using a hot wire pen system (MX4, Devices Ltd., London) (Figure B). Three preparations were run simultaneously. After each test and washing of the bath, a 15 minute rest was allowed. Good responses could usually be obtained for about 8 hours with these

Figure A. Equine bronchial strips mounted in 50 ml organ baths.

Figure B. The organ baths and recording system used in these *in vitro* studies.





tissues.

DRUGS, ANTIGEN AND CONTROL SOLUTIONS.

The tissues' responses were tested to: histamine, 5-HT, acetylcholine, carbachol, adrenaline, isoprenaline, mepyramine, M. faeni antigen and a control which consisted of nutrient broth (Oxoid CMI) with 1% dextrose. The M. faeni antigen and the control were prepared by the Department of Veterinary Pathology, University of Edinburgh, as described by Lawson, et al., (1979). The antigen was diluted in saline to a final concentration of 2.4 mg/ml.

RESULTS.

Histamine, 5-HT, acetylcholine and carbachol caused contraction in all four tissue types from both normal and COPD affected horses. Isoprenaline and adrenaline caused relaxation of all tissues when partially contracted in response to carbachol. Typical responses of the horses' bronchial strips to the above agents are shown in Figure C. In all tissues, mepyramine (10^{-7} to 5×10^{-5} M) selectively antagonised histamine in a dose-dependant fashion.

The nutrient broth control (at doses ranging from 0.2 to 1.0 ml) failed to cause contraction in any of the tissues. Doses of nutrient broth control solution ranging from 1.0 to 2.0 ml caused very slight

Figure C. Responses of bronchial strips from COPD affected horses in Krebs-Henseleit solution mixed with 5% CO_2 in O_2 at 37°C, under a resting tension of 3 grams. Contractions are taken from resting state. Time markers indicate minutes.

Panel (i) shows contractile responses of a bronchial strip to histamine (H), 5-hydroxytryptamine (5-HT), acetylcholine (ACH) and carbachol (CARB) and relaxation to isoprenaline (ISO). Doses are expressed in molar bath concentration.

Panel (ii). A bronchial strip from a COPD affected horse showed no response to increasing concentrations of nutrient broth control (C) but contracted on initial exposure to *M. faeni* (MF). Repeated *M. faeni* challenge at increased concentrations produced no further response from the tissue.

Panel (iii). A bronchial strip from a COPD affected horse showing repeated contractions to *M. faeni*, with contractions becoming progressively weaker.

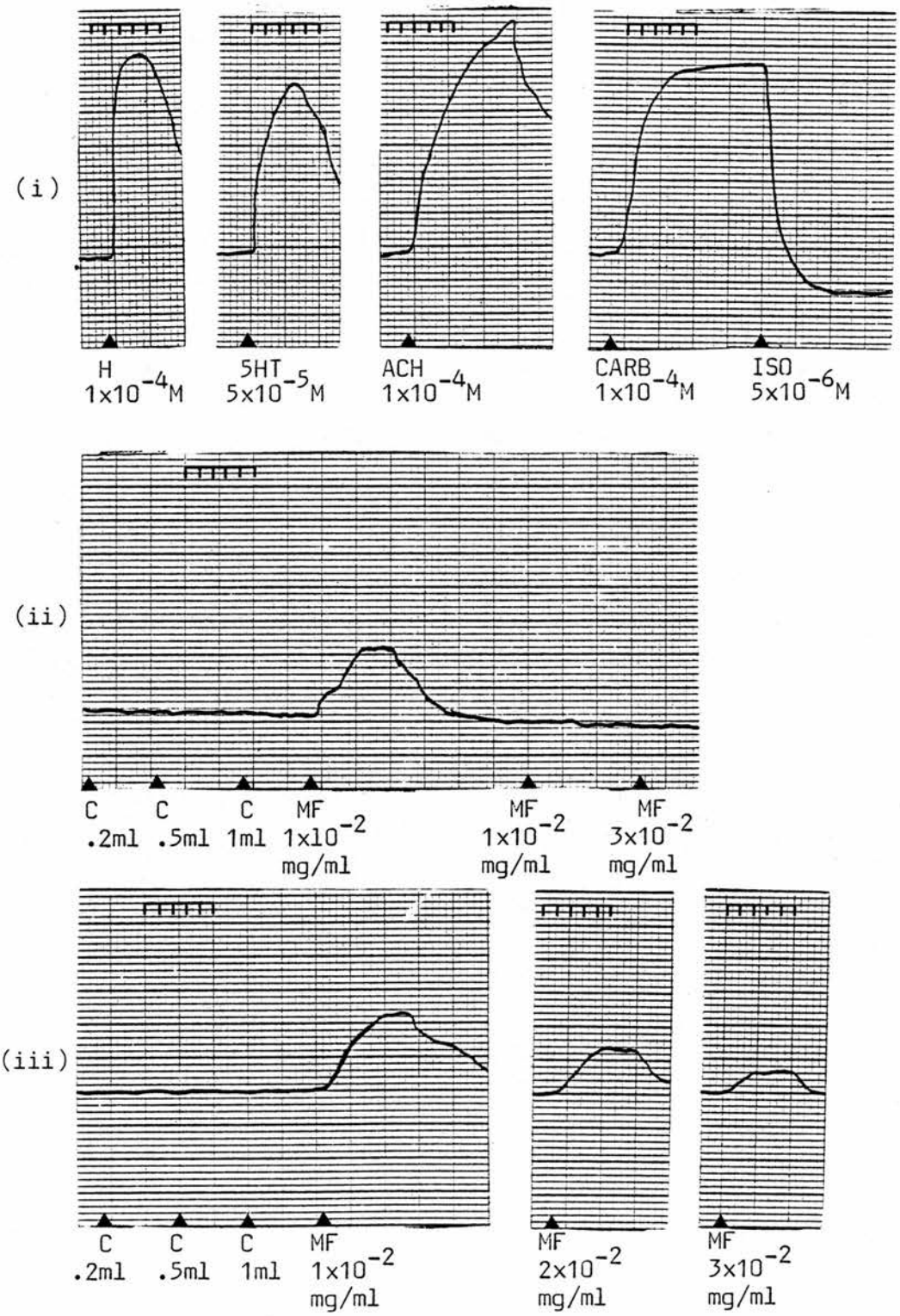

(i)
H
1x10-4M
5HT
5x10-5M
ACH
1x10-4M
CARB
1x10-4M
ISO
5x10-6M
(ii)
C
.2ml
C
.5ml
C
1ml
MF
1x10-2
mg/ml
MF
1x10-2
mg/ml
MF
3x10-2
mg/ml
(iii)
C
.2ml
C
.5ml
C
1ml
MF
1x10-2
mg/ml
MF
2x10-2
mg/ml
MF
3x10-2
mg/ml

Figure C.

contractions in the bronchial strips and lung parenchymal strips of normal horse No. E6 and COPD affected horse No. D4 and in the lung parenchymal strip only of COPD affected horse No. D9. No other tissues from any other horses contracted to this concentration of nutrient broth control. All 4 tissues from 8 out of the 10 COPD affected horses contracted in the presence of M. faeni antigen at concentrations of 0.01 to 0.03 mg M. faeni extract/ml tissue bath fluid. Increasing the M. faeni concentration did not further enhance the strength of contractions in these tissues. Some tissues only responded to a single challenge dose whilst other tissues contracted more than once but rapidly showed tachyphylaxis. The pulmonary vein and bronchial strips from COPD affected horse No. D2 showed very weak contractions to M. faeni whilst the other two tissues from the same animal failed to respond at all to antigen challenge. Tissues from COPD affected horse No. D5 failed to respond to antigen challenge and only showed weak contractions in the presence of histamine, 5-HT and acetylcholine. In this animal, there was a delay of 20 to 30 minutes between death and the excision of the pulmonary tissues which could possibly have reduced the viability of the tissues, resulting in poorer in vitro responses. Tissues from 2 normal horses, Nos. E6 and E9 showed slight contractions to M. faeni at concentrations of 0.01 to

0.03 mg/ml. Increasing the M. faeni concentration did not cause contraction in any of the tissues from the control horses. The contractions to M. faeni in tissues from normal horse No. E6 were only slightly larger in magnitude than those recorded from the same tissues when tested to the nutrient broth control.

CONCLUSIONS.

The isolated tissues from the majority of COPD affected horses with in vivo respiratory hypersensitivity to M. faeni contracted in vitro when challenged with that antigen. This indicated in vitro hypersensitivity of pulmonary vascular and airway tissues in these horses.

APPENDIX 5.2.

Effects of isoprenaline inhalation on some respiratory function parameters in normal and COPD affected horses.

Respiratory Rate (/min.)

Group	Horse Number	0	10	20	30	60	120	240	360
		Time after treatment (min.)							
NORMAL HORSES	A21	12	9	6	9	10	10	14	12
	A22	9	16	14	12	14	12	14	12
	A23	12	12	12	10	12	12	11	12
	A24	13	24	26	18	12	12	14	9
	A25	9	8	9	9	8	9	10	10
	A26	10	9	9	12	10	10	14	11
	A27	10	12	14	12	10	12	9	12
	A28	12	12	12	12	12	12	10	12
MEAN ± S.D.		10.88	12.75	12.75	11.75	11.0	11.13	12.0	11.25
		1.55	5.20	6.02	2.87	1.85	1.25	2.20	1.16
Statistical difference of post treatment values from resting values			NS	NS	NS	NS	NS	NS	NS
COPD AFFECTED HORSES	B21	18	14	10	12	18	18	22	20
	B22	20	18	10	14	16	18	16	16
	B23	18	12	9	10	18	20	16	18
	B24	16	16	10	12	18	18	14	16
	B25	18	14	10	9	18	20	16	16
	B26	9	12	12	9	9	10	10	9
	B27	9	12	18	16	10	9	12	10
	B28	12	18	16	10	12	12	12	10
	B29	12	16	12	10	12	14	14	16
	B30	20	18	14	12	20	20	18	17
	B31	33	27	24	18	20	30	28	30
	B32	20	20	16	12	20	18	18	19
MEAN ± S.D.		17.08	16.42	13.42	12.00	15.92	17.25	16.33	16.42
		6.50	4.29	4.44	2.80	4.06	5.61	4.89	5.60
Statistical difference of post treatment values from resting values			NS	NS	••	NS	NS	NS	NS
Statistical difference from corresponding values in normal horses		•	NS	NS	NS	••	••	•	•

NS = not significant.
• = P < 0.05
•• = P < 0.01
••• = P < 0.001

Max. Δ Ppl (cm Hg).

Group	Horse Number	0	10	20	30	60	120	240	Maximum Response
		Time after treatment (min.)							
NORMAL HORSES	A21	4.0	2.5	3.2	3.5	3.0	3.5	4.1	
	A22	3.5	2.0	2.4	3.7	2.5	3.1	2.8	
	A23	3.6	5.1	2.5	4.2	3.5	3.4	3.9	
	A24	3.0	3.4	2.8	3.0	3.7	3.0	4.2	
	A25	3.0	3.0	3.0	2.6	3.6	3.5	3.0	
	A26	3.4	3.1	3.5	3.8	3.0	3.2	3.8	
	A27	3.7	3.5	3.2	4.7	3.2	3.5	4.0	
	A28	2.8	2.9	4.1	3.5	4.0	3.1	3.7	
MEAN ± S.D.		3.38	3.19	3.09	3.63	3.31	3.29	3.69	
		0.41	0.91	0.55	0.65	0.48	0.21	0.51	
Statistical difference of post treatment values from resting values			NS	NS	NS	NS	NS	NS	
COPD AFFECTED HORSES	B21	10.5	3.5	4.2	4.5	9.0	10.5	12.1	3.5
	B22	23.2	5.0	5.5	6.6	12.6	23.0	21.7	4.9
	B23	14.5	5.0	5.0	5.6	12.1	14.6	16.0	4.1
	B24	17.0	4.6	5.0	5.3	13.5	17.1	16.9	4.2
	B25	13.5	6.1	5.0	7.0	14.3	13.5	14.7	4.5
	B26	16.5	5.0	4.3	5.0	14.0	17.0	15.8	4.3
	B27	18.0	5.2	4.5	5.8	16.3	18.5	19.2	4.0
	B28	26.4	6.0	6.9	9.5	19.0	26.0	24.3	5.6
	B29	15.6	7.1	6.5	11.4	15.3	15.7	17.0	6.0
	B30	13.5	5.7	5.2	6.5	13.0	14.0	14.2	5.0
	B31	33.2	13.0	13.4	13.0	29.5	35.5	32.9	13.0
	B32	26.0	12.1	10.0	10.4	26.2	26.0	25.3	9.3
MEAN ± S.D.		18.99	6.53	6.29	7.55	16.23	19.28	19.18	5.70
		6.73	2.96	2.75	2.80	5.98	7.08	5.91	2.75
Statistical difference of post treatment values from resting values			•••	•••	•••	•	NS	NS	•••

NS = not significant
• = P<0.05
•• = P<0.01
••• = P<0.001

PaO_2 (mm Hg)

Group	Horse Number	0	10	20	60	120	240
		Time after treatment (min.)					
NORMAL HORSES	A21	87.2	82.0	81.9	88.0	89.5	89.3
	A22	94.5	93.9	90.1	92.0	90.0	96.8
	A23	93.0	90.5	84.5	89.3	94.1	90.9
	A24	88.1	80.1	79.3	87.5	86.4	90.6
	A25	91.2	80.0	80.4	93.1	96.0	96.9
	A26	92.1	90.4	86.3	95.4	96.1	84.0
	A27	92.1	83.2	84.8	90.5	90.6	93.0
	A28	89.0	88.2	82.9	92.1	86.4	90.9
MEAN ± S.D.		90.90	86.04	83.78	90.99	91.14	91.55
		2.55	5.37	3.46	2.68	3.90	4.18
Statistical difference of post treatment values from resting values			•	•••	NS	NS	NS
COPD AFFECTED HORSES	B21	80.1	75.0	72.9	86.5	85.0	78.4
	B22	79.1	78.5	66.4	84.3	85.0	80.4
	B23	73.0	75.2	67.9	77.0	75.1	71.4
	B24	72.0	65.6	65.8	76.9	73.0	70.1
	B25	67.2	61.0	60.4	74.9	69.1	70.0
	B26	69.4	62.2	63.1	64.2	65.1	68.9
	B27	72.0	70.4	64.9	76.1	70.4	69.5
	B28	64.7	63.9	74.4	74.2	72.9	64.5
	B29	63.9	60.1	59.9	71.0	66.0	71.6
	B30	76.0	64.2	60.0	70.9	80.2	77.3
	B31	62.8	61.6	58.1	68.0	66.4	63.9
	B32	58.5	51.0	51.5	66.0	62.1	60.7
MEAN ± S.D.		69.89	65.56	63.78	74.17	72.53	70.56
		6.71	7.84	6.39	6.74	7.61	5.94
Statistical difference of post treatment values from resting values			••	••	••	•	NS

$PaCO_2$ (mm Hg).

Group	Horse Number	0	10	20	60	120	240
		Time after treatment (min.)					
NORMAL HORSES	A21	37.6	39.0	37.0	38.2	38.0	37.8
	A22	31.2	33.8	33.0	30.9	34.0	32.5
	A23	32.7	33.4	32.9	34.0	35.0	34.5
	A24	34.0	36.0	34.9	30.3	32.0	30.9
	A25	32.1	32.5	31.0	30.8	31.2	30.4
	A26	34.6	32.2	33.0	37.9	34.6	32.1
	A27	35.4	34.3	34.2	33.9	34.0	34.1
	A28	35.0	35.9	35.1	33.2	31.0	37.6
MEAN ± S.D.		34.08	34.53	33.89	33.65	33.73	33.74
		2.05	2.20	1.82	3.07	2.32	2.82
Statistical difference of post treatment values from resting values			NS	NS	NS	NS	NS
COPD AFFECTED HORSES	B21	40.1	39.0	38.3	39.2	39.0	40.1
	B22	36.0	36.2	35.6	34.0	33.2	34.0
	B23	38.0	38.3	37.2	36.4	36.0	38.3
	B24	41.2	41.6	40.0	40.8	40.6	40.1
	B25	36.1	36.2	35.3	35.0	34.2	39.5
	B26	37.3	37.8	39.1	40.0	38.2	38.1
	B27	39.2	40.4	42.9	38.1	39.4	40.1
	B28	36.3	37.0	41.0	35.0	36.0	37.3
	B29	44.3	42.8	42.6	41.4	42.8	40.0
	B30	40.5	40.0	38.3	39.0	40.1	39.6
	B31	43.2	43.5	42.1	40.3	40.5	41.0
	B32	38.2	39.1	38.0	36.3	37.1	37.6
MEAN ± S.D.		39.22	39.33	39.2	37.96	38.09	38.55
		2.73	2.43	2.57	2.53	2.85	2.04
Statistical difference of post treatment values from resting values			NS	NS	•	••	NS

NS = not significant
• = P<0.05
•• = P<0.01
••• = P<0.001

Heart Rate (/min.)

Group	Horse Number	0	10	20	30	60	120	240
		Time after treatment (min.)						
NORMAL HORSES	A21	38	88	72	62	48	36	34
	A22	40	86	66	54	40	36	36
	A23	34	66	70	66	50	36	36
	A24	38	68	48	40	38	36	32
	A25	32	66	68	60	42	32	34
	A26	34	44	64	52	36	36	36
	A27	36	48	68	66	38	36	32
	A28	32	46	72	62	42	32	32
MEAN ± S.D.		35.50	59.0	66.0	57.75	41.75	35.00	34.00
		2.98	18.61	7.78	8.78	4.95	1.85	1.85
Statistical difference of post treatment values from resting values			••	•••	•••	•	NS	NS
COPD AFFECTED HORSES	B21	36	44	48	48	36	36	34
	B22	36	48	52	40	36	36	34
	B23	34	48	68	56	36	36	32
	B24	32	44	62	62	38	34	34
	B25	42	56	68	56	36	34	36
	B26	34	46	56	48	36	34	32
	B27	32	42	56	48	36	36	34
	B28	36	56	70	64	56	36	32
	B29	36	52	44	42	38	32	36
	B30	32	48	68	64	48	34	36
	B31	32	44	62	50	40	32	34
	B32	34	54	76	66	54	36	32
MEAN ± S.D.		34.67	43.67	60.83	53.67	42.50	34.67	33.83
		2.87	4.50	9.74	8.94	8.88	1.56	1.59
Statistical difference of post treatment values from resting values			•••	•••	•••	•	NS	NS

APPENDIX 5.3.

Effects of atropine inhalation on some respiratory function parameters in normal and COPD affected horses.

Respiratory Rate (/mins.)

Group	Horse Number	Time after treatment (min.) 0	10	20	30	60	120	240	360
NORMAL HORSES	A29	24	24	22	18	16	18	18	20
	A30	18	26	24	18	18	16	18	14
	A31	9	10	12	12	10	10	12	9
	A32	9	9	18	10	9	10	9	12
	A33	8	8	9	8	8	9	8	10
	A34	10	12	12	10	10	10	9	11
	A35	10	12	12	12	10	12	12	12
	A36	14	9	12	14	12	12	14	10
MEAN ± S.D.		12.75 ± 5.63	13.75 ± 7.11	15.13 ± 5.49	12.75 ± 3.69	11.63 ± 3.54	12.13 ± 3.23	12.50 ± 3.93	12.25 ± 3.49
Statistical difference of post treatment values from resting values.			NS	NS	NS	NS	NS	NS	NS
COPD AFFECTED HORSES	B33	28	22	12	12	14	14	20	18
	B34	18	12	9	12	14	18	18	20
	B35	12	9	9	10	14	20	20	20
	B36	24	22	16	16	18	20	22	20
	B37	20	16	10	14	16	20	18	18
	B38	19	14	10	12	14	18	18	16
	B39	28	20	12	11	14	18	26	25
	B40	20	20	16	14	16	18	20	22
	B41	12	16	14	12	12	12	12	16
	B42	14	12	12	16	12	12	14	16
	B43	12	14	12	10	10	9	10	14
	B44	16	16	12	12	10	12	14	12
MEAN ± S.D.		18.58 ± 5.79	16.08 ± 4.19	12.00 ± 2.37	12.58 ± 2.02	13.67 ± 2.39	15.92 ± 3.87	17.67 ± 4.50	18.08 ± 3.58
Statistical difference of post treatment values from resting values.			•	••	••	••	NS	NS	NS
Statistical difference from corresponding values in normal horses.		•	NS	NS	NS	NS	•	•	••

NS = not significant
• = P<0.05
•• = P<0.01
••• = P<0.001

Max. Δ Ppl (mm Hg)

Group	Horse Number	Time after treatment (min.) 0	10	20	30	60	120	240	Maximum Response
NORMAL HORSES	A29	3.0	3.2	3.0	3.5	3.0	2.9	3.5	
	A30	3.0	3.1	2.9	3.0	3.0	3.5	3.0	
	A31	3.3	3.0	3.5	3.6	3.1	3.6	3.3	
	A32	3.1	3.0	4.1	3.0	3.4	3.0	3.7	
	A33	4.5	4.5	3.9	4.1	3.5	3.9	4.2	
	A34	2.7	3.0	3.1	2.8	3.0	3.5	3.5	
	A35	3.9	3.4	3.5	3.6	3.5	4.1	3.3	
	A36	3.5	3.6	3.5	3.4	3.5	2.5	3.5	
MEAN ± S.D.		3.38 ± 0.58	3.35 ± 0.51	3.44 ± 0.42	3.38 ± 0.42	3.25 ± 0.25	3.38 ± 0.54	3.5 ± 0.35	
Statistical difference of post treatment values from resting values.			NS	NS	NS	NS	NS	NS	
COPD AFFECTED HORSES	B33	18.4	12.0	5.1	5.0	8.5	16.1	17.5	5.0
	B34	20.1	9.1	5.0	7.4	15.2	20.5	21.2	5.0
	B35	23.5	10.7	7.2	8.1	9.0	19.3	23.8	7.0
	B36	14.0	10.5	7.3	6.9	7.3	13.8	16.0	7.3
	B37	12.5	9.3	4.0	4.5	9.5	12.0	11.4	3.5
	B38	15.5	11.2	5.0	6.0	10.0	15.0	16.6	5.0
	B39	21.5	16.4	7.1	7.8	11.9	15.1	21.0	7.1
	B40	19.0	15.0	9.3	10.0	12.7	16.4	20.3	9.2
	B41	13.5	6.0	3.5	4.3	7.2	9.5	11.7	3.5
	B42	15.0	11.3	7.0	7.0	7.5	9.6	14.5	6.4
	B43	17.4	9.6	8.1	9.2	10.3	15.0	18.1	8.1
	B44	14.5	11.0	10.4	10.5	11.2	13.3	13.4	10.4
MEAN ± S.D.		17.08 ± 3.47	11.01 ± 2.70	6.58 ± 2.11	7.23 ± 2.05	10.03 ± 2.42	14.63 ± 3.34	17.13 ± 3.95	6.46 ± 2.15
Statistical difference of post treatment values from resting values.			•••	•••	•••	•••	••	NS	•••
Statistical difference from corresponding values in normal horses.		•••	•••	•••	•••	•••	•••	•••	

NS = not significant
• = P<0.05
•• = P<0.01
••• = P<0.001

PaO_2 (mm Hg).

Group	Horse Number	Time after treatment (min.) 0	10	20	60	120	240
NORMAL HORSES	A29	94.1	90.0	90.5	95.8	94.2	93.1
	A30	85.5	80.6	84.7	90.5	92.0	89.8
	A31	87.0	85.8	86.1	88.6	90.3	92.2
	A32	95.7	90.9	92.1	94.9	95.3	90.5
	A33	91.3	89.4	89.0	92.5	90.9	95.6
	A34	90.2	86.0	86.4	93.1	94.0	94.0
	A35	92.5	93.0	86.4	90.6	90.9	96.0
	A36	96.0	90.5	96.1	94.0	95.5	90.2
MEAN ± S.D.		91.54 ± 3.85	88.28 ± 3.94	88.91 ± 3.83	92.5 ± 2.45	92.89 ± 2.10	92.68 ± 2.42
Statistical difference of post treatment values from resting values.			••	••	NS	NS	NS
COPD AFFECTED HORSES	B33	72.9	70.1	85.1	89.8	96.5	74.1
	B34	65.6	66.0	71.1	81.0	69.5	77.6
	B35	66.7	60.2	66.7	68.2	71.4	64.2
	B36	85.1	87.3	86.4	89.3	86.0	84.1
	B37	66.4	60.9	60.0	73.9	70.2	72.1
	B38	71.5	69.4	70.9	78.4	82.1	75.4
	B39	68.9	68.0	70.2	76.4	75.0	70.1
	B40	70.4	66.8	68.1	76.9	75.0	76.2
	B41	72.6	70.1	68.4	74.6	78.0	76.3
	B42	68.5	65.0	60.4	67.0	70.3	72.4
	B43	70.0	71.4	69.3	75.0	75.9	72.6
	B44	71.0	62.5	62.0	71.6	75.9	70.4
MEAN ± S.D.		70.8 ± 5.10	68.37 ± 6.92	69.88 ± 8.38	76.84 ± 7.13	77.15 ± 7.85	73.79 ± 4.85
Statistical difference of post treatment values from resting values.			•	NS	••	••	•
Statistical difference from corresponding values in normal horses.		•••	•••	•••	•••	•••	•••

NS = not significant
• = P<0.05
•• = P<0.01
••• = P<0.001

$PaCO_2$ (mm Hg).

Group	Horse Number	Time after treatment (min.) 0	10	20	60	120	240
NORMAL HORSES	A29	36.0	36.2	36.3	36.1	34.3	35.0
	A30	37.2	36.5	33.5	34.0	34.9	34.1
	A31	36.3	36.8	35.9	35.5	36.0	36.2
	A32	36.0	35.4	38.2	35.1	36.0	35.9
	A33	37.0	36.5	36.3	37.1	36.8	37.0
	A34	35.4	35.9	33.0	34.9	35.0	35.5
	A35	34.6	34.0	34.2	34.3	34.7	35.0
	A36	32.1	33.5	34.0	32.0	33.5	33.1
MEAN ± S.D.		35.58 ± 1.63	35.60 ± 1.23	35.18 ± 1.78	34.88 ± 1.52	35.15 ± 1.06	35.23 ± 1.23
Statistical difference of post treatment values from resting values.			NS	NS	NS	NS	NS
COPD AFFECTED HORSES	B33	37.2	37.3	39.5	42.1	37.1	38.3
	B34	41.2	40.5	37.6	35.7	38.8	38.9
	B35	43.9	42.4	40.4	40.9	39.8	42.0
	B36	37.1	37.0	34.1	34.9	35.0	37.3
	B37	38.3	37.9	37.0	36.5	36.9	37.0
	B38	39.0	40.2	39.2	37.5	38.0	38.3
	B39	40.1	38.9	39.4	38.0	38.5	39.2
	B40	39.9	40.0	39.2	38.0	38.1	38.8
	B41	37.0	37.5	37.4	38.9	37.6	37.2
	B42	39.3	38.0	37.5	39.0	38.5	38.0
	B43	40.2	39.4	38.5	38.0	40.3	41.0
	B44	37.3	37.0	38.6	37.5	38.0	36.4
MEAN ± S.D.		39.21 ± 2.05	38.84 ± 1.69	38.2 ± 1.65	38.08 ± 2.01	38.05 ± 1.63	38.53 ± 1.63
Statistical difference of post treatment values from resting values.			NS	NS	NS	•	•
Statistical difference from corresponding values in normal horses.		•••	•••	••	••	•••	•••

Heart Rate (/min.)

Group	Horse Number	Time after treatment (min.) 0	10	20	30	60	120	240
NORMAL HORSES	A29	38	50	48	38	37	38	38
	A30	38	48	48	40	38	36	38
	A31	32	38	52	48	36	32	34
	A32	32	38	52	42	36	32	32
	A33	34	40	48	40	36	32	32
	A34	36	38	42	38	36	36	36
	A35	32	36	60	62	52	36	32
	A36	36	40	60	58	36	34	36
MEAN ± S.D.		34.75 ± 2.6	41.00 ± 5.13	51.25 ± 6.23	45.75 ± 9.41	38.5 ± 5.53	34.5 ± 2.33	34.75 ± 2.6
Statistical difference of post treatment values from resting values.			••	•••	•	NS	NS	NS
COPD AFFECTED HORSES	B33	36	36	48	44	36	36	36
	B34	36	40	40	40	36	34	36
	B35	32	36	34	30	32	32	30
	B36	32	34	32	32	32	32	32
	B37	38	46	58	50	42	36	36
	B38	34	48	48	40	36	36	36
	B39	34	38	48	48	36	36	36
	B40	36	48	60	56	40	36	36
	B41	36	48	52	52	46	38	36
	B42	36	46	56	54	42	36	36
	B43	36	48	50	50	42	38	36
	B44	38	52	56	54	40	36	36
MEAN ± S.D.		35.33 ± 1.97	43.33 ± 6.11	48.5 ± 9.07	45.83 ± 8.67	38.33 ± 4.33	35.5 ± 1.93	35.17 ± 1.99
Statistical difference of post treatment values from resting values.			•••	•••	•••	••	NS	NS
Statistical difference from corresponding values in normal horses.		NS	NS	NS	NS	NS	NS	NS

APPENDIX 5.4.

Effects of terbutaline inhalation on some respiratory function parameters in normal and COPD affected horses.

Respiratory Rate (/mins.)

Group	Horse Number	0	10	20	30	60	120	240	360
		Time after treatment (mins.)							
NORMAL HORSES	A37	9	12	12	12	10	10	12	10
	A38	12	12	10	9	12	10	9	10
	A39	18	24	20	16	16	12	12	12
	A40	8	10	9	10	9	9	10	9
	A41	8	10	8	10	8	9	8	8
	A42	8	7	8	8	8	8	8	9
	A43	14	16	12	12	12	9	8	10
	A44	10	12	10	9	10	9	9	10
MEAN ± S.D.		10.88 3.60	12.88 5.17	11.13 3.91	10.75 2.55	10.63 2.67	9.50 1.20	9.50 1.69	9.75 1.16
Statistical difference of post treatment values from resting values.			NS	NS	NS	NS	NS	NS	NS
COPD AFFECTED HORSES	B45	12	8	10	9	10	10	12	8
	B46	14	12	10	10	10	10	12	12
	B47	16	10	6	9	10	10	12	10
	B48	18	12	12	10	10	12	12	12
	B49	12	12	12	12	12	12	12	12
	B50	10	9	8	9	10	9	12	9
	B51	24	22	12	12	10	9	18	22
	B52	23	14	13	10	12	10	20	14
	B53	24	26	26	24	24	20	22	26
	B54	16	24	20	10	10	12	12	24
	B55	23	18	12	12	14	12	16	18
	B56	18	14	14	11	12	12	14	14
MEAN ± S.D.		17.50 5.04	15.08 6.02	12.92 5.35	11.50 4.10	12.00 4.00	11.50 2.94	14.50 3.63	15.08 6.02
Statistical difference of post treatment values from resting values.			NS	NS	•••	••	••	••	NS
Statistical difference from comparative values in normal horses.		••	NS	NS	NS	NS	NS	NS	•

Max. Δ Ppl (mm Hg).

Group	Horse Number	0	10	20	30	60	120	240	360	Maximum Response
		Time after treatment (mins.)								
NORMAL HORSES	A37	3.6	3.1	3.2	3.0	3.5	3.0	2.9	3.0	
	A38	4.5	4.2	5.0	4.4	4.7	4.0	3.6	3.5	
	A39	3.0	2.7	3.1	2.6	3.0	3.7	3.5	3.8	
	A40	3.2	3.5	3.3	3.5	4.0	3.3	4.2	3.2	
	A41	3.6	3.0	2.7	3.0	2.8	4.5	3.5	3.5	
	A42	3.4	3.3	3.1	3.0	3.1	3.5	2.8	4.1	
	A43	4.0	3.7	3.1	3.2	3.4	4.4	5.0	3.4	
	A44	3.8	3.0	3.0	3.8	3.5	3.6	4.5	4.0	
MEAN ± S.D.		3.64 0.47	3.31 0.48	3.31 0.70	3.31 0.57	3.5 0.61	3.75 0.52	3.75 0.77	3.56 0.38	
Statistical difference of post treatment values from resting values.			NS	NS	NS	NS	NS	NS	NS	
COPD AFFECTED HORSES	B45	19.5	9.5	5.1	5.2	7.2	8.0	11.1	12.5	5.0
	B46	12.6	9.0	4.6	5.0	5.0	6.1	8.9	7.4	4.5
	B47	14.5	10.2	5.9	5.3	5.8	7.0	10.5	10.7	5.1
	B48	18.0	11.5	7.0	6.5	7.4	9.4	12.0	13.4	6.5
	B49	14.5	9.3	9.4	9.6	9.1	10.0	11.6	13.0	9.1
	B50	26.1	8.7	9.0	11.0	12.0	14.2	10.1	18.5	8.0
	B51	30.0	13.8	3.8	5.9	6.4	7.8	12.8	20.5	3.7
	B52	27.0	14.9	13.2	15.1	15.1	16.3	15.0	19.2	13.0
	B53	20.3	13.0	16.0	17.4	18.0	18.3	14.3	18.2	13.0
	B54	12.7	8.8	5.1	6.3	6.0	7.6	9.7	9.1	5.1
	B55	10.5	7.0	4.4	4.7	5.2	6.0	7.5	6.5	4.3
	B56	12.0	9.1	3.5	4.0	4.3	5.0	10.1	7.5	3.5
MEAN ± S.D.		18.14 6.55	10.40 2.38	7.25 3.95	8.0 4.38	8.49 4.35	9.64 4.32	11.13 2.16	13.04 5.02	6.73 3.37
Statistical difference of post treatment values from resting values.			•••	•••	•••	•••	•••	•••	•••	•••
Statistical difference from comparative values in normal horses.		•••	•••	•••	•	•	••	••	•••	

PaO_2 (mm Hg)

Group	Horse Number	0	10	20	30	60	120	240	360
		Time after treatment (mins.)							
NORMAL HORSES	A37	84.0	83.0	74.8	85.0	86.7	87.2	84.9	
	A38	90.6	85.6	84.7	88.4	92.1	90.9	92.6	
	A39	89.7	86.4	89.7	88.0	90.4	87.3	95.3	
	A40	92.0	92.8	85.4	87.0	90.5	93.1	88.0	
	A41	91.3	87.7	85.4	89.2	95.0	94.9	87.9	
	A42	96.0	95.3	90.4	95.9	96.8	97.0	90.2	
	A43	97.9	92.1	94.4	95.3	96.0	97.8	94.9	
	A44	94.4	90.4	83.1	84.5	80.1	89.1	93.6	
MEAN ± S.D.		91.99 4.28	89.16 4.17	85.99 5.85	89.16 4.29	90.95 5.53	92.16 4.19	90.93 3.77	
Statistical difference of post treatment values from resting values.			•	••	•	NS	NS	NS	
COPD AFFECTED HORSES	B45	66.1	64.9	61.2	62.9	70.8	69.7	72.4	
	B46	73.0	68.0	67.9	77.5	74.9	70.3	68.9	
	B47	71.0	70.2	63.8	70.2	76.9	70.1	74.2	
	B48	66.0	67.4	62.3	81.9	88.4	77.1	72.5	
	B49	66.9	58.0	57.0	63.2	79.0	80.3	80.5	
	B50	65.8	63.2	68.8	76.2	83.1	76.9	82.3	
	B51	63.2	64.5	69.9	76.2	85.0	80.9	77.3	
	B52	54.8	55.1	51.0	60.4	65.7	70.5	62.1	
	B53	66.4	63.0	62.9	72.4	79.2	74.5	62.4	
	B54	80.4	81.2	76.3	85.2	87.0	82.4	78.3	
	B55	76.4	69.3	67.8	78.3	72.0	75.4	77.9	
	B56	82.4	84.5	80.8	87.5	88.0	87.2	83.1	
MEAN ± S.D.		69.37 7.73	67.44 8.45	65.81 8.04	74.33 8.80	79.17 7.39	76.28 5.64	74.33 7.03	
Statistical difference of post treatment values from resting values.			NS	•	•	••	•••	•	
Statistical difference from comparative values in normal horses.		•••	•••	•••	•••	••	•••	•••	

$PaCO_2$ (mm Hg)

Group	Horse Number	0	10	20	30	60	120	240	360
		Time after treatment (mins.)							
NORMAL HORSES	A37	33.8	35.0	35.5	34.2	34.0	34.2	36.8	
	A38	34.5	32.5	32.8	33.0	33.5	33.1	35.1	
	A39	33.5	35.6	35.5	35.2	35.5	34.0	32.7	
	A40	33.4	35.0	36.1	35.0	34.3	32.9	36.3	
	A41	33.0	30.6	35.2	31.0	32.1	32.8	35.4	
	A42	32.1	33.8	34.5	31.0	31.9	32.4	30.7	
	A43	30.2	31.3	30.0	31.9	30.0	32.3	34.0	
	A44	39.3	40.5	37.9	40.5	41.0	43.3	37.6	
MEAN ± S.D.		33.73 2.60	34.29 3.1	34.69 2.38	33.98 3.12	34.04 3.29	34.39 3.66	34.83 2.28	36.5 5.53
Statistical difference of post treatment values from resting values.			NS	NS	NS	NS	NS	NS	NS
COPD AFFECTED HORSES	B45	38.5	38.4	37.2	37.7	38.3	38.5	39.6	
	B46	38.0	39.5	41.2	37.9	36.4	38.1	35.7	
	B47	39.6	39.2	41.4	38.2	37.9	40.4	38.0	
	B48	42.9	42.1	42.0	37.4	38.0	40.3	36.2	
	B49	42.9	41.5	41.8	43.7	40.3	39.4	42.1	
	B50	44.4	44.0	44.1	42.3	39.0	37.0	40.1	
	B51	38.9	39.2	37.2	35.4	35.3	36.0	34.9	
	B52	42.9	40.1	38.1	37.3	36.8	37.0	39.5	
	B53	32.6	34.0	34.7	35.2	35.0	36.3	34.9	
	B54	34.1	34.5	32.1	33.0	32.9	33.5	37.3	
	B55	40.6	41. 0	39.9	37.8	38.0	38.2	35.3	
	B56	44.8	44.6	42.2	40.1	39.0	39.5	36.7	
MEAN ± S.D.		39.97 3.88	39.84 3.23	39.33 3.52	38.00 2.95	37.24 2.06	37.85 2.01	37.53 2.35	35.00 1.81
Statistical difference of post treatment values from resting values.			NS	NS	•	••	•	•	
Statistical difference from comparative values in normal horses.		••	NS	NS	NS	NS	NS	NS	NS

Heart Rate

Group	Horse Number	0	10	20	30	60	120	240	360
		Time after treatment (mins.)							
NORMAL HORSES	A37	60	48	48	44	44	38	36	36
	A38	48	54	48	48	42	36	34	36
	A39	48	42	54	42	40	38	36	36
	A40	36	48	52	46	38	36	36	36
	A41	48	44	52	42	32	32	32	34
	A42	48	44	52	42	32	32	32	34
	A43	38	42	46	36	36	34	34	36
	A44	32	56	46	36	36	32	32	36
MEAN ± S.D.		47.0 5.53	49.75 3.11	42.0 4.28	37.5 4.38	35.0 2.62	34.0 1.86	35.50 0.93	
Statistical difference of post treatment values from resting values.			•	•••	NS	NS	NS	NS	NS
COPD AFFECTED HORSES	B45	36	42	46	54	36	36	36	36
	B46	32	40	46	56	36	34	36	36
	B47	36	46	52	42	36	40	34	32
	B48	36	40	46	42	38	40	34	32
	B49	36	42	50	46	36	36	36	36
	B50	32	44	56	44	38	36	36	36
	B51	36	60	42	46	42	38	34	36
	B52	32	42	49	52	46	36	36	34
	B53	36	42	42	46	40	36	36	36
	B54	36	42	48	42	38	36	36	36
	B55	36	46	56	56	46	36	36	36
	B56	32	42	52	50	40	32	32	34
MEAN ± S.D.		35.00 1.81	42.33 2.06	48.75 4.69	48.0 5.39	39.33 3.65	35.17 1.80	34.83 1.80	35.0 1.60
Statistical difference of post treatment values from resting values.			•••	•••	•••	••	NS	NS	NS
Statistical difference from comparative values in normal horses.		•	NS	•	NS	NS	NS	NS	

NS = not significant
• = P<0.05
•• = P<0.01
••• = P<0.001

APPENDIX 5.5.

Effects of Atropine (by intravenous injection) on some respiratory function parameters in normal and COPD affected horses.

Respiratory Rate (/mins.)

Group	Horse Number	Time after treatment (mins.) 0	10	20	30	60	120	240	360
NORMAL HORSES	A45	13	14	15	12	13	13	12	12
	A46	16	14	12	14	12	12	14	10
	A47	10	12	10	12	10	10	10	12
	A48	13	12	10	12	12	14	12	9
	A49	16	12	12	14	12	12	14	11
	A50	12	16	14	12	14	12	12	14
	A51	14	12	14	12	12	14	12	12
	A52	24	30	24	20	16	18	18	16
MEAN ± S.D.		14.75 ± 4.23	15.25 ± 6.14	13.88 ± 4.49	13.5 ± 2.78	12.63 ± 1.77	13.13 ± 2.36	13.0 ± 2.39	12.00 ± 2.20
Statistical difference of post treatment values from resting values			NS	NS	NS	NS	NS	•	NS
COPD AFFECTED HORSES	B57	21	16	16	17	18	20	20	18
	B58	21	14	14	16	18	20	20	19
	B59	17	12	10	9	9	12	20	18
	B60	18	14	14	13	16	16	18	16
	B61	20	16	12	14	16	18	21	22
	B62	18	20	10	12	12	14	17	20
	B63	24	16	13	18	18	20	22	20
	B64	19	12	10	10	12	18	18	16
	B65	30	30	24	18	18	20	24	28
	B66	20	18	10	12	16	18	20	18
	B67	18	14	16	14	15	18	20	18
	B68	24	16	14	16	16	16	22	20
MEAN ± S.D.		20.83 ± 3.66	16.5 ± 4.83	13.58 ± 3.99	14.08 ± 3.00	15.33 ± 2.90	17.50 ± 2.58	20.17 ± 1.95	19.42 ± 3.20
Statistical difference of post treatment values from resting values.			•••	•••	•••	•••	••	NS	NS
Statistical difference from comparative values in normal horses.		•••	NS	NS	NS	•	••	•••	

NS = not significant
• = P<0.05
•• = P<0.01
••• = P<0.001

Max. Δ Ppl (cm Hg)

	Time after treatment (mins.) 0	10	20	30	60	120	240	Maximum Response
	4.4	3.5	3.2	3.5	3.8	4.0	3.4	
	2.5	3.6	3.0	3.1	2.2	3.3	2.9	
	4.5	4.0	4.1	3.5	3.5	4.2	3.5	
	3.0	3.8	2.8	2.9	3.9	3.2	3.5	
	3.0	2.7	3.0	3.5	4.5	3.0	3.5	
	5.4	3.0	2.4	3.9	4.0	2.8	3.1	
	3.7	3.2	3.7	2.6	3.5	2.7	3.7	
	3.0	3.3	3.4	3.5	2.6	3.0	2.8	
	3.69	3.39	3.20	3.31	3.50	3.28	3.30	
	1.00	0.43	0.53	0.42	0.76	0.55	0.33	
		NS	NS	NS	NS	NS	NS	
	19.5	9.6	8.7	9.5	12.3	17.4	21.0	8.1
	12.2	5.2	6.5	8.1	8.0	9.6	11.4	5.0
	18.4	7.1	9.0	8.0	8.5	10.5	16.1	7.0
	14.5	4.9	3.8	5.2	7.2	10.7	14.0	3.5
	22.0	8.2	8.0	8.3	10.0	13.8	20.2	8.0
	15.3	6.5	6.2	6.1	7.5	14.0	15.3	5.6
	13.0	3.5	4.0	5.1	5.3	6.0	10.5	3.5
	26.6	9.2	9.4	10.0	13.7	16.4	24.4	9.2
	22.2	8.8	9.0	10.2	10.9	18.1	23.1	8.5
	19.0	15.7	7.3	8.0	9.0	17.5	20.0	6.2
	23.6	8.5	3.3	3.9	4.8	12.5	20.6	3.1
	12.5	7.7	6.9	7.5	7.9	9.3	11.4	6.9
	18.23	7.91	6.84	7.49	8.76	12.98	17.33	6.22
	4.77	3.09	2.16	2.02	2.63	3.87	4.83	2.10
		•••	•••	•••	•••	•••	NS	•••
	•••	•••	•••	•••	•••	•••	•••	

PaO_2 (mm Hg)

	Time after treatment (min.) 0	10	20	30	60	120	240
	92.1	88.4	89.3	92.5	89.1	93.4	
	96.9	90.3	92.9	97.0	95.6	93.5	
	88.3	86.0	84.1	92.4	89.9	90.4	
	98.4	92.1	96.9	95.0	97.2	96.5	
	93.4	90.6	90.5	95.3	95.0	92.6	
	88.4	82.6	84.1	90.5	87.9	93.5	
	89.5	90.5	86.2	90.4	84.5	90.1	
	89.8	92.6	82.1	90.5	94.6	91.9	
	92.1	89.14	88.01	92.95	91.73	92.74	
	3.87	3.36	4.60	2.54	4.49	2.03	
		•	•••	NS	NS	NS	
	69.5	65.0	66.9	73.4	72.0	68.0	
	72.5	64.9	71.6	78.5	74.0	70.5	
	63.0	58.2	63.5	69.9	68.5	65.2	
	76.1	69.4	73.8	86.2	78.5	75.4	
	68.2	66.4	70.6	76.9	75.3	70.0	
	73.8	73.2	75.9	79.6	80.4	72.0	
	77.5	70.6	74.1	82.5	86.9	78.5	
	61.0	57.3	58.4	67.9	64.2	60.5	
	59.9	53.5	59.7	60.1	52.4	57.0	
	63.0	58.0	60.5	66.4	65.9	64.6	
	79.1	75.2	69.2	88.0	76.4	72.9	
	66.1	60.5	54.4	70.9	69.0	68.3	
	69.14	64.35	66.55	75.03	71.96	68.58	
	6.66	6.91	7.09	8.42	8.91	6.09	
		•••	NS	•••	NS	NS	
	•••	•••	••	•••	•••	•••	

$PaCO_2$ (mm Hg)

	Time after treatment (min.) 0	10	20	30	60	120	240
	34.6	34.0	35.0	35.6	35.2	34.1	
	36.2	37.4	37.0	35.5	35.6	36.0	
	37.5	37.6	36.0	36.4	36.5	36.3	
	34.1	35.0	35.5	35.9	34.8	35.0	
	37.6	36.5	36.9	37.8	37.0	37.0	
	37.6	38.5	38.0	36.4	37.0	36.5	
	40.6	40.0	41.5	39.2	43.2	41.4	
	35.4	31.4	32.9	33.5	34.0	34.6	
	36.70	36.3	36.6	36.29	36.66	36.36	
	2.09	2.74	2.51	1.68	2.85	2.27	
	NS	NS	NS	NS	NS	NS	
	38.5	38.4	38.5	38.2	38.1	38.6	
	36.1	36.5	36.4	36.0	36.1	35.9	
	39.1	38.5	38.6	38.0	37.9	37.9	
	37.1	38.5	38.6	38.0	37.9	37.0	
	37.9	39.2	38.5	38.3	37.4	37.5	
	41.2	41.6	40.8	38.4	38.5	39.9	
	40.1	41.5	41.4	39.8	38.2	40.8	
	42.8	42.4	40.2	39.0	38.5	40.1	
	42.7	43.0	41.9	41.0	40.0	38.3	
	42.0	42.5	42.8	38.8	38.5	41.0	
	36.0	36.4	35.9	37.0	36.5	36.9	
	36.1	36.4	36.9	35.4	35.9	36.0	
	39.13	39.49	39.16	38.16	37.78	38.32	
	2.59	2.54	2.25	1.53	1.17	1.77	
		NS	NS	NS	•	NS	
	•	•	•	•	NS	•	

Heart Rate (/Min.)

	Time after treatment (min.) 0	10	20	30	60	120	240
	36	76	66	58	40	36	36
	38	80	66	60	42	36	36
	34	66	60	50	36	34	34
	36	70	60	42	36	36	36
	36	84	72	58	48	36	36
	38	90	76	60	42	38	36
	36	64	60	52	40	36	36
	36	96	90	76	56	36	36
	36.25	78.25	68.75	57.00	42.75	36.00	35.75
	1.28	11.44	10.42	9.86	5.58	1.07	0.71
		•••	•••	•••	•	NS	NS
	36	66	60	54	42	36	36
	34	70	64	54	48	38	32
	36	78	56	50	38	36	34
	32	80	66	50	42	34	34
	36	66	54	42	40	36	36
	34	86	70	56	48	36	36
	36	60	54	42	38	36	36
	36	78	56	50	36	36	36
	38	72	66	66	48	36	36
	36	72	62	60	48	36	36
	36	70	50	46	38	36	36
	36	75	65	60	52	38	36
	35.5	72.75	60.25	52.50	43.17	36.17	35.33
	1.51	7.12	6.18	7.34	5.36	1.03	1.30
		•••	•••	•••	••	NS	NS
	•	NS	NS	NS	NS	NS	NS

NS = not significant
• = P<0.05
•• = P<0.01
••• = P<0.001

APPENDIX 5.6.

Effects of Clenbuterol (by intravenous injection) on some respiratory function parameters in normal and COPD affected horses.

Respiratory Rate (/mins.) — Time after treatment (mins.)

Group	Horse Number	0	10	20	30	60	120	240	360
NORMAL HORSES	A53	14	16	14	12	12	10	12	12
	A54	16	10	12	10	10	14	12	16
	A55	9	12	10	16	12	12	14	14
	A56	12	12	12	12	10	12	12	12
	A57	10	18	16	20	18	12	12	10
	A58	14	16	14	14	12	16	10	9
	A59	16	12	12	10	14	10	12	12
	A60	11	14	11	10	10	12	10	10
MEAN ± S.D.		12.75	13.75	12.63	13.00	12.25	12.25	11.75	11.88
		2.66	2.71	1.92	3.55	2.71	1.98	1.28	2.30
Statistical difference of post treatment values from resting values.			NS	NS	NS	NS	NS	NS	NS
	B69	16	13	12	12	13	12	14	16
	B70	16	14	13	15	14	16	18	16
	B71	16	11	11	9	14	12	16	16
	B72	14	12	9	9	9	10	12	12
COPD AFFECTED HORSES	B73	13	10	9	11	10	12	12	12
	B74	12	8	9	9	9	9	10	10
	B75	22	16	12	13	12	16	18	18
	B76	16	12	12	12	12	12	16	16
	B77	14	12	10	12	12	12	12	12
	B78	13	10	9	10	11	10	12	12
	B79	18	18	12	14	14	16	18	18
	B80	26	18	14	14	16	16	20	28
MEAN ± S.D.		16.33	12.83	11.00	11.67	12.17	12.75	14.83	15.5
		4.05	3.16	1.76	2.10	2.17	2.60	3.24	4.76
Statistical difference of post treatment values from resting values.			•••	•••	•••	•••	•••	•	NS
Statistical difference from comparative values in normal horses.		•	NS	NS	NS	NS	NS	•	NS

Max. Δ Ppl (cm Hg). — Time after treatment (mins.)

Group	Horse Number	0	10	20	30	60	120	240	360	Maximum Response
NORMAL HORSES	A53	3.1	3.4	3.0	3.2	3.9	4.5	3.4	3.3	
	A54	4.3	5.0	3.9	3.8	4.0	4.0	5.1	3.9	
	A55	3.0	3.0	3.1	3.6	2.9	3.8	4.7	3.0	
	A56	3.0	2.7	3.5	4.0	3.6	3.0	2.[illegible]	4.0	
	A57	4.5	4.1	5.2	3.9	6.1	4.7	3.5	3.8	
	A58	3.7	3.5	2.8	2.6	3.0	3.2	2.8	3.5	
	A59	2.8	3.0	3.0	4.7	4.4	3.9	3.7	3.9	
	A60	3.7	3.1	4.0	3.7	3.3	2.7	3.2	2.6	
MEAN ± S.D.		3.51	3.48	3.56	3.69	3.90	3.73	3.61	3.50	
		0.64	0.75	0.80	0.61	1.03	0.71	0.89	0.50	
Statistical difference of post treatment values from resting values.			NS	NS	NS	NS	NS	NS	NS	
COPD AFFECTED HORSES	B69	15.1	9.2	7.0	8.6	8.1	9.5	14.2	16.7	7.0
	B70	28.0	12.2	10.3	12.0	14.6	17.1	26.3	25.2	10.1
	B71	11.4	6.8	4.0	4.2	4.0	6.3	9.1	12.0	4.0
	B72	18.3	9.3	6.1	6.4	6.5	7.0	12.6	14.0	6.0
	B73	8.5	4.1	3.0	3.3	4.0	4.4	6.2	7.4	3.0
	B74	12.0	6.6	5.5	6.0	6.6	8.0	8.5	10.1	5.3
	B75	23.1	12.3	9.5	10.2	10.5	12.4	20.0	24.6	9.5
	B76	16.6	8.6	7.0	7.2	8.4	9.1	14.4	13.0	7.0
	B77	14.5	5.3	5.4	6.0	7.6	6.3	8.4	12.2	5.3
	B78	13.7	4.3	4.5	5.0	6.2	9.3	9.0	12.5	4.0
	B79	21.0	13.7	10.3	11.0	12.5	16.0	20.7	22.8	10.2
	B80	32.7	14.1	9.0	10.2	11.1	14.7	26.0	31.2	9.3
MEAN ± S.D.		17.91	8.89	6.80	7.51	8.34	10.01	14.62	16.81	6.73
		7.15	3.57	2.49	2.84	3.28	4.13	7.04	7.34	2.55
Statistical difference of post treatment values from resting values.			•••	•••	•••	•••	•••	•••	NS	•••
Statistical difference from comparative values in normal horses.		•••	•••	••	••	••	•••	•••	•••	

PaO_2 (mm Hg) — Time after treatment (mins.)

Group	Horse Number	0	10	20	60	120	240	360
NORMAL HORSES	A53	84.9	86.0	80.2	81.9	74.7	83.5	90.1
	A54	86.5	82.0	75.5	77.0	82.1	78.4	85.5
	A55	92.1	94.3	87.3	80.2	86.5	84.3	88.9
	A56	90.5	84.1	86.2	80.9	72.4	83.7	87.0
	A57	83.9	84.0	80.1	86.2	84.6	90.7	88.5
	A58	96.2	90.5	92.4	87.0	89.3	90.7	94.5
	A59	90.2	78.3	80.2	84.6	87.4	91.5	88.2
	A60	84.0	82.5	74.9	78.0	82.0	90.6	87.5
MEAN ± S.D.		88.54	85.21	82.10	81.98	82.38	86.68	88.78
		4.43	5.06	6.05	3.68	6.02	4.84	2.69
Statistical difference of post treatment values from resting values.			NS	•••	••	•	NS	NS
COPD AFFECTED HORSES	B69	70.8	77.3	68.3	71.0	83.5	80.7	72.4
	B70	70.5	69.5	68.4	72.1	78.2	73.1	69.8
	B71	73.4	73.2	70.9	75.4	76.9	79.4	70.8
	B72	65.4	68.7	62.3	67.7	72.1	70.7	61.9
	B73	78.3	75.4	72.0	76.9	84.3	80.2	76.3
	B74	64.4	70.2	64.3	67.7	75.9	70.6	70.7
	B75	71.0	68.6	65.4	60.9	69.0	72.7	67.5
	B76	72.9	70.7	65.9	66.0	73.0	77.1	75.0
	B77	75.7	76.0	70.9	70.0	81.6	78.5	77.7
	B78	70.6	74.9	66.7	66.4	72.0	76.7	72.8
	B79	64.9	66.2	65.1	68.3	70.9	68.4	61.0
	B80	60.3	58.7	56.4	59.5	63.7	68.2	59.4
MEAN ± S.D.		69.85	70.78	66.38	68.49	75.09	74.69	69.61
		5.19	5.15	4.30	5.14	6.17	4.61	6.05
Statistical difference of post treatment values from resting values.			NS	•••	NS	••	•••	NS
Statistical difference from comparative values in normal horses.		•••	•••	•••	•••	•	•••	•••

$PaCO_2$ (mm Hg) — Time after treatment (mins.)

Group	Horse Number	0	10	20	60	120	240	360
NORMAL HORSES	A53	36.5	37.2	36.3	38.4	34.1	36.2	37.5
	A54	38.1	36.4	36.0	37.8	37.2	38.9	39.5
	A55	35.3	37.4	36.1	38.4	38.7	36.7	34.9
	A56	38.9	35.2	33.1	37.2	36.5	37.4	35.5
	A57	40.1	39.3	38.6	36.4	39.5	39.7	38.4
	A58	31.3	34.5	35.9	34.3	35.8	32.1	33.9
	A59	37.7	39.6	40.4	39.8	42.6	38.7	39.4
	A60	36.4	38.2	37.4	35.5	34.1	35.1	33.2
MEAN ± S.D.		36.79	37.23	36.73	37.23	37.31	36.85	36.54
		2.69	1.82	2.15	1.77	2.89	2.45	2.49
Statistical difference of post treatment values from resting values.			NS	NS	NS	NS	NS	NS
COPD AFFECTED HORSES	B69	39.9	36.1	38.8	38.2	37.5	37.1	38.4
	B70	43.3	34.7	36.5	36.7	33.0	39.2	42.7
	B71	39.8	40.1	38.6	32.9	36.6	37.1	37.8
	B72	39.6	40.1	40.7	39.7	38.3	39.5	40.7
	B73	42.9	39.6	38.7	38.2	38.8	39.7	38.4
	B74	41.8	40.7	37.2	37.8	38.1	38.5	39.0
	B75	43.3	43.5	44.9	43.1	40.7	40.5	41.0
	B76	38.7	39.0	39.1	40.7	38.2	37.5	37.9
	B77	37.9	38.2	37.5	36.3	37.9	38.4	37.5
	B78	38.9	38.2	39.7	39.0	37.6	37.5	37.2
	B79	43.7	43.0	42.5	41.0	42.6	42.9	44.1
	B80	43.9	44.5	44.2	44.0	42.7	43.6	43.0
MEAN ± S.D.		41.14	39.81	39.87	38.96	38.50	39.29	39.81
		2.20	2.90	2.71	3.04	2.62	2.15	2.41
Statistical difference of post treatment values from resting values.			NS	NS	•	••	••	•
Statistical difference from comparative values in normal horses.		•••	•	•	NS	NS	•	••

Heart Rate — Time after treatment (mins.)

Group	Horse Number	0	10	20	30	60	120	240	360
NORMAL HORSES	A53	32	36	36	38	36	36	32	32
	A54	32	36	46	48	44	36	36	36
	A55	34	40	48	44	38	34	36	32
	A56	32	38	46	52	42	36	32	34
	A57	36	42	44	40	36	32	34	34
	A58	36	38	46	42	38	32	32	36
	A59	32	38	38	40	34	32	32	32
	A60	36	44	42	40	36	34	36	32
MEAN ± S.D.		33.75	39.00	43.25	43.00	38.00	34.00	33.75	33.50
		1.98	2.83	4.27	4.78	3.38	1.85	1.98	1.77
Statistical difference of post treatment values from resting values.			•••	•••	••	•	NS	NS	NS
COPD AFFECTED HORSES	B69	36	48	46	40	36	34	36	32
	B70	38	42	48	42	36	38	36	36
	B71	40	48	48	42	38	36	36	36
	B72	32	42	44	40	36	32	32	32
	B73	32	48	46	40	36	32	34	34
	B74	36	48	48	42	38	36	36	34
	B75	32	46	46	38	36	32	32	34
	B76	36	40	44	40	36	32	34	32
	B77	34	48	44	42	38	36	36	32
	B78	36	48	46	40	40	36	38	36
	B79	34	46	46	40	40	38	36	36
	B80	32	44	46	38	36	32	32	32
MEAN ± S.D.		34.84	45.67	46.17	40.33	37.17	34.50	34.83	33.67
		2.62	2.93	1.34	1.44	1.59	2.43	1.99	1.88
Statistical difference of post treatment values from resting values.			•••	•••	•••	•	NS	NS	NS
Statistical difference from comparative values in normal horses.			•••	•	NS	NS	NS	NS	NS

NS = not significant
• = P < 0.05
•• = P < 0.01
••• = P < 0.001

APPENDIX 5.7.

Effects of Etamiphylline camsylate (by intravenous injection) on some respiratory function parameters in normal and COPD affected horses.

Respiratory Rate (/mins.)

Group	Horse Number	Time after treatment (mins.) 0	10	20	30	60	120	240	360
NORMAL HORSES	A61	10	14	12	12	9	12	13	11
	A62	12	12	14	12	10	10	9	12
	A63	8	12	9	10	12	9	8	9
	A64	12	16	14	16	12	14	14	14
	A65	11	12	12	12	12	10	13	12
	A66	14	10	10	14	14	11	10	10
	A67	12	9	11	9	10	13	12	12
	A68	10	12	12	11	11	12	14	14
MEAN ± S.D.		11.13 1.81	12.13 2.17	11.75 1.75	12.0 2.20	11.25 1.58	11.38 1.69	11.63 2.33	11.75 1.75
Statistical difference of post treatment values from resting values.			NS	NS	NS	NS	NS	NS	NS
COPD AFFECTED HORSES	B81	16	14	14	12	12	16	14	16
	B82	14	18	16	12	10	12	12	12
	B83	12	10	9	9	8	10	10	12
	B84	10	12	12	12	12	10	12	10
	B85	13	16	12	8	10	9	9	11
	B86	12	9	10	10	14	14	16	16
	B87	18	20	16	16	11	11	13	12
	B88	14	10	8	14	10	12	14	16
	B89	16	12	14	10	16	16	14	12
	B90	11	9	9	8	9	7	10	12
	B91	20	14	12	12	10	12	18	24
	B92	16	12	8	7	12	10	12	12
MEAN ± S.D.		14.33 2.96	13.0 3.54	11.67 2.90	10.83 2.66	11.17 2.21	11.58 2.71	12.83 2.59	13.75 3.82
Statistical difference of post treatment values from resting values.			NS	*	**	**	*	NS	NS
Statistical difference from comparative values in normal horses.		*	NS	NS	NS	NS	NS	NS	NS

Max. Δ Ppl (mm Hg)

Group	Horse Number	Time after treatment (mins.) 0	10	20	30	60	120	240	360	Maximum Response
NORMAL HORSES	A61	3.9	4.4	3.5	3.2	3.9	2.9	4.5	3.1	
	A62	5.2	5.7	4.0	4.5	3.8	4.6	3.2	4.9	
	A63	3.0	2.5	2.9	4.0	3.1	3.5	3.0	3.0	
	A64	4.0	3.0	3.8	4.4	5.1	3.2	2.4	3.1	
	A65	3.5	5.0	4.1	3.9	2.8	2.5	3.7	3.5	
	A66	2.8	2.5	3.0	3.0	3.0	3.5	3.4	3.0	
	A67	4.4	3.9	4.8	3.2	4.2	4.0	4.7	4.6	
	A68	4.1	3.8	3.5	2.9	3.1	3.9	3.5	3.6	
MEAN ± S.D.		3.86 0.77	3.85 1.16	3.70 0.62	3.64 0.64	3.63 0.78	3.51 0.66	3.55 0.76	3.6 0.75	
Statistical difference of post treatment values from resting values.			NS	NS	NS	NS	NS	NS	NS	
COPD AFFECTED HORSES	B81	12.2	8.4	5.2	6.7	7.5	9.4	11.9	13.5	5.0
	B82	15.0	7.3	6.0	8.2	12.1	12.6	14.0	14.8	6.0
	B83	9.5	4.0	3.2	3.5	4.8	7.1	10.3	10.8	3.1
	B84	10.1	6.4	5.0	7.2	8.0	8.5	9.8	10.5	5.0
	B85	17.6	12.1	10.1	12.0	14.4	15.6	13.2	14.8	9.8
	B86	13.8	8.5	6.4	6.0	7.5	9.2	9.0	9.0	5.8
	B87	9.0	7.5	3.5	3.6	3.8	4.5	6.8	10.8	3.5
	B88	23.6	12.2	9.5	12.8	12.5	16.3	24.9	26.7	9.2
	B89	16.0	10.9	8.7	7.0	10.0	12.7	17.4	16.8	7.0
	B90	10.0	7.3	4.0	5.6	6.0	8.3	8.5	13.5	3.7
	B91	12.9	5.2	3.0	3.4	6.5	8.2	9.5	10.1	3.0
	B92	18.3	13.3	10.7	12.2	15.9	18.0	19.6	18.5	10.5
MEAN ± S.D.		14.0 4.37	8.59 2.94	6.28 2.80	7.35 3.38	9.08 3.87	10.87 4.14	12.91 5.32	14.15 4.90	5.97 2.64
Statistical difference of post treatment values from resting values.			***	***	***	***	***	NS	NS	***
Statistical difference from comparative values in normal horses.		***	***	*	**	**	***	***	***	

PaO_2 (mm Hg)

Group	Horse Number	Time after treatment (mins.) 0	10	20	60	120	240	360
NORMAL HORSES	A61	87.3	85.4	92.1	87.4	86.5	92.4	90.7
	A62	91.7	93.2	88.4	86.3	82.1	87.9	94.3
	A63	85.0	87.4	84.5	89.3	85.0	89.0	86.3
	A64	85.9	87.6	80.3	85.7	83.0	88.4	95.7
	A65	87.2	80.5	78.3	84.4	82.0	87.0	84.0
	A66	92.3	90.1	90.5	90.9	95.5	94.1	96.7
	A67	88.3	88.0	85.0	91.7	90.6	96.6	93.7
	A68	86.8	82.4	77.6	87.6	82.3	85.2	90.7
MEAN ± S.D.		88.06 2.63	86.83 4.06	84.59 5.51	87.91 2.55	85.93 4.99	90.08 3.89	91.51 4.50
Statistical difference of post treatment values from resting values.			NS	NS	NS	NS	NS	*
COPD AFFECTED HORSES	B81	72.9	68.4	68.0	72.7	74.5	70.8	75.5
	B82	69.3	67.2	63.2	61.0	66.2	73.9	70.7
	B83	74.6	76.2	70.9	68.3	67.5	72.4	76.0
	B84	77.8	70.9	72.1	75.1	80.9	86.3	82.8
	B85	67.2	63.1	64.4	66.2	70.2	72.1	69.3
	B86	73.0	70.9	68.0	70.9	75.2	71.7	69.4
	B87	76.1	68.5	70.0	67.5	74.9	78.3	74.3
	B88	65.5	66.0	59.8	62.4	68.4	72.5	62.4
	B89	70.9	67.7	66.0	68.0	69.0	70.2	67.5
	B90	70.2	73.6	67.2	65.5	76.4	79.9	80.1
	B91	72.5	74.0	70.7	67.0	75.7	73.0	78.4
	B92	68.0	66.3	69.2	70.4	72.1	65.5	69.0
MEAN ± S.D.		71.5 3.68	69.4 3.81	67.46 3.60	67.92 4.02	72.58 4.39	73.88 5.36	72.95 5.90
Statistical difference of post treatment values from resting values.			*	**	**	NS	NS	NS
Statistical difference from comparative values in normal horses.		***	***	***	***	***	***	***

$PaCO_2$ (mm Hg)

Group	Horse Number	Time after treatment (mins.) 0	10	20	60	120	240	360
NORMAL HORSES	A61	32.1	35.7	34.0	37.6	35.9	33.7	36.4
	A62	34.3	37.6	37.0	35.1	36.6	38.2	38.2
	A63	36.1	34.2	33.2	36.4	35.5	35.8	37.2
	A64	36.4	38.2	34.1	33.6	34.0	33.9	34.7
	A65	43.0	40.4	39.5	38.2	40.5	40.9	41.7
	A66	38.6	40.6	41.3	40.7	39.7	39.4	39.9
	A67	34.1	36.7	35.2	37.7	37.3	37.9	35.2
	A68	32.4	31.4	33.5	36.5	37.2	37.0	34.8
MEAN ± S.D.		35.88 3.60	36.85 3.10	35.98 3.02	36.98 2.13	37.09 2.15	37.1 2.54	37.26 2.54
Statistical difference of post treatment values from resting values.			NS	NS	NS	NS	NS	NS
COPD AFFECTED HORSES	B81	34.5	36.0	35.2	32.1	34.9	33.2	35.4
	B82	36.2	35.3	35.5	37.6	38.4	36.2	34.9
	B83	40.7	38.2	40.4	41.9	42.0	38.4	37.6
	B84	33.0	35.0	32.9	34.7	37.5	37.6	35.0
	B85	39.2	40.7	39.0	38.3	42.5	38.4	37.9
	B86	35.1	36.9	36.3	36.2	35.1	37.3	35.0
	B87	35.9	37.4	39.2	40.7	36.2	38.4	38.9
	B88	37.2	35.0	37.4	34.1	37.6	37.9	38.3
	B89	36.0	34.2	35.6	38.4	36.1	36.7	32.5
	B90	39.9	42.4	39.3	36.4	39.0	37.5	41.7
	B91	42.1	38.0	41.7	38.2	39.9	44.7	40.6
	B92	34.3	36.5	33.1	34.7	36.9	33.2	32.0
MEAN ± S.D.		37.01 2.84	37.13 2.44	37.13 2.82	36.94 2.81	38.01 2.48	37.46 2.92	36.65 3.02
Statistical difference of post treatment values from resting values.			NS	NS	NS	NS	NS	NS
Statistical difference from comparative values in normal horses.		NS	NS	NS	NS	NS	NS	NS

Heart Rate

Group	Horse Number	Time after treatment (mins.) 0	10	20	30	60	120	240	360
NORMAL HORSES	A61	32	44	48	56	46	36	32	32
	A62	36	42	54	54	42	33	34	32
	A63	34	46	58	60	46	32	32	32
	A64	32	40	44	44	36	34	36	32
	A65	32	42	48	48	40	36	36	36
	A66	34	40	42	44	37	34	36	34
	A67	36	46	50	48	44	34	32	36
	A68	32	45	48	50	40	35	32	36
MEAN ± S.D.		33.50 1.77	43.13 2.48	49.25 4.89	50.5 5.73	41.38 3.82	34.88 1.81	33.5 1.77	33.75 1.98
Statistical difference of post treatment values from resting values.			***	***	***	***	NS	NS	NS
COPD AFFECTED HORSES	B81	34	44	48	52	40	32	32	32
	B82	32	50	56	54	46	36	36	32
	B83	32	48	56	56	48	32	34	34
	B84	36	46	54	48	36	36	34	36
	B85	32	46	50	50	44	32	32	32
	B86	36	48	52	50	48	40	32	34
	B87	36	44	50	44	40	36	36	36
	B88	32	40	54	58	50	38	32	32
	B89	34	48	48	42	36	34	36	34
	B90	32	48	50	52	44	36	32	32
	B91	32	46	56	50	44	34	36	36
	B92	36	42	46	48	40	34	32	32
MEAN ± S.D.		33.83 1.80	45.83 2.89	51.67 3.50	51.17 4.22	43.0 4.63	35.50 2.43	34.17 2.17	33.50 1.73
Statistical difference of post treatment values from resting values.			***	***	***	***	*	NS	NS
Statistical difference from comparative values in normal horses.		*	NS	NS	NS	NS	NS	NS	NS

NS = not significant
* = P<0.05
** = P<0.01
*** = P<0.001

APPENDIX 6.1.

Effects of oral Clenbuterol treatment on some respiratory function parameters in COPD affected horses housed in the natural challenge environment.

Trial	Horse Number	Respiratory Rate (/min.) Days 0	2	4	7	9	11	14	Max. ΔPpl (cm Hg) Days 0	2	4	7	9	11	14	PaO_2 (mm Hg) Days 0	2	4	7	9	11	14	$PaCO_2$ (mm Hg) Days 0	2	4	7	9	11	14	Arterial pH Days 0	2	4	7	9	11	14
	B93	14	11	12	14	13	14	12	15.5	15.0	12.5	18.4	16.5	18.6	14.5	73.8	75.6	74.0	70.8	76.1	73.9	78.5	39.4	38.6	39.5	37.6	38.4	39.9	37.0	7.412	7.430	7.417	7.410	7.415	7.422	7.437
	B94	16	16	24	24	24	22	22	23.3	25.5	32.5	31.0	33.5	30.4	27.5	62.9	61.3	58.9	64.7	60.2	62.1	66.3	41.7	42.6	44.6	40.3	44.0	45.6	42.4	7.376	7.377	7.362	7.402	7.371	7.351	7.390
	B95	12	16	18	18	16	16	16	9.6	13.4	10.1	12.5	11.3	13.5	12.1	76.3	70.5	72.4	68.7	75.9	73.0	70.6	40.7	38.3	37.9	39.2	39.0	38.2	38.9	7.411	7.426	7.421	7.401	7.412	7.416	7.414
	B96	20	20	24	24	24	24	22	29.5	28.0	33.5	35.5	31.8	27.5	32.6	63.6	67.1	62.5	59.7	63.8	64.0	61.9	44.4	45.9	46.0	45.2	45.3	44.2	44.8	7.371	7.392	7.382	7.390	7.400	7.406	7.385
HORSES	B97	12	10	12	11	13	12	12	12.5	13.5	18.6	17.6	18.5	16.5	18.7	74.3	72.6	68.3	75.4	72.3	71.0	73.0	38.4	37.9	40.1	37.7	39.9	37.8	38.4	7.437	7.422	7.416	7.419	7.406	7.410	7.400
UNTREATED	B98	16	16	16	16	12	16	14	18.5	27.7	25.5	22.5	20.8	26.0	24.0	70.1	60.7	62.3	65.5	69.4	59.7	63.9	42.1	43.7	41.6	44.0	40.8	44.5	44.0	7.402	7.381	7.385	7.389	7.402	7.380	7.376
	B99	12	12	10	14	12	13	12	9.2	9.5	10.5	8.5	7.5	9.5	8.2	75.7	76.3	70.6	74.2	78.3	70.4	78.3	38.1	37.3	38.3	38.0	39.0	38.3	37.4	7.417	7.427	7.422	7.420	7.441	7.416	7.428
	B100	12	10	10	14	12	12	12	13.0	10.5	12.2	15.9	13.5	16.5	17.9	78.3	79.1	75.5	72.0	74.6	70.9	69.2	37.9	36.1	36.5	37.2	36.8	37.2	38.4	7.412	7.420	7.415	7.410	7.426	7.418	7.415
MEAN		14.25	13.88	15.75	16.88	15.75	16.13	15.25	16.39	17.89	19.43	20.24	19.18	19.81	19.44	71.88	70.4	68.06	68.88	71.33	68.13	70.21	40.34	40.05	40.56	39.9	40.4	40.71	40.16	7.405	7.409	7.403	7.405	7.409	7.402	7.406
S.D.		2.92	3.64	5.80	4.82	5.26	4.55	4.40	7.06	7.83	9.81	9.12	9.30	7.36	8.16	5.82	6.87	6.14	5.31	6.42	5.38	6.18	2.30	3.53	3.32	3.08	2.88	3.46	3.09	0.022	0.022	0.023	0.019	0.020	0.025	0.021
	B93	12	12	13	12	13	12	11	14.5	16.5	15.3	13.0	18.5	15.5	13.5	78.5	72.4	73.4	75.0	71.6	72.8	72.9	37.0	37.5	37.9	38.4	39.6	40.6	37.6	7.437	7.424	7.420	7.428	7.408	7.412	7.426
	B94	22	26	26	20	15	14	18	27.5	33.1	30.5	23.5	20.5	18.5	17.5	66.3	58.3	65.2	67.0	64.9	67.8	65.3	42.4	44.9	43.1	43.6	42.1	42.5	41.8	7.390	7.360	7.392	7.382	7.400	7.392	7.406
	B95	16	16	16	16	16	16	20	12.1	8.8	5.5	3.8	5.5	7.5	9.7	70.6	76.4	84.7	90.1	83.0	70.7	71.3	38.9	36.0	34.3	30.6	35.7	34.4	37.4	7.414	7.436	7.430	7.439	7.436	7.418	7.421
HORSES	B96	22	26	30	12	18	16	20	32.6	43.4	33.5	35.2	33.0	21.4	28.2	61.9	62.5	66.4	60.9	61.5	63.6	64.0	44.8	46.7	40.2	42.6	44.0	44.7	44.8	7.385	7.362	7.390	7.384	7.38	7.389	7.376
TREATED	B97	12	11	10	13	10	12	10	18.7	15.5	13.6	10.5	12.1	10.6	14.0	73.0	70.8	75.5	76.4	74.3	78.4	75.0	38.4	39.5	37.6	37.0	37.4	36.3	38.7	7.400	7.42	7.423	7.435	7.428	7.425	7.430
WITH	B98	14	16	15	14	14	15	16	24.0	25.2	29.5	27.4	23.4	24.5	28.4	63.9	68.0	65.4	66.0	68.0	60.0	61.2	44.0	44.0	44.4	43.2	43.0	43.2	40.8	7.376	7.382	7.380	7.390	7.396	7.402	7.412
CLENBUTEROL	B99	12	7	10	10	8	9	12	8.2	3.4	7.1	7.5	8.9	8.8	6.5	78.3	78.3	76.2	75.9	76.1	72.1	76.3	37.4	39.0	37.1	38.6	40.9	39.0	37.4	7.428	7.421	7.430	7.410	7.388	7.420	7.431
	B100	12	10	9	9	10	10	10	17.9	11.6	10.5	10.5	12.0	9.5	12.5	69.2	77.9	80.2	76.4	74.1	81.6	78.4	38.4	37.0	36.4	37.2	36.3	35.7	36.0	7.415	7.420	7.437	7.420	7.441	7.445	7.438
MEAN		15.25	15.5	16.13	13.25	13.0	13.0	14.63	19.44	19.69	18.19	16.43	16.74	14.54	16.29	70.21	70.58	73.38	73.46	71.69	70.88	70.55	40.16	40.58	38.88	38.9	39.88	39.55	38.31	7.406	7.403	7.413	7.411	7.410	7.413	7.418
S.D.		4.40	7.13	7.81	3.50	3.42	2.67	4.37	8.16	13.36	11.25	10.98	8.93	6.40	8.09	6.18	7.30	7.23	8.93	6.80	7.15	6.31	3.09	4.05	3.44	4.31	3.14	3.81	2.93	0.021	0.030	0.022	0.024	0.023	0.018	0.020
Statistical difference from corresponding values in untreated horses.		NS	NS	NS	*	NS	*	NS	*	NS	NS	NS	NS	**	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

** = P<0.01
* = P<0.05
NS = not significant.

APPENDIX 6.2.

Effects of oral Clenbuterol treatment on some respiratory function parameters in COPD affected horses housed in the controlled environment.

Trial	Horse Number	Respiratory Rate (/mins.) Days 0	2	4	7	9	11	14	Max. Δ Ppl (mm Hg) Days 0	2	4	7	9	11	14	PaO_2 (mm Hg) Days 0	2	4	7	9	11	14	$PaCO_2$ (mm Hg) Days 0	2	4	7	9	11	14	Arterial pH Days 0	2	4	7	9	11	14
	B93	14	12	11	10	10	9	9	15.4	8.0	8.2	6.1	4.0	3.5	4.7	68.5	72.1	78.3	80.6	87.1	88.6	84.9	39.7	38.6	38.4	37.2	37.9	38.5	36.3	7.408	7.412	7.416	7.409	7.427	7.430	7.432
	B94	22	16	12	12	12	12	12	29.2	14.2	8.7	5.0	4.4	4.2	3.6	65.7	74.0	78.6	84.7	82.0	86.5	90.6	42.4	40.6	39.7	37.6	38.2	37.3	37.5	7.385	7.396	7.408	7.420	7.424	7.426	7.415
	B95	18	16	16	12	16	16	14	14.5	9.5	7.4	3.4	3.4	3.1	3.5	73.9	76.8	82.1	86.7	84.3	92.4	90.0	38.4	38.3	36.7	37.9	35.8	34.2	36.7	7.413	7.425	7.430	7.422	7.416	7.445	7.430
	B96	22	20	20	16	16	16	14	28.3	22.6	18.3	15.7	14.5	10.7	8.7	64.0	65.5	69.6	72.7	70.8	74.1	76.5	44.8	42.1	43.7	39.7	41.0	39.7	39.9	7.376	7.396	7.401	7.385	7.408	7.417	7.430
HORSES	B97	13	10	12	13	10	11	12	14.5	9.5	5.2	3.5	4.2	3.5	4.6	71.6	77.8	80.1	87.9	84.0	87.6	88.4	36.2	37.9	36.1	38.4	37.5	38.1	37.2	7.420	7.416	7.434	7.419	7.438	7.440	7.435
UNTREATED.	B98	16	14	12	12	12	12	12	22.7	18.4	15.6	13.6	10.5	6.3	4.4	62.1	66.3	70.1	74.5	75.0	82.4	86.1	43.9	40.6	39.7	38.2	39.4	38.9	39.9	7.382	7.396	7.410	7.412	7.420	7.418	7.422
	B99	13	11	10	11	10	11	10	17.5	10.5	6.1	3.1	3.7	3.0	3.2	73.9	80.6	79.8	88.5	90.1	92.6	88.2	39.5	38.9	37.5	35.4	36.1	36.4	38.0	7.416	7.418	7.428	7.434	7.439	7.440	7.432
	B100	10	12	9	9	10	8	10	12.9	8.3	6.4	3.1	3.3	4.1	3.8	80.7	87.9	89.1	92.1	96.4	90.1	86.5	33.2	34.0	30.5	36.5	35.9	34.7	36.5	7.428	7.442	7.439	7.421	7.437	7.428	7.420
MEAN		16.0	13.88	12.75	11.88	12.00	11.88	11.63	19.38	12.63	9.49	6.69	6.10	4.80	4.56	70.05	75.13	78.46	83.46	83.71	86.79	86.4	39.76	38.88	37.79	37.61	37.73	37.23	37.75	7.404	7.413	7.421	7.415	7.426	7.431	7.427
S.D.		4.38	3.31	3.58	2.10	2.62	2.90	1.85	6.50	5.34	4.80	5.06	4.20	2.60	1.76	6.17	7.41	6.32	6.93	8.10	6.10	4.44	3.91	2.43	3.78	1.29	1.83	1.98	1.44	0.020	0.016	0.014	0.014	0.011	0.010	0.007
	B93	16	12	12	12	12	10	12	18.2	10.6	6.5	3.5	3.6	4.6	3.0	70.1	75.7	79.0	82.1	86.7	80.9	87.6	37.5	39.8	36.7	38.3	37.4	38.5	36.6	7.419	7.415	7.428	7.420	7.434	7.427	7.420
	B94	20	12	12	10	10	10	9	22.5	5.5	4.7	4.1	3.4	3.5	3.2	64.5	78.6	84.0	89.7	87.1	85.4	92.4	39.9	37.5	38.9	37.6	36.4	35.4	38.0	7.382	7.420	7.416	7.424	7.436	7.426	7.435
	B95	20	16	16	14	12	16	12	16.4	9.5	5.2	4.0	3.1	3.1	3.4	70.9	72.9	84.9	88.1	90.7	86.0	80.5	38.4	37.6	36.3	37.9	38.4	39.1	40.7	7.415	7.412	7.428	7.415	7.422	7.420	7.439
HORSES	B96	24	22	20	20	16	14	12	32.5	24.2	19.1	15.2	10.5	5.5	4.1	60.1	66.4	70.9	75.7	72.3	77.7	83.1	44.8	43.0	38.6	37.5	39.3	40.4	38.0	7.370	7.390	7.401	7.412	7.416	7.408	7.415
TREATED	B97	12	12	12	12	11	12	9	18.0	8.3	6.2	3.0	3.0	3.2	3.3	67.4	86.8	80.9	88.7	88.9	81.6	90.7	39.5	36.3	39.5	38.2	35.4	36.4	38.9	7.406	7.438	7.420	7.442	7.438	7.420	7.434
WITH	B98	16	16	12	12	12	12	12	24.1	16.1	13.5	7.3	5.2	5.5	4.0	58.9	70.9	76.8	82.3	84.6	78.6	86.4	43.9	39.9	38.5	39.1	38.5	40.1	38.2	7.378	7.400	7.410	7.412	7.419	7.415	7.419
CLENBUTEROL.	B99	16	16	12	12	10	12	12	15.0	6.3	4.4	3.3	3.4	3.4	4.2	74.3	81.9	89.4	94.1	90.5	93.6	87.7	37.9	36.3	36.3	38.2	37.0	38.1	37.1	7.420	7.423	7.435	7.420	7.434	7.438	7.424
	B100	12	12	11	9	9	9	9	15.2	9.5	6.5	3.0	3.5	5.1	4.4	78.5	88.1	86.5	90.2	78.4	86.0	83.9	34.5	35.7	32.5	35.7	38.3	36.2	33.0	7.430	7.430	7.442	7.430	7.435	7.445	7.438
MEAN		17.0	14.75	13.38	12.63	11.50	11.88	10.88	20.24	11.25	8.26	5.43	4.46	4.24	3.7	68.09	77.66	81.55	86.36	84.9	83.73	86.54	39.55	38.26	37.16	37.81	37.59	38.03	37.56	7.403	7.416	7.423	7.422	7.429	7.425	7.428
S.D.		4.14	3.54	3.07	3.34	2.14	2.30	1.55	5.94	6.14	5.24	4.19	2.53	1.05	0.53	6.77	7.66	5.93	5.89	6.44	5.15	3.95	3.39	2.47	2.26	0.99	1.28	1.86	2.22	0.023	0.016	0.013	0.010	0.009	0.012	0.010
Statistical difference from corresponding values in untreated horses.		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = not significant.

APPENDIX 6.3.

Effects of oral etamiphylline camsylate treatment on some respiratory function parameters in COPD affected horses housed in the natural challenge environment.

Trial	Horse Number	Respiratory Rate (/min.) Days 0	2	4	7	9	11	14	Max. Δ Ppl (cm Hg) Days 0	2	4	7	9	11	14	PaO_2 (mm Hg) Days 0	2	4	7	9	11	14	$PaCO_2$ (mm Hg) Days 0	2	4	7	9	11	14	Arterial pH Days 0	2	4	7	9	11	14
	B101	12	12	12	12	12	12	12	14.4	16.0	15.3	13.5	13.4	14.8	12.6	74.5	70.2	75.4	73.9	78.6	77.9	79.2	38.6	38.4	39.5	36.4	37.6	37.1	37.6	7.417	7.412	7.407	7.425	7.421	7.436	7.422
	B102	11	12	12	10	12	12	12	9.5	10.5	9.5	9.6	12.0	10.5	8.8	78.1	79.2	79.0	75.3	72.1	78.4	80.9	37.9	37.5	37.0	38.9	36.7	37.2	36.4	7.432	7.422	7.434	7.429	7.415	7.434	7.425
	B103	12	14	16	16	16	16	18	13.7	15.5	12.6	17.0	18.2	14.5	16.3	77.1	71.9	70.0	72.5	74.9	75.0	71.8	36.4	38.5	36.3	36.0	37.2	38.9	36.6	7.425	7.427	7.424	7.422	7.420	7.420	7.416
	B104	12	12	10	10	12	12	10	14.8	15.2	16.5	17.4	15.9	16.2	17.5	76.2	77.3	73.4	70.9	71.2	73.7	75.6	37.1	38.5	37.7	37.0	39.6	38.4	37.2	7.417	7.422	7.420	7.416	7.420	7.415	7.412
HORSES	B105	16	18	16	16	20	16	16	18.2	18.5	16.7	22.3	24.5	20.7	16.5	68.9	67.0	72.1	64.4	65.0	68.9	72.5	39.9	43.4	40.9	44.5	44.1	42.9	40.4	7.401	7.400	7.412	7.396	7.380	7.372	7.396
UNTREATED.	B106	16	14	14	18	18	18	18	15.6	14.9	12.2	13.2	11.7	12.5	13.3	72.1	74.9	73.2	76.1	73.9	80.2	72.8	37.6	38.1	38.4	37.5	37.0	39.3	38.9	7.412	7.426	7.422	7.430	7.418	7.415	7.424
	B107	18	16	18	18	18	24	18	21.4	21.0	18.5	24.1	22.5	27.1	20.5	66.2	68.4	66.2	62.7	65.5	59.7	61.9	43.7	40.9	42.5	43.8	42.7	44.5	44.0	7.374	7.390	7.381	7.378	7.390	7.370	7.385
	B108	12	12	12	12	14	12	12	12.0	11.4	10.8	13.0	16.4	13.5	12.4	72.9	75.6	81.0	77.9	72.4	75.8	79.2	38.4	38.0	36.3	39.4	38.1	37.6	39.0	7.41	7.415	7.425	7.414	7.415	7.422	7.409
MEAN		13.63	13.75	13.75	14.00	15.25	15.25	14.50	14.95	15.38	14.01	16.26	16.83	16.23	14.74	73.25	73.06	73.79	71.71	71.7	73.7	74.24	38.70	39.16	38.58	39.19	39.13	39.49	38.76	7.411	7.414	7.416	7.413	7.410	7.411	7.411
S.D.		2.62	2.25	2.71	3.38	3.20	4.27	3.34	3.64	3.41	3.19	4.95	4.71	5.31	3.66	4.12	4.37	4.74	5.49	4.58	6.62	6.08	2.28	1.99	2.25	3.28	2.81	2.75	2.51	0.018	0.013	0.016	0.020	0.016	0.026	0.014
	B101	12	10	8	9	10	10	12	12.1	12.6	10.6	6.0	5.5	6.7	8.5	79.2	74.1	78.5	81.9	80.2	82.9	80.0	37.6	36.5	35.3	37.9	36.5	37.0	36.3	7.422	7.428	7.439	7.420	7.428	7.419	7.432
	B102	12	12	9	12	10	9	10	8.5	10.2	9.5	10.6	12.8	8.5	10.7	80.9	77.2	82.5	80.1	81.7	83.2	79.4	36.4	38.2	34.1	36.7	37.5	35.8	37.6	7.425	7.431	7.425	7.446	7.432	7.428	7.418
	B103	18	16	18	16	14	16	14	16.2	15.3	17.2	12.2	15.4	16.6	12.0	71.8	73.2	75.1	79.2	73.3	69.0	73.0	36.6	38.6	38.0	36.5	37.0	40.7	38.4	7.416	7.428	7.420	7.425	7.418	7.410	7.416
HORSES	B104	10	12	10	9	12	9	10	17.5	12.1	11.5	12.4	10.5	8.1	8.2	75.6	73.9	78.2	77.5	80.6	82.1	79.9	37.2	38.5	36.2	38.5	39.7	38.0	36.4	7.412	7.416	7.412	7.428	7.420	7.436	7.430
TREATED	B105	16	18	20	22	22	22	26	16.7	19.5	22.5	27.0	28.6	32.5	27.4	72.5	70.9	65.5	62.1	63.0	61.2	64.4	40.4	39.8	42.7	43.9	41.8	40.9	43.5	7.396	7.390	7.388	7.385	7.401	7.392	7.386
WITH	B106	18	16	20	18	20	18	16	13.0	16.5	18.9	18.5	17.6	18.8	16.5	72.8	72.4	68.9	66.3	72.1	69.9	75.0	38.9	39.1	39.5	39.9	38.2	37.5	39.0	7.424	7.425	7.40	7.410	7.415	7.427	7.418
ETAMIPHYLLINE	B107	18	16	16	14	20	16	16	20.3	18.1	15.2	20.9	18.5	19.4	24.0	61.9	69.7	68.3	65.1	69.9	68.0	67.9	44.0	41.6	40.9	41.7	42.6	39.9	38.5	7.385	7.390	7.412	7.390	7.398	7.401	7.410
CAMSYLATE.	B108	12	12	14	12	14	14	14	12.4	14.4	16.0	13.7	17.3	16.5	17.1	79.2	76.0	74.5	78.3	73.9	68.3	70.7	39.0	37.1	39.5	38.6	40.4	39.3	37.4	7.409	7.419	7.400	7.415	7.408	7.414	7.426
MEAN		14.50	14.00	14.38	14.00	15.25	14.25	14.75	14.59	14.84	15.18	15.16	15.78	15.89	15.55	74.24	73.43	73.94	73.81	74.34	73.08	73.79	38.76	38.68	38.28	39.21	39.21	38.64	38.39	7.411	7.416	7.413	7.415	7.415	7.416	7.417
S.D.		3.34	2.83	4.90	4.50	4.77	4.68	5.12	3.76	3.15	4.46	6.63	6.76	8.43	7.12	6.08	2.48	5.88	7.90	6.35	8.42	5.88	2.51	1.58	2.93	2.53	2.27	1.85	2.28	0.014	0.017	0.016	0.020	0.012	0.015	0.015
Statistical difference from corresponding values in untreated horses.		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = not significant.

APPENDIX 6.4.

Effects of oral etamiphylline camsylate treatment on some respiratory function parameters in COPD affected horses housed in the controlled environment.

Trial	Horse Number	Respiratory Rate (/min.) Days 0	2	4	7	9	11	14	Max. Δ Ppl (cm Hg). Days 0	2	4	7	9	11	14	PaO_2 (mm Hg) Days 0	2	4	7	9	11	14	$PaCO_2$ (mm Hg) Days 0	2	4	7	9	11	14	Arterial pH Days 0	2	4	7	9	11	14
	B101	16	12	12	10	10	9	12	18.1	12.4	8.5	4.2	3.5	3.0	3.7	69.7	72.6	80.4	85.4	82.7	90.1	96.3	39.9	37.6	38.0	37.5	36.4	35.5	37.0	7.408	7.425	7.419	7.441	7.446	7.442	7.440
	B102	10	12	9	12	10	9	10	11.5	5.6	3.5	4.4	3.5	3.1	3.5	72.5	79.3	84.5	86.0	93.2	86.2	79.5	40.9	40.5	37.6	38.0	36.4	36.2	39.5	7.416	7.414	7.420	7.426	7.430	7.440	7.436
	B103	16	16	12	12	12	12	14	15.5	15.2	10.1	5.5	4.6	4.4	3.6	69.3	75.7	80.9	81.2	86.9	85.9	88.2	37.4	37.1	38.5	36.3	37.0	37.0	35.4	7.409	7.422	7.426	7.435	7.430	7.438	7.428
HORSES	B104	12	12	12	12	12	12	12	14.4	9.0	8.8	3.5	3.2	4.5	3.5	76.9	79.8	79.5	87.0	90.1	85.9	92.7	36.9	35.4	38.2	38.7	37.6	38.0	36.5	7.430	7.445	7.419	7.418	7.425	7.422	7.438
UNTREATED.	B105	20	20	16	12	14	12	12	25.2	16.4	10.2	8.5	6.5	5.0	4.2	63.0	69.7	74.6	76.0	80.9	84.6	87.8	43.9	42.5	40.7	38.6	39.2	38.5	39.4	7.400	7.380	7.412	7.410	7.425	7.428	7.436
	B106	18	16	18	18	14	16	14	17.5	9.5	5.5	4.5	4.5	4.0	4.0	71.2	79.0	78.6	76.5	84.5	95.3	89.2	38.4	35.3	36.0	37.8	34.3	34.6	37.5	7.412	7.432	7.428	7.436	7.440	7.446	7.438
	B107	18	18	16	14	16	12	12	20.5	19.5	14.7	16.5	10.5	7.9	4.5	59.7	68.5	77.6	70.4	75.9	77.3	86.0	43.4	44.7	39.2	40.7	39.6	37.5	38.9	7.402	7.378	7.401	7.400	7.417	7.410	7.406
	B108	12	12	9	10	10	12	10	10.4	5.5	4.3	3.7	3.5	3.2	3.8	76.3	80.6	78.4	90.4	88.2	94.1	90.7	39.6	37.0	39.0	37.3	39.4	37.8	37.4	7.415	7.430	7.426	7.419	7.422	7.439	7.430
MEAN		15.25	14.75	13.00	12.50	12.25	11.75	12.00	16.64	11.64	8.20	6.35	4.98	4.39	3.85	69.83	75.65	79.31	81.5	85.3	87.43	88.66	40.05	38.76	38.4	38.11	37.49	36.89	37.70	7.412	7.416	7.419	7.423	7.429	7.433	7.432
S.D.		3.54	3.20	3.39	2.56	2.25	2.19	1.51	4.82	5.12	3.68	4.40	2.48	1.60	0.36	5.98	4.81	2.86	6.7	5.50	5.74	5.00	2.58	3.44	1.36	1.29	1.85	1.35	1.46	0.009	0.024	0.009	0.014	0.010	0.012	0.011
	B101	14	10	10	9	12	10	9	16.4	7.1	5.2	3.1	3.4	3.7	3.5	72.6	80.3	85.1	89.3	90.1	94.5	92.7	38.3	39.0	36.3	34.3	36.4	36.1	35.5	7.410	7.412	7.434	7.440	7.432	7.445	7.451
	B102	12	12	10	9	12	10	12	11.0	4.2	3.3	3.5	3.2	3.5	4.1	78.9	84.0	87.6	81.5	90.7	82.6	92.8	38.0	37.9	39.2	40.4	37.3	36.9	37.4	7.415	7.443	7.421	7.409	7.426	7.445	7.438
HORSES	B103	16	16	12	12	16	16	16	13.1	10.5	7.5	4.5	3.5	3.1	3.5	72.0	75.1	78.2	82.0	87.5	93.6	85.9	36.2	39.1	37.1	36.4	39.7	37.0	38.4	7.405	7.425	7.420	7.434	7.413	7.428	7.416
TREATED	B104	12	14	12	14	12	16	14	17.6	14.5	9.5	5.5	5.6	5.2	4.9	69.0	70.1	75.4	82.3	84.5	86.0	85.3	39.5	40.4	38.6	39.9	38.9	40.9	39.5	7.401	7.398	7.405	7.416	7.420	7.418	7.419
WITH	B105	24	18	16	14	12	16	12	26.5	13.5	9.4	4.0	3.5	4.5	4.8	62.9	72.6	79.9	88.0	94.5	90.7	87.1	38.6	40.4	37.2	36.4	37.2	37.5	38.9	7.381	7.398	7.408	7.406	7.415	7.434	7.422
ETAMIPHYLLINE	B106	22	22	18	16	18	16	16	19.0	16.0	12.5	5.5	5.5	5.5	4.1	64.9	70.9	72.6	82.9	84.9	76.4	85.9	40.6	41.7	39.9	40.7	38.7	40.4	38.7	7.402	7.392	7.404	7.409	7.412	7.415	7.422
CAMSYLATE.	B107	18	16	16	12	14	16	12	18.2	13.5	10.9	6.5	5.8	5.5	4.0	61.2	70.9	74.6	80.2	75.3	81.9	86.7	40.2	43.1	37.3	38.6	40.1	39.2	40.2	7.400	7.396	7.410	7.410	7.408	7.420	7.409
	B108	12	14	12	9	12	14	9	13.6	9.5	7.5	5.5	3.1	5.0	5.5	74.5	74.0	79.3	87.3	92.1	84.0	87.0	39.0	38.5	41.5	37.3	36.4	42.0	38.6	7.420	7.420	7.402	7.425	7.436	7.400	7.410
MEAN		16.25	15.25	13.25	11.88	13.50	14.25	12.5	16.93	11.1	8.23	4.67	4.20	4.50	4.30	69.50	74.74	79.09	84.19	87.45	86.21	87.93	38.8	40.01	38.39	38.0	38.09	38.75	38.4	7.404	7.411	7.413	7.419	7.420	7.426	7.423
S.D.		4.71	3.69	3.01	2.70	2.33	2.71	2.73	4.77	4.03	3.00	1.17	1.20	0.95	0.71	6.13	4.97	5.15	3.45	6.00	6.28	3.04	1.38	1.74	1.74	2.28	1.45	2.18	1.43	0.012	0.018	0.010	0.013	0.010	0.016	0.014
Statistical difference from corresponding values in untreated horses.		NS	NS	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

** = P<0.01
NS = not significant.

APPENDIX 8.1.

Respiratory function parameters recorded from COPD affected horses before and after 1 sodium cromoglycate treatment (Group 1).

Horse Number	Challenge environment No drug treatment				Controlled environment No drug treatment				Challenge environment after sodium cromoglycate treatment.				End of trial				Days asymptomatic after sodium cromoglycate treatment
	R.R. (/min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	R.R. (/min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	R.R. (/min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	R.R. (/min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	
B109	18	14.5	63.4	40.2	12	4.1	87.3	38.0	13	4.8	80.2	38.6	16	8.7	70.9	38.6	2
B110	18	8.1	76.1	37.6	12	4.0	86.4	36.0	12	4.0	86.4	37.0	14	7.3	83.9	37.4	4
B111	16	13.0	72.4	39.0	10	3.1	91.6	35.7	11	3.8	84.9	36.6	12	6.5	79.2	38.3	5
B112	20	10.4	75.9	38.2	12	3.4	88.0	37.0	12	3.8	85.7	37.2	16	8.0	78.2	37.6	2
B113	14	9.2	75.2	38.5	9	3.7	93.4	35.3	10	3.7	86.7	36.0	12	6.1	81.6	36.9	4
B114	14	6.0	82.9	39.9	7	3.2	86.2	39.0	11	4.0	85.4	40.2	13	6.4	78.3	42.9	3
B115	18	6.9	74.1	41.0	12	3.4	88.9	37.5	11	3.5	85.7	39.2	12	5.5	79.6	40.4	3
B116	12	12.2	64.4	41.8	10	4.1	87.2	38.5	9	4.0	85.0	38.3	12	7.9	82.3	39.5	4
B117	15	13.5	71.0	43.3	9	3.0	92.0	31.6	12	3.7	86.8	37.5	12	5.5	77.4	40.3	5
Mean	16.11	10.42	72.82	39.94	10.33	3.56	89.00	36.51	11.22	3.92	85.40	37.82	13.22	6.88	79.04	39.10	3.56
± S.D.	±2.57	±3.06	±6.04	±1.85	±1.80	±0.43	±2.67	±2.24	±1.20	±0.37	±2.27	±1.34	±1.72	±1.15	±3.73	±1.89	±1.13

R.R. - Respiratory rate

Challenge environment, no drug treatment - Mean values recorded after 24 hours in challenge environment.

Controlled environment, no drug treatment - Mean values recorded when horses were clinically asymptomatic.

Challenge environment after sodium cromoglycate treatment - Mean values recorded on a composite of days at 1 and 2 day intervals throughout each horse's period of protection

End of trial - Mean values recorded at the end of the sodium cromoglycate protective period.

APPENDIX 8.2.

Respiratory function parameters recorded from COPD affected horses before and after sodium cromoglycate treatment on 2 successive days (Group 2).

Horse Number	Challenge environment, No drug treatment				Controlled environment, No drug treatment				Challenge environment after sodium cromoglycate treatment				End of trial				Days asymptomatic after sodium cromoglycate treatment
	R.R. (/min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	R.R. (/min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	R.R. (/min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	R.R. (/min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	
B118	15	10.5	73.0	38.3	10	3.0	89.7	36.5	10	3.8	88.6	37.0	11	7.3	79.9	37.3	8
B119	16	8.0	78.6	44.7	10	3.0	89.7	36.5	11	3.5	89.2	37.0	14	6.1	82.4	37.8	10
B120	15	13.2	60.9	39.2	8	4.3	88.1	36.4	10	4.5	87.5	38.1	14	7.9	72.5	38.6	7
B121	18	15.0	68.5	39.1	12	4.1	89.5	36.4	10	4.5	87.0	37.2	14	6.0	80.3	38.4	7
B122	13	7.6	78.1	38.5	10	3.1	88.9	36.5	11	3.4	90.0	36.3	15	6.0	83.0	34.9	19
B123	16	8.3	82.3	39.0	10	3.5	92.4	37.0	11	3.8	91.1	36.6	12	6.2	81.0	37.8	9
B124	12	6.1	62.5	40.5	8	3.0	91.2	34.0	11	4.2	88.4	35.9	12	7.4	62.0	41.9	5
B125	20	7.0	61.9	40.0	10	3.0	84.5	38.7	11	3.7	86.0	37.7	12	6.1	74.3	43.8	7
B126	18	20.3	65.6	41.2	12	4.5	87.4	37.9	11	4.5	85.5	38.9	14	7.5	76.2	40.4	8
B127	16	8.5	59.7	44.5	10	4.1	88.4	35.3	10	4.4	86.5	35.8	12	6.4	76.3	40.5	7
B128	19	22.9	61.0	45.9	12	3.4	82.3	39.6	10	4.3	82.6	38.5	12	8.8	72.7	40.6	4
B129	12	8.0	74.6	39.2	7	3.0	90.0	33.1	8	4.0	86.6	34.9	12	8.5	72.0	36.3	7
B130	18	22.2	63.8	36.4	12	3.5	89.2	34.0	10	3.8	86.8	35.7	12	6.1	78.2	37.9	10
B131	10	8.5	72.3	37.6	8	3.6	89.0	32.5	10	4.1	88.5	35.3	12	8.7	73.4	38.0	11
B132	18	11.8	67.5	42.6	10	3.5	87.0	36.1	10	4.2	87.9	36.2	12	6.5	82.4	40.9	8
B133	18	6.0	83.6	30.0	8	3.3	91.6	37.6	9	3.8	90.8	35.3	12	6.6	82.6	36.9	10
B134	14	9.2	71.9	36.1	8	3.5	92.5	33.4	10	3.7	86.5	34.6	12	5.9	84.3	36.5	7
B135	14	27.3	58.0	35.0	12	4.3	87.0	36.5	12	4.0	86.5	37.0	13	8.5	70.1	39.0	2
B136	12	10.1	75.0	34.1	9	3.0	90.5	35.2	9	3.4	92.7	35.5	12	7.2	80.1	37.2	10
B137	18	22.0	59.4	44.6	12	3.6	86.4	37.9	11	4.3	83.1	38.2	14	9.5	74.1	40.5	2
B138	16	12.5	72.6	37.4	9	3.1	86.3	36.4	9	3.6	90.7	35.6	12	9.3	78.4	38.9	9
B139	15	6.8	83.0	34.9	8	3.3	90.5	34.0	10	3.5	89.9	36.1	12	13.0	73.4	38.0	7
B140	15	12.9	73.4	38.0	10	3.0	87.6	35.9	11	3.4	92.5	36.8	12	10.5	63.8	35.7	11
Mean	15.56	12.38	69.88	38.99	9.78	3.47	88.68	35.97	10.20	3.92	88.03	36.52	12.56	7.65	76.24	38.60	8.04
± S.D.	±2.61	±6.25	±8.09	±3.83	±1.62	±0.48	±2.46	±1.82	±0.90	±0.38	±2.64	±1.18	±1.04	±1.77	±5.91	±2.12	±3.44

R.R. - Respiratory rate.

Challenge environment, no drug treatment - Mean values recorded after 24 hours in challenge environment.

Controlled environment, no drug treatment - Mean values recorded when horses were clinically asymptomatic.

Challenge environment after sodium cromoglycate treatment - Mean values recorded on a composite of days at 2 and 3 day intervals throughout each horse's period of protection.

End of trial - Mean values recorded at the end of the sodium cromoglycate protective period.

APPENDIX 8.3.

Respiratory function parameters recorded from COPD affected horses before and after sodium cromoglycate treatment on 3 successive days (Group 3).

Horse Number	Challenge environment No drug treatment				Controlled environment No drug treatment				Challenge environment after sodium cromoglycate treatment				End of trial				Days asymptomatic after sodium cromoglycate treatment
	R.R. (/min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	R.R. (/min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	R.R. (/min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	R.R. (/min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	
B141	20	9.5	69.5	43.2	12	4.1	84.4	41.0	10	3.8	85.7	38.5	12	6.7	74.6	38.7	12
B142	18	34.0	64.4	47.0	9	4.6	88.7	37.6	10	4.5	85.1	38.3	11	8.5	76.5	39.0	14
B143	14	18.0	63.9	44.3	12	4.0	87.0	38.5	12	4.2	82.6	39.2	14	7.1	82.1	39.2	11
B144	18	11.5	69.4	38.2	10	3.4	87.6	34.0	10	3.5	88.9	36.8	12	9.7	72.9	37.6	15
B145	16	7.0	62.0	41.9	12	4.9	87.3	37.9	10	4.5	86.6	37.0	12	8.5	79.7	38.5	7
B146	15	8.5	72.0	36.3	13	3.0	83.7	43.6	11	3.6	84.5	38.0	13	10.4	60.6	37.4	7
B147	12	15.5	58.4	42.1	12	4.2	89.9	38.6	12	4.4	85.4	38.4	12	6.6	62.6	36.2	8
B148	15	12.2	66.1	34.8	10	3.0	93.5	36.5	10	3.9	88.3	36.6	13	8.0	80.6	33.1	12
B149	15	14.0	81.9	36.1	9	3.2	94.6	36.2	11	3.7	88.6	36.7	16	6.5	83.2	38.6	16
B150	18	12.7	74.1	38.2	10	3.5	88.4	37.4	10	3.5	90.5	35.9	12	6.2	82.1	38.5	12
B151	15	13.0	63.1	46.0	11	4.0	87.9	37.3	10	3.8	86.6	36.9	12	6.7	79.6	37.8	13
B152	14	13.4	62.8	42.3	10	3.8	88.1	37.3	10	3.6	89.3	37.2	12	6.0	81.3	39.8	12
B153	12	8.3	82.3	29.7	11	3.3	94.6	31.7	10	3.5	90.1	34.8	14	6.1	78.2	37.5	15
B154	11	10.0	76.5	40.8	8	3.0	88.5	33.9	9	3.5	86.8	36.4	12	6.0	79.6	37.3	14
B155	20	10.5	76.2	39.0	12	3.5	90.5	37.1	12	3.7	86.4	38.1	18	7.2	78.1	38.5	14
B156	14	8.2	79.4	39.3	10	3.4	84.9	34.9	11	3.6	86.0	37.0	14	6.1	62.5	40.5	9
Mean	15.44	12.89	70.13	39.95	10.69	3.68	88.73	37.09	10.50	3.83	86.95	37.24	13.06	7.27	75.89	38.01	11.94
± S.D.	±2.73	±6.35	±7.59	±4.46	±1.40	±0.58	±3.29	±2.80	±0.89	±0.36	±2.18	±1.12	±1.81	±1.37	±7.46	±1.68	±2.86

R.R. - Respiratory rate.

Challenge environment, no drug treatment - Mean values recorded after 24 hours in challenge environment.

Controlled environment, no drug treatment - Mean values recorded when horses were clinically asymptomatic.

Challenge environment after sodium cromoglycate treatment - Mean values recorded on a composite of days at 2 and 3 day intervals throughout each horse's period of protection.

End of trial - Mean values recorded at the end of the sodium cromoglycate protective period.

APPENDIX 8.4.

Respiratory function parameters recorded from COPD affected horses before and after sodium cromoglycate treatment on 4 successive days (Group 4).

Horse Number	Challenge environment No drug treatment				Controlled environment No drug treatment				Challenge environment after sodium cromoglycate treatment				End of trial				Days asymptomatic after sodium cromoglycate treatment
	R.R. (/min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	R.R. (/min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	R.R. (/min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	R.R. (/min.)	Max. Δ Ppl (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	
B157	15	9.5	70.6	39.0	10	3.7	88.1	35.0	11	3.9	82.9	37.5	12	6.2	80.0	38.2	18
B158	18	14.2	68.1	39.2	12	5.0	89.9	36.2	12	4.1	85.0	37.3	14	7.3	78.2	37.4	26
B159	16	10.5	73.9	38.4	12	3.2	92.1	34.6	11	4.0	84.2	37.6	18	8.0	76.2	39.5	17
B160	24	25.0	64.4	39.2	12	4.0	89.9	35.6	13	5.1	84.3	37.9	14	9.1	80.7	39.0	12
B161	16	12.5	74.3	37.5	12	4.4	85.6	32.1	13	4.1	83.4	33.7	10	7.9	79.7	30.9	17
B162	12	15.5	68.2	36.1	12	3.1	90.6	37.2	10	3.5	85.4	36.2	8	12.6	77.6	37.1	39
B163	12	10.1	77.0	38.2	12	3.0	92.5	37.1	11	3.2	89.1	33.8	10	6.6	88.3	32.0	50
B164	18	22.5	63.0	40.7	12	4.5	87.3	37.4	13	4.1	84.0	35.7	14	8.3	76.3	36.1	15
Mean	16.38	14.98	69.94	38.54	11.75	3.86	89.50	35.65	11.75	4.00	84.78	36.22	12.50	8.25	79.63	36.28	24.25
± S.D.	±3.82	±5.83	±4.93	±1.36	±0.71	±0.74	±2.37	±1.77	±1.17	±0.57	±1.93	±1.71	±3.16	±1.99	±3.88	±3.18	±13.44

R.R. - Respiratory rate.

Challenge environment, no drug treatment - Mean values recorded after 24 hours in challenge environment.

Controlled environment, no drug treatment - Mean values recorded when horses were clinically asymptomatic.

Challenge environment after sodium cromoglycate treatment - Mean values recorded on a composite of days at 2 and 3 day intervals throughout each horse's period of protection.

End of trial - Mean values recorded at the end of the sodium cromoglycate protective period.

APPENDIX 9.1.

Prophylactic effects of intermittent sodium cromoglycate treatment on respiratory rate and max. Δ Ppl in COPD affected horses housed in the natural challenge environment.

Trial	Horse Number	Respiratory Rate (/min.) Days 0	1	2	4	7	9	11	14	16	18	21	23	25	28
HORSES UNTREATED	B117	10	10	12	12	12	14	14	12	10	12	14	12	12	12
	B121	12	12	14	12	12	12	16	20	20	18	18	20	18	18
	B126	12	12	13	12	13	12	11	15	16	13	16	14	15	14
	B130	14	14	16	16	16	16	20	16	18	18	16	18	18	16
	B140	12	14	16	16	22	18	16	20	18	24	18	16	16	18
	B154	14	14	16	16	18	24	20	20	18	18	20	18	18	20
	B162	10	10	12	12	12	10	12	14	12	12	14	12	12	14
	B163	10	12	12	12	9	10	12	12	10	12	11	12	12	12
Mean value		11.75	12.25	13.88	13.50	14.25	14.50	15.13	16.13	15.25	15.88	15.88	15.25	15.13	15.50
± S.D.		1.67	1.67	1.89	2.07	4.17	4.75	3.52	3.48	3.99	4.36	2.85	3.20	2.80	2.98
Statistical difference of values recorded during the trial from baseline values (Day 0).			NS	***	**	NS	NS	**	**	**	*	***	**	***	**
HORSES TREATED WITH SODIUM CROMOGLYCATE.	B117	9	9	9	10	12	12	10	12	10	9	9	11	10	10
	B121	12	12	11	11	12	12	12	10	12	10	12	11	12	10
	B126	10	10	12	12	12	10	10	9	9	12	10	12	13	14
	B130	20	20	14	16	20	16	16	13	18	14	13	14	16	14
	B140	11	12	12	14	16	14	14	14	18	12	12	14	16	13
	B154	16	16	14	14	12	12	14	12	14	12	16	14	16	14
	B162	12	13	12	10	12	10	12	12	10	12	10	8	10	10
	B163	16	14	16	12	12	14	12	12	10	12	10	12	10	9
Mean value		13.25	13.25	12.50	12.38	13.5	12.5	12.5	11.75	12.63	11.63	11.50	12.00	12.88	11.75
± S.D.		3.73	3.49	2.14	2.13	2.98	2.07	2.07	1.58	3.66	1.51	2.27	2.07	2.80	2.19
Statistical difference of values recorded during the trial from baseline values (Day 0).			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Statistical difference from corresponding values in untreated horses.		NS	NS	NS	*	NS	NS	*	*	NS	*	***	*	NS	**

Trial	Horse Number	Max. Δ Ppl (mm Hg). Days 0	1	2	4	7	9	11	14	16	18	21	23	25	28
HORSES UNTREATED	B117	3.3	9.5	11.2	12.5	10.5	10.7	15.4	12.2	13.9	15.4	14.6	12.5	12.8	13.1
	B121	3.5	14.8	16.7	15.8	16.6	14.5	17.4	22.5	25.1	20.2	17.5	21.5	24.3	19.7
	B126	3.5	9.2	10.4	13.5	13.6	16.8	14.4	18.7	22.5	20.8	24.1	21.2	23.9	19.6
	B130	3.5	9.5	12.2	10.4	13.5	15.6	15.9	18.5	15.4	20.5	17.8	19.4	15.3	18.5
	B140	4.1	15.5	22.7	25.5	32.6	28.2	22.5	35.6	28.7	34.0	30.5	32.2	25.7	33.9
	B154	4.9	10.2	13.5	13.4	15.8	18.1	16.5	15.4	17.6	15.2	12.5	17.6	20.8	17.3
	B162	3.7	8.5	9.1	10.5	9.6	12.5	15.3	10.8	14.9	13.5	16.3	14.5	15.7	12.6
	B163	3.4	7.5	9.2	8.5	10.8	9.1	11.5	13.4	15.5	12.6	10.8	14.4	12.0	12.5
Mean value		3.74	10.59	13.13	13.76	15.38	15.69	16.11	18.39	19.20	19.03	18.01	19.16	18.81	18.40
± S.D.		0.53	2.93	4.60	5.26	7.40	5.90	3.12	7.95	5.52	6.87	6.44	6.22	5.50	6.98
Statistical difference of values recorded during the trial from baseline values (Day 0).			***	***	***	***	***	***	***	***	***	***	***	***	***
HORSES TREATED WITH SODIUM CROMOGLYCATE.	B117	3.5	3.9	4.3	3.9	3.5	3.7	4.2	4.5	4.0	3.5	3.6	4.2	3.5	4.6
	B121	3.3	4.5	3.6	4.7	3.8	3.4	6.1	5.7	4.5	4.9	3.5	4.7	5.2	6.5
	B126	3.5	3.2	4.4	3.5	3.5	4.2	5.5	4.9	3.5	5.6	3.2	3.5	6.5	5.5
	B130	3.2	4.0	3.5	3.8	3.1	4.5	5.1	4.6	3.5	4.4	5.2	3.4	5.5	3.6
	B140	5.8	5.3	4.7	4.5	4.6	4.0	5.7	3.5	4.3	4.5	6.8	6.2	8.5	10.9
	B154	3.4	3.5	3.8	4.9	3.5	3.5	4.4	3.1	3.8	5.8	4.2	3.5	5.1	5.7
	B162	3.0	3.7	3.9	3.5	3.2	3.8	4.5	5.6	5.1	5.6	3.5	3.4	5.2	6.8
	B163	5.1	4.5	3.5	3.3	4.4	6.2	4.9	3.2	3.9	4.5	3.1	4.9	6.5	3.4
Mean value		3.85	4.08	3.96	4.01	3.70	4.16	5.05	4.39	4.08	4.85	4.39	4.23	5.75	5.88
± S.D.		1.02	0.67	0.45	0.61	0.54	0.90	0.68	1.03	0.54	0.78	1.26	1.00	1.46	2.38
Statistical difference of values recorded during the trial from baseline values (Day 0).			NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	***	*
Statistical difference from corresponding values in untreated horses.		NS	***	***	***	**	**	***	**	***	***	***	***	***	***

N.S. = not significant

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

APPENDIX 9.2.

Prophylactic effects of intermittent sodium cromoglycate treatment on PaO_2 and $PaCO_2$ in COPD affected horses housed in the natural challenge environment.

PaO_2 (mm Hg).

Trial	Horse Number	Days 0	1	2	4	7	9	11	14	16	18	21	23	25	28
HORSES UNTREATED.	B117	88.9	80.7	80.9	78.4	76.7	80.5	72.1	73.0	75.4	79.5	72.5	68.1	70.7	72.5
	B121	84.9	77.0	74.3	70.6	73.1	75.5	71.9	66.0	61.3	60.7	64.5	64.0	68.1	69.5
	B126	92.1	80.9	76.5	72.4	70.9	72.1	67.5	68.3	64.1	66.6	62.5	63.0	68.5	70.9
	B130	87.0	80.6	78.2	72.5	70.6	74.1	72.0	66.4	69.7	67.3	75.0	70.8	73.1	70.4
	B140	84.6	72.9	68.0	65.4	62.0	66.3	65.4	59.7	62.3	64.5	66.1	60.8	61.9	58.5
	B154	89.5	82.1	72.5	78.9	68.4	73.5	70.6	76.1	70.9	68.4	74.3	69.5	66.1	69.0
	B162	88.4	82.1	78.2	74.0	78.1	75.5	76.2	75.0	69.4	73.0	74.1	76.0	71.9	72.7
	B163	92.7	84.6	83.0	77.2	74.1	78.5	79.0	79.0	74.1	70.7	75.3	70.9	72.1	73.2
Mean value		88.51	80.11	76.45	73.68	71.74	74.50	71.84	70.44	68.40	68.84	70.54	67.89	69.05	69.59
± S.D.		2.98	3.60	4.79	4.52	5.08	4.28	4.35	6.42	5.30	5.70	5.26	5.00	3.73	4.73
Statistical difference of values recorded during the trial from baseline values (Day 0).			xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
HORSES TREATED WITH SODIUM CROMOGLYCATE.	B117	88.0	84.5	87.9	90.6	86.3	84.1	82.7	86.0	86.4	78.9	84.5	89.7	80.5	83.4
	B121	87.6	85.0	88.1	83.5	89.0	90.1	85.5	76.0	85.3	84.2	90.7	86.3	80.2	78.9
	B126	86.3	88.4	82.5	83.0	87.6	78.1	80.5	85.1	90.7	75.2	79.3	84.1	85.9	83.7
	B130	86.3	85.3	84.5	87.1	91.3	82.3	80.9	82.7	88.3	84.7	86.2	80.8	78.1	86.6
	B140	80.3	79.5	82.1	76.4	71.1	82.1	75.4	80.9	83.7	82.1	71.9	76.9	68.7	69.7
	B154	90.1	86.4	87.5	82.0	92.6	88.4	83.9	84.7	91.5	87.9	85.3	84.0	77.5	80.6
	B162	81.0	86.4	88.4	82.1	84.7	77.8	79.5	82.9	84.8	78.7	86.8	86.9	79.2	80.8
	B163	87.5	90.4	92.6	89.3	86.6	86.7	83.3	88.7	98.6	86.2	88.1	87.2	85.7	86.5
Mean value		85.89	85.74	86.70	84.25	86.15	83.7	81.46	83.38	88.66	82.24	84.1	84.49	79.48	81.28
± S.D.		3.45	3.18	3.49	4.59	6.62	4.52	3.14	3.80	4.89	4.32	5.91	4.06	5.39	5.43
Statistical difference of values recorded during the trial from baseline values (Day 0).			NS	NS	NS	NS	NS	xxx	NS	NS	x	NS	NS	xx	x
Statistical difference from corresponding values in untreated horses.		NS	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx

$PaCO_2$ (mm Hg)

Trial	Horse Number	Days 0	1	2	4	7	9	11	14	16	18	21	23	25	28
HORSES UNTREATED.	B117	36.1	37.5	36.4	38.5	39.1	36.5	40.4	37.9	38.5	38.0	39.2	41.2	39.0	38.7
	B121	37.6	37.8	38.5	39.7	39.1	38.4	39.3	39.4	39.6	40.5	42.3	41.5	44.0	40.2
	B126	33.5	35.7	37.9	38.4	39.6	38.5	37.0	38.7	40.4	44.9	42.1	39.5	42.6	43.0
	B130	35.3	37.5	36.1	38.4	37.1	36.3	39.2	39.9	37.1	40.7	36.1	38.3	38.0	39.5
	B140	36.3	37.0	39.4	37.9	40.9	41.0	39.7	40.5	37.9	39.7	37.3	36.1	37.8	39.0
	B154	38.4	37.3	40.0	39.5	38.4	37.7	36.7	39.2	38.9	37.5	40.9	39.5	41.5	40.4
	B162	37.6	38.2	36.4	37.3	36.4	38.0	39.3	38.2	39.8	37.2	38.2	39.0	36.3	39.0
	B163	33.1	35.7	35.0	37.6	38.7	34.0	36.5	34.1	35.6	37.9	36.4	38.1	35.9	36.3
Mean value		35.99	37.09	37.46	38.41	38.66	37.55	38.51	38.49	38.48	39.55	39.06	39.15	39.39	39.51
± S.D.		1.93	0.92	1.76	0.85	1.41	2.04	1.53	1.97	1.58	2.56	2.47	1.74	2.98	1.89
Statistical difference of values recorded during the trial from baseline values (Day 0).			x	NS	xx	x	NS	xx	xx	xx	x	x	xx	x	xx
HORSES TREATED WITH SODIUM CROMOGLYCATE.	B117	38.9	37.6	39.0	36.4	37.0	37.4	37.2	38.5	36.1	37.4	34.4	36.9	38.5	37.3
	B121	36.7	37.1	37.0	38.5	37.2	34.6	36.0	38.7	34.3	35.7	35.5	36.1	38.3	37.7
	B126	39.3	39.2	39.0	37.3	38.4	36.1	38.5	38.9	37.3	39.6	37.8	38.0	38.7	39.4
	B130	38.7	37.6	39.3	37.5	36.6	37.4	36.3	35.5	37.6	36.1	37.8	36.8	37.7	35.4
	B140	37.0	37.6	35.7	37.8	40.0	37.5	39.9	40.7	38.6	36.5	40.7	43.4	36.4	37.2
	B154	35.4	37.8	36.0	38.4	35.2	36.9	34.5	32.7	36.9	35.4	39.7	36.3	37.6	35.8
	B162	44.0	37.9	37.6	37.9	39.8	38.4	38.4	37.2	27.1	30.9	36.7	33.5	31.9	34.7
	B163	38.6	37.5	35.3	37.0	36.5	38.1	39.0	39.1	27.6	33.3	32.2	33.4	30.1	39.1
Mean value		38.58	37.79	37.36	37.60	37.59	37.05	37.48	37.55	34.44	35.61	38.85	36.80	36.15	37.08
± S.D.		2.57	0.62	1.61	0.71	1.69	1.21	1.80	2.48	4.55	2.61	2.78	3.12	3.29	1.69
Statistical difference of values recorded during the trial from baseline values (Day 0).			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Statistical difference from corresponding values in untreated horses.		NS	NS	NS	x	NS	NS	NS	NS	x	xxx	NS	NS	xx	x

NS = not significant
x = $P < 0.05$
xx = $P < 0.01$
xxx = $P < 0.001$

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Short Communication

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

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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 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 ($Pa0_2$). 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 ± 2.84	8.00 ± 4.50	4.67 ± 1.16
	$Pa0_2$ (mmHg)	82.93 ± 2.15	77.70 ± 4.29	84.87 ± 5.26
5 hours post-challenge	max. Δ Ppl (mmHg)	13.67 ± 2.30	16.00 ± 4.27	12.50 ± 2.00
	$Pa0_2$ (mmHg)	76.80 ± 4.25	74.47 ± 9.17	80.23 ± 2.15
$\bar{d}$	max. Δ Ppl (mmHg)	7.17 ± 1.15	7.67 ± 0.29	7.83 ± 0.76
	$Pa0_2$ (mmHg)	-6.13 ± 5.64	-3.43 ± 5.75	-4.63 ± 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 ($Pa0_2$). 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 ± 1.89	4.67 ± 1.61
	$Pa0_2$ (mmHg)	81.97 ± 3.61	85.43 ± 2.64
5 hours post-challenge	max. Δ Ppl (mmHg)	4.66 ± 2.08	4.50 ± 1.50
	$Pa0_2$ (mmHg)	85.30 ± 4.85	86.90 ± 4.35
$\bar{d}$	max. Δ Ppl (mmHg)	0.33 ± 0.29	3.33 ± 5.12
	$Pa0_2$ (mmHg)	-0.17 ± 0.58	1.47 ± 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 ± 1.55	3.50 ± 0.5
		Day 4	3.5	3.5
		Day 5	9.0	4.0
	PaO_2 (mmHg)	Days 1-3	85.50 ± 1.55	87.37 ± 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 ± 0.58	3.16 ± 0.29
		Day 4	10.0	4.5
		Day 5	14.5	9.5
	PaO_2 (mmHg)	Days 1-3	85.77 ± 3.93	89.27 ± 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. 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.

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

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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 Rossdale 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 observation 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 $\triangle$ Ppl) of $\geqslant$ 6 mm Hg and resting arterial oxygen partial pressures (PaO_2) of $\leqslant$ 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 $\triangle$ 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 PaO_2 and carbon dioxide partial pressure ($PaCO_2$) 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 PaO_2 and $PaCO_2$ estimations using this technique is reported to be less than 2 per cent.

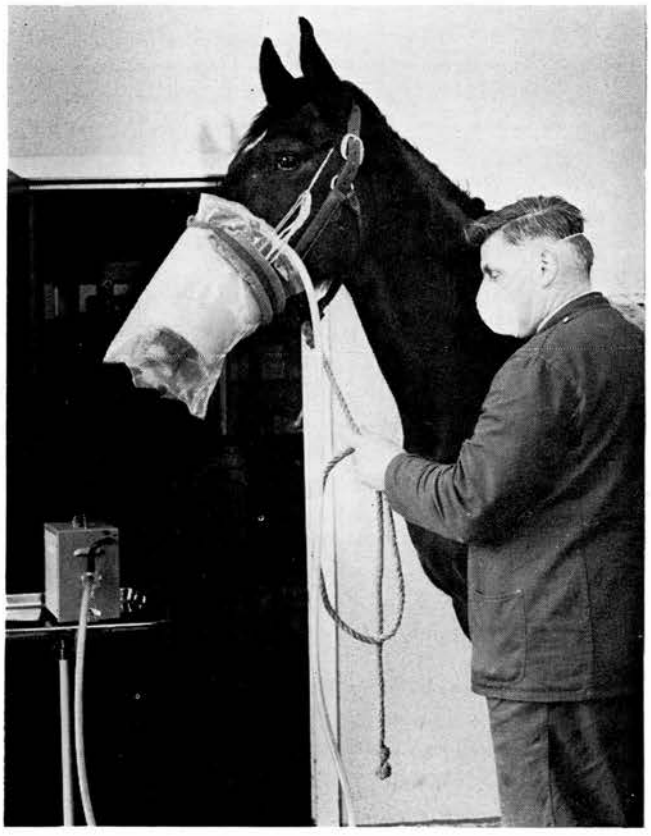

Fig 1. Method of drug inhalation in the horse using a face mask and Wright's nebuliser.

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 △ Ppl and PaO_2 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.

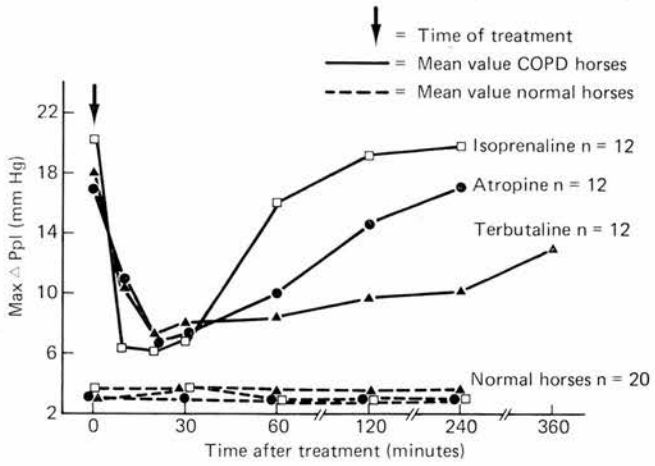

Fig 2. Effect of bronchodilator drugs on intrathoracic pressure in COPD affected and normal horses.

Intrathoracic pressure changes

Max △ Ppl decreased significantly ($P<0.001$) in COPD affected horses after administration of all 3 drugs. Max △ 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 △ Ppl after all drugs, max △ 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 △ 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.

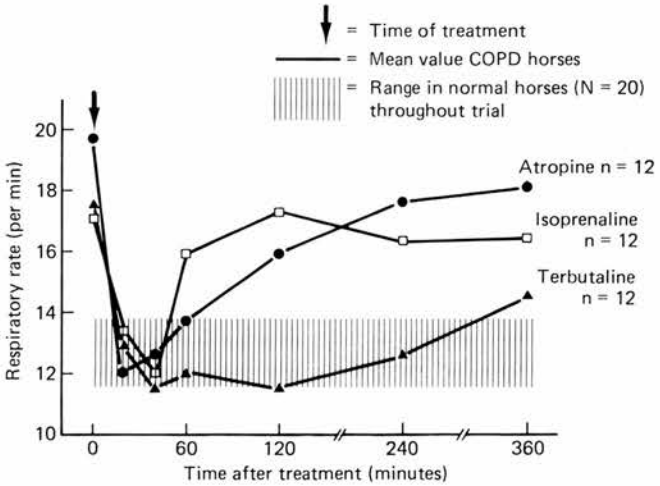

Fig 3. Effect of bronchodilator drugs on respiratory rate in COPD affected and normal horses.

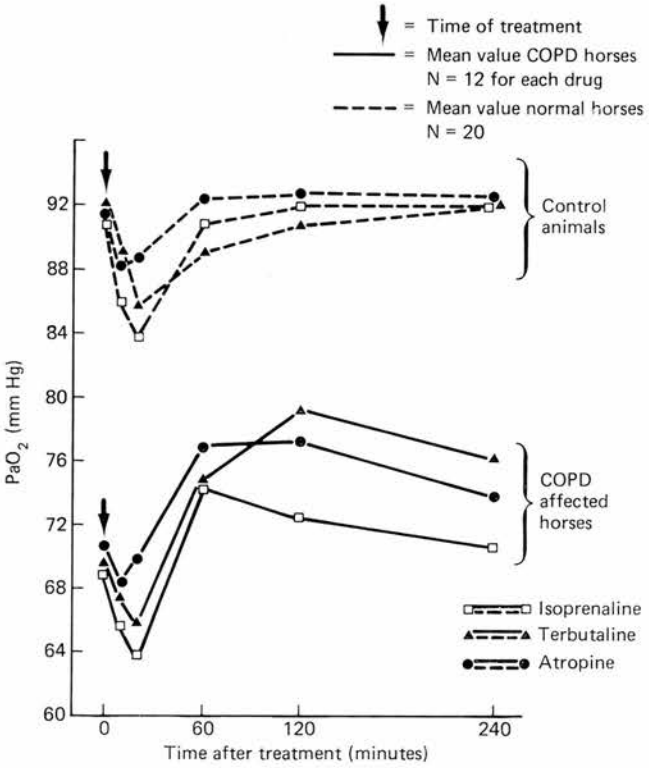

Fig 4. Effect of bronchodilator drugs on PaO_2 in normal and COPD affected horses.

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 ($\bar{\times}$ 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 $\triangle$ Ppl with terbutaline was not as great as with isoprenaline, terbutaline's action was more prolonged with max $\triangle$ 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 $\triangle$ 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 $\triangle$ Ppl was found to decrease greatly after all drugs. While this max $\triangle$ 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 $\triangle$ Ppl decrease; it is also possible that a decrease in tidal volume could have caused a decrease in max $\triangle$ 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 $\triangle$ 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 $\triangle$ 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 $\triangle$ 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 △ 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 △ Ppl in COPD horses after bronchodilator administration, their max △ Ppl and PaO_2 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 PaO_2 levels in both normal and COPD animals occurred, without a simultaneous increase in $PaCO_2$ 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, Krumpe, Duffell and Maniscalco 1970; Chick, Nicholson and Johnson 1973). This fall in PaO_2 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

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Résumé

Les effets d'agents bronchodilateurs 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 Bronchodilatatoren (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

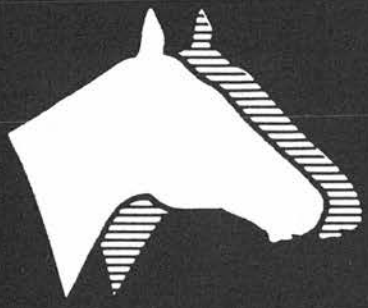
ELLIS, R. N. W. and LAWRENCE, T. L. J. (1979). *Br. vet. J.* **135,** 331-337.

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



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Prophylactic effects of sodium cromoglycate on chronic obstructive pulmonary disease in the horse

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Summary

When stabled in a controlled environment, horses affected with chronic obstructive pulmonary disease (COPD) became clinically asymptomatic in 4 to 32 days (mean [± sd] 9.1 ± 4.9 days), the time being influenced most by the severity of the disease judged on maximum intrathoracic pressure change (Max Δ Ppl) and the age of the animal.

Sodium cromoglycate, a drug widely used for prophylaxis of allergic respiratory disease in man, was administered by inhalation to 56 COPD-affected horses. The results showed that a linear response existed between the number of successive days treatment with this drug and the duration of remission of clinical signs of COPD while horses were exposed to natural antigen challenge (poor quality straw bedding). Sodium cromoglycate given as a single daily dose (80 mg) on one day and on 4 successive days prevented clinical signs for mean 3.6 days and mean 24.3 days, respectively. These results indicate that prophylactic treatment with inhaled sodium cromoglycate is an effective method of controlling the clinical signs of COPD.

Introduction

CHRONIC obstructive pulmonary disease (COPD) ("heaves") is usually associated with exposure of susceptible animals to poor quality (dusty, mould-contaminated) hay and straw bedding and inadequate stable ventilation. Most horses affected with COPD show respiratory hypersensitivity to inhaled antigens, eg, *Micropolyspora faeni* and *Aspergillus fumigatus*, mainly as a delayed response at 4 to 8 h after challenge (McPherson *et al* 1979).

Usually, the condition can be controlled by removal of the affected horses from contact with the aetiological agents (Thurlbeck and Lowell 1964; Cook 1976). However, strict environmental control is not always possible and additional means of prophylaxis are therefore required. Atropine, sympathomimetic bronchodilators and corticosteroids cause a temporary clinical improvement, but these compounds are short-acting and some have side effects (Obel and Schmitterlöw 1948; Muylle and Oyaert 1973; Gerber 1973; Sasse and Hajer 1977; Murphy, McPherson and Dixon 1980).

Inhaled sodium cromoglycate is widely used in the prophylaxis of human bronchial asthma because it inhibits immediate and delayed hypersensitivity reactions to inhaled allergens in affected individuals (Altounyan 1967; Pepys, Hargreave, Chan and McCarthy 1968). It is believed to act by maintaining membrane stability of sensitised pulmonary mast cells, so inhibiting cellular degranulation after exposure to the allergen and thus preventing release of the pharmacological mediators which cause constriction of smooth muscle in the airways.

The role of mast cells in the pathogenesis of equine COPD has not been established but pulmonary mast cell hyperplasia has been noted in COPD-affected horses (Nicholls 1978). In preliminary studies on 2 COPD-affected horses hypersensitive to *M faeni*, inhalation of sodium cromoglycate 20 to 30 mins before antigen inhalation challenge prevented the exacerbation of respiratory disease normally observed 4 to 8 h after challenge. After a single sodium cromoglycate treatment, horses failed to respond to antigen challenge for 4 to 5 days (Murphy, McPherson and Lawson 1979).

A clinical trial to evaluate the efficacy of sodium cromoglycate on a larger number of COPD-affected horses is described here.

Materials and methods

Animals

Fifty-six horses, referred for respiratory disease investigation, were divided into 4 groups according to duration of sodium cromoglycate treatment. Their breed type, age and duration of illness before the trial are shown in Table 1.

Diagnosis and protocol of antigenic challenge

The criteria of McPherson *et al* (1978) were used to diagnose COPD. Affected animals showed many of the following signs: coughing, dyspnoea, double expiratory effort, increased harsh chest sounds with wheezing or crepitant sounds. All horses showed a maximum intrathoracic pressure change (Max Δ Ppl) of 6 mmHg or more and a resting arterial oxygen partial pressure (Pa_{O_2}) of 82 mmHg or less.

Horses were housed in loose-boxes (dimensions 5 × 4 × 3 m) and fed a diet of hay *ad libitum* and oats. A natural antigenic challenge was achieved by bedding the horses on wheat straw, visibly contaminated with moulds.

Horses affected with COPD were exposed to this environment for 4 to 5 days to establish that an exacerbation of COPD signs occurred.

The horses were then bedded on peat moss and fed a complete cubed diet (Spillers Agriculture Ltd, London) to minimise exposure to aetiological antigens, until clinical signs abated and their Max Δ Ppl and Pa_{O_2} levels were within normal ranges (Max Δ Ppl < 5 mmHg, $Pa_{O_2} \geqslant 86$ mmHg).

TABLE 1: The type, age (mean ± sd) and duration of illness (mean ± sd) of COPD-affected horses used in the clinical trial

Number of days treatment (group number)	Number of horses	Type			Age (years)	Duration of illness (months)
		Thoroughbred	Hunter	Pony		
1	9	4	4	1	9.33 ± 3.20	22.11 ± 17.57
2	23	10	7	6	10.65 ± 5.27	22.17 ± 25.56
3	16	7	6	3	8.56 ± 2.96	14.50 ± 12.03
4	8	1	6	1	13.25 ± 4.40	40.50 ± 18.07

Treatment

The horses were then given sodium cromoglycate 1 per cent w/v solution (Cromovet; Fisons Ltd, Loughborough) nebulised into a face mask applied over the horse's mouth and nose. The solution was converted into an aerosol using a Wright's nebuliser or a Pulmo-Aide series 561 Portable Compressor (Devilbiss, Pennsylvania) and a Cromovet nebuliser (Fisons Ltd, Loughborough). Eighty milligrams of sodium cromoglycate was administered as a single daily dose for one, 2, 3 or 4 successive days to 9, 23, 16 and 8 horses respectively (**Groups 1, 2, 3** and **4** — Table 1).

Parameters used to assess response

On completion of the treatment schedule, horses were returned to the challenge environment and were subjected to daily clinical examination. Particular attention was paid to the presence and degree of double expiratory effort, coughing, respiratory rate and chest sounds. Max Δ Ppl, Pa_{O_2} and carbon dioxide partial pressures (Pa_{CO_2}) were monitored at 2 to 3 day intervals and all measurements and observations were made at rest. Maximum expiratory, minimum inspiratory and Max Δ Ppl were measured using an intra-oesophageal balloon (McPherson and Lawson 1974). These values were estimated from the mean of 10 consecutive and representative respiratory cycles (Sasse 1971). Samples for blood gas determinations were collected and analysed using a previously described technique (Murphy *et al* 1980).

End point of trial

The end point of the post treatment asymptomatic period was established by clinical assessment and when values for Max Δ Ppl and Pa_{O_2} became abnormal (ie, Max Δ Ppl > 6 mmHg, Pa_{O_2} < 82 mmHg).

Statistical analysis of results

The correlation between the number of sodium cromoglycate treatments and the time horses remained asymptomatic after treatment was calculated by regression analysis using all the horses (Snedecor and Cochran 1967).

Max Δ Ppl, Pa_{O_2} and Pa_{CO_2} values for untreated symptomatic horses and for symptomatic horses at the end point of the trial were compared to values for clinically normal horses in a controlled environment and sodium cromoglycate treated horses exposed to antigen challenge by Student's *t* test.

The significance of the correlation between age, breed type, body weight (bwt), duration of illness, Max Δ Ppl, Pa_{O_2} and Pa_{CO_2} values for untreated symptomatic horses, and the time taken for horses to become asymptomatic in the controlled environment and the time horses remained asymptomatic after sodium cromoglycate treatment, was tested.

Results

Diagnostic phase

On admission, exposure to antigen challenge (without sodium cromoglycate treatment) induced clinical signs of COPD in all horses after 24 h. Mean values for Max Δ Ppl and blood gases (Table 2) also indicate that the horses became symptomatic. Horses remained symptomatic throughout the 4 to 5 days that they were maintained in this environment.

Pre-treatment (asymptomatic) phase

When placed in a controlled environment, COPD-affected horses became asymptomatic in 4 to 32 days (mean [± sd] 9.1 ± 4.9 days). Mean values for Max Δ Ppl and blood gases in asymptomatic horses are shown in Table 2.

The decrease in Max Δ Ppl and increase in Pa_{O_2} was highly

TABLE 2: Efficacy of sodium cromoglycate as a prophylactic treatment for equine COPD. Maximum change in intrathoracic pressure (mm Hg) and blood gases (mm Hg) (mean value ± sd) before and after one to 4 days inhaled sodium cromoglycate treatment

Number of days treatment (group number)	Number of horses	Diagnostic phase* Horses untreated and exposed to antigen challenge			Pre-treatment (asymptomatic) phase† Horses in a controlled environment and clinically normal before sodium cromoglycate treatment			Sodium cromoglycate inhalations	Post treatment (asymptomatic) phase‡ Horses exposed to antigen challenge protected by preceding sodium cromoglycate treatment			Endpoint of trial§ Horses in challenge environment at end of sodium cromoglycate protective period		
		Max Δ Ppl	Pa_{O_2}	Pa_{CO_2}	Max Δ Ppl	Pa_{O_2}	Pa_{CO_2}		Max Δ Ppl	Pa_{O_2}	Pa_{CO_2}	Max Δ Ppl	Pa_{O_2}	Pa_{CO_2}
1	9	± 10.3 3.1	± 72.8 6.0	± 39.9 1.9	± 3.6 0.4	± 89.0 2.7	± 36.5 2.2		± 3.9 0.4	± 85.4 2.3	± 37.8 1.3	± 6.8 1.1	± 79.0 3.7	± 39.1 1.9
2	23	± 12.3 6.3	± 69.9 8.1	± 39.0 3.8	± 3.5 0.5	± 89.1 3.1	± 36.0 1.8		± 3.9 0.4	± 88.0 2.7	± 36.5 1.2	± 7.6 1.9	± 76.5 6.3	± 38.5 2.3
3	16	± 12.6 6.4	± 71.1 7.6	± 39.3 4.5	± 3.6 0.6	± 89.5 4.4	± 36.8 3.1		± 3.8 0.4	± 87.0 2.2	± 37.2 1.1	± 7.3 1.4	± 75.9 7.5	± 38.0 1.7
4	8	± 15.0 5.8	± 69.9 4.9	± 38.5 1.4	± 3.6 0.6	± 89.5 2.4	± 35.7 1.8		± 4.0 0.6	± 84.8 1.9	± 36.2 1.7	± 8.9 2.2	± 77.8 2.8	± 37.0 2.7

* Means recorded on one day
† Means recorded on one day
‡ Means recorded on a composite of days, at 2 and 3 day intervals throughout period of remission
§ Means recorded on one day

significant in all groups ($P < 0.001$). A significant decrease in $PaCO_2$ ($P < 0.05$) was recorded in all groups. Significant linear and positive correlations existed between the time taken for horses to become asymptomatic and 2 variables, namely age ($r[54] = 0.375$; $P < 0.01$) and symptomatic Max Δ Ppl ($r[54] = 0.583$; $P < 0.01$). There was no correlation with breed, bwt, duration of illness or symptomatic PaO_2 and $PaCO_2$ values.

Post treatment (asymptomatic) phase

The duration of the asymptomatic period after various treatment schedules ranged from a mean of 3.6 days for a single day's treatment to a mean of 24.3 days for treatments on 4 successive days (Fig 1). The correlation between the number of treatments and the asymptomatic time was highly significant (correlation coefficient $r[54] = +0.709$; $P < 0.001$). The line of best fit was linear (Fig 1). The mean values for Max Δ Ppl and blood gases after completion of the sodium cromoglycate treatment and during the period of remission are shown in Table 2. During the asymptomatic period, an average of 3 recordings/horse were made in **Group 1,** 5/horse in **Group 2,** 7/horse in **Group 3** and 12/horse in **Group 4.** The values expressed are a mean of these recordings for all horses.

The differences in Max Δ Ppl and PaO_2 between the diagnostic and post treatment phases (Table 2) were highly significant ($P < 0.001$), whereas the decrease in $PaCO_2$ was only just significant ($P < 0.05$). There was no correlation between the time horses remained asymptomatic after sodium cromoglycate treatment and age, breed type, bwt, duration of illness, symptomatic Max Δ Ppl, PaO_2 and $PaCO_2$ values, or the time taken for horses to become asymptomatic in the controlled environment.

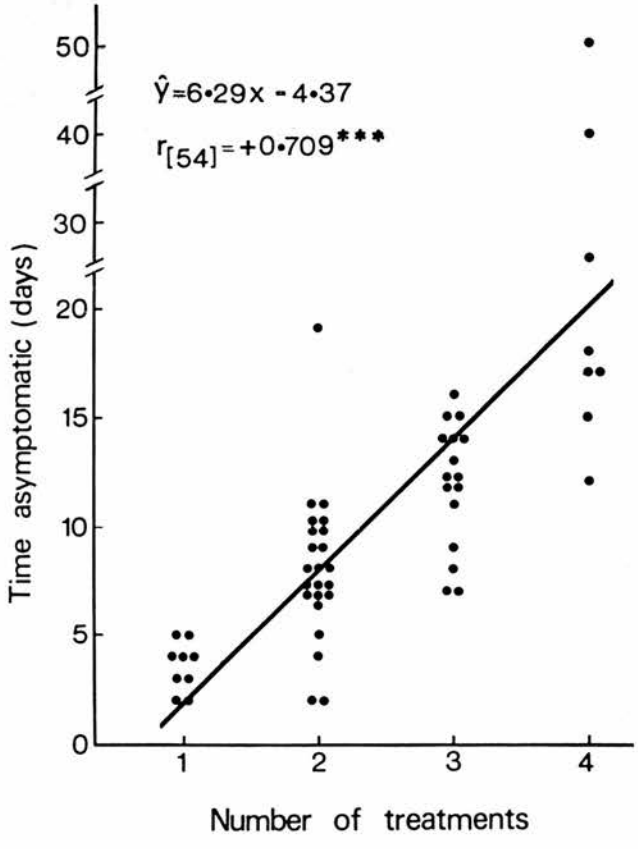

Fig 1. *Relationship between the number of sodium cromoglycate treatments and the time horses remained asymptomatic after treatment, in the face of continuous antigen challenge (*** $P < 0.001$)*

End point of trial

Towards the end of the post treatment asymptomatic period, faint harsh vesicular respiratory sounds became audible on auscultation and these rapidly increased in intensity over one to 3 days. This was accompanied by a slight double expiratory effort which became increasingly more pronounced and, in some cases, an increase in respiratory rate and coughing. Mean values for Max Δ Ppl and blood gases when symptoms became re-established at the end of the period of efficacy are shown in Table 2. The increase in Max Δ Ppl and decrease in PaO_2 were significant ($P < 0.001$ and $P < 0.01$, respectively) compared with values for the previous phase but there was no significant change in $PaCO_2$.

No untoward side effects were observed in any of the horses as a result of sodium cromoglycate treatment.

Discussion

For many years, authors have recognised that removing COPD-affected horses from dusty, mould-contaminated hay and straw will usually alleviate clinical signs of the disease. The use of a controlled environment enabled us to render affected horses clinically asymptomatic with values for Max Δ Ppl and PaO_2 within our range for normal horses. The time taken for horses to show remission of COPD signs varied greatly and was most affected by Max Δ Ppl when symptomatic (ie, the severity of the horse's condition) and by the age of the animal.

From this study, it is apparent that sodium cromoglycate administration to asymptomatic COPD-affected horses is effective as a short-term prophylactic measure, the protective period varying according to the number of daily treatments before antigen challenge. Each horse acted as its own control by virtue of its response to changes of environment. Normal horses kept in a similar environment have shown no changes in their respiratory pattern.

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

Like Muylle and Oyaert (1973), we have found no significant difference in tidal volume between COPD-affected and normal horses. These authors showed that the ratio of tidal volume:Max Δ Ppl was significantly lower in COPD-affected horses than in normal horses and we can conclude that Max Δ Ppl is the important basic measurement. Sasse (1971) attached great importance to determination of Max Δ Ppl in COPD-affected horses. He regarded "minute viscous work" values as being the most important data. Sasse could not demonstrate any significant difference in the minute respiratory volume between COPD-affected and normal horses and it is apparent that differences in Max Δ Ppl accounted for the highly significant differences in "minute viscous work" between normal and affected horses.

COPD-affected animals which are asymptomatic usually show signs of COPD within 4 to 8 h of antigen challenge (McPherson *et al* 1979) but increased Max Δ Ppl values have been recorded within 2 h of antigen challenge (J. R. Murphy and E. A. McPherson, unpublished data). Although the pathogenesis of COPD is not completely known, it is thought to consist of a dual response, including a Type I reaction, producing bronchospasm, and a Type III-like reaction with the

main pathological findings being an exudative bronchiolitis (Nicholls 1978). In human bronchial asthma, sodium cromoglycate is essentially used in the prophylaxis of Type I hypersensitivities, but it has also been found effective in the control of Type III reactions.

In man and other species, the half-life of sodium cromoglycate is very short (45 to 90 mins) (Cox 1976) and, in the treatment of asthma, repeated administration (3 to 4 times daily) is necessary to obtain adequate protection. The duration of protection in the horse after treatment is surprisingly prolonged compared to that in man.

COPD-affected horses are usually capable of working normally as long as they remain asymptomatic and this can be achieved by ensuring strict environmental control. In some situations, this regime is impractical, eg, during transportation or when horses are moved temporarily away from the home environment. Minimal antigen exposure may provoke clinical signs of COPD and render the horse incapable of performing to its full potential. In such cases, prophylactic sodium cromoglycate administration may prove beneficial to COPD-affected horses during periods of unavoidable challenge.

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Résumé

Lorsqu'ils sont placés dans des conditions hygièniques favorables, les chevaux atteints d'affection pulmonaires obstructive chronique, deviennent cliniquement asymptomatiques dans un délai de 4 à 32 jours (moy. 9,1 ± 4,9 jours), ce délai dépend surtout de la sévérité de l'affection (appréciée par la variation maximale de la pression intrathoracique) et de l'âge de l'animal.

Le cromoglycate de sodium utilisé fréquemment dans la prophylaxie des allergies respiratoires chez l'homme fut utilisé en inhalation chez 52 chevaux atteints de maladie pulmonaire obstructive chronique (MPOC). Les résultats montrèrent qu'il existe une réponse linéaire entre le nombre de jours consécutifs d'administration de ce traitement et la durée de rémission des signes cliniques de la MPOC, quand les chevaux sont exposés à des antigènes naturels (litière de mauvaise qualité). Le cromoglycate de sodium donné chaque jour à dose unique (80 mg) durant 4 jours, prévenait les signes cliniques pour environ 24,3 jours, mais donné une seule journée à la même dose, il prévenait les signes cliniques pour 3,6 jours seulement. Ces résultats indiquent que le traitement prophylactique avec le cromoglycate de sodium par inhalation est une méthode efficace de contrôle des formes cliniques de la MPOC.

Zusammenfassung

In einer kontrollierten Umgebung wurden Pferde, die an chronisch obstruktiven Lungenkrankheiten (COPD) litten, innert 4 bis 32 Tagen (Durchschnitt 9.1 ± 4.9 Tage) symptomenfrei; diese Zeit hängt vor allem vom Grad der Erkrankung — beurteilt nach der maximalen intrathorakalen Druckveränderung max. Δ Ppl — ab und auch vom Alter des Tieres.

Natrium-Chromoglykat, ein in der Prophylaxe allergischer Atemwegserkrankungen des Menschen häufig verwendetes Medikament, wurde per inhalationem 56 COPD-Patienten verabreicht. Die Resultate zeigen, dass zwischen der Anzahl sich folgender Behandlungstage und der Remissionsdauer während natürlicher Antigenexposition (Strohstreu schlechter Qualität) eine lineare Beziehung besteht. Na-Chromoglykat verabreicht als eine einzige Tagesdosis von 80 mg, beziehungsweise während vier aufeinanderfolgenden Behandlungstagen, vermochte die klinischen Symptome 3,6 Tage, beziehungsweise 24.3 Tage lang zu unterdrücken. Diese Resultate deuten daraufhin, dass eine prophylaktische Behandlung mit inhaliertem Na-Chromoglykat eine wirksame Methode zur Beherrschung der klinischen Manifestation von COPD darstellt.

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