

## STUDIES ON THE PHYSIOPATHOLOGY OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN THE HORSE. IV. BLOOD GAS AND ACID-BASE VALUES AT REST

A. LITTLEJOHN and FELICITY BOWLES, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110

### ABSTRACT

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Radiometer Blood Micro-system 2 was used in studies designed to, (a) compare the mean blood gas and acid-base values of 38 normal horses and 20 horses with chronic obstructive pulmonary disease (COPD), (b) determine the means and standard deviations of blood gas and acid-base values of Thoroughbred horses in training, and (c) investigate the relationships between clinical data, blood gas values, intracardiac and pulmonary arterial pressures in subjects with COPD.

There were significant differences between the mean values for partial pressure of arterial oxygen ( $\text{PaO}_2$ ), arterial carbon dioxide ( $\text{PaCO}_2$ ) and mixed venous carbon dioxide ( $\text{PvCO}_2$ ) in normal and COPD subjects.

The mean values and standard deviations for determinations of blood gases and acid-base status in Thoroughbred horses in training were as follows:  $\text{PaO}_2 = 77,4 \pm 4,3$  mm Hg;  $\text{PvO}_2$  (mixed venous oxygen partial pressure) =  $36,2 \pm 4,1$  mm Hg;  $\text{PaCO}_2 = 40,9 \pm 5,8$  mm Hg;  $\text{PvCO}_2 = 49,4 \pm 5,0$  mm Hg;  $\text{pHa}$  (arterial) =  $7,358 \pm 0,051$ ;  $\text{pHv}$  (venous) =  $7,343 \pm 0,027$ ; standard bicarbonate =  $22,7$  mM/l.

The  $\text{PaO}_2$ , the  $\text{PaCO}_2$  and the arterial pH were significantly correlated to the respiratory frequency in COPD subjects.

The correlations of pulmonary diastolic pressure to both  $\text{PaO}_2$  and  $\text{pHa}$  were of probable significance ( $P < 0,05$ ) in COPD subjects.

$\text{PaCO}_2$  was highly significantly correlated to  $\text{PaO}_2$  and  $\text{pHa}$  in COPD subjects.

### Résumé

*ETUDES SUR LA PHYSIOPATHOLOGIE DE LA MALADIE OBSTRUCTIVE PULMONAIRE CHRONIQUE DU CHEVAL. IV. GAZ DU SANG ET VALEURS ACIDE-BASE AU REPOS*

Un radiomètre de sang à micro-système 2 a été utilisé dans des études visant à: (a) comparer le gaz moyen du sang et les valeurs acide-base de 38 chevaux normaux et de 20 chevaux atteints de la maladie obstructive pulmonaire chronique (COPD); (b) déterminer les déviations moyennes et standards de gaz du sang et les valeurs acide-base de chevaux pur-sang à l'entraînement; et (c) rechercher la relation entre les données cliniques, les valeurs gaz du sang, les pressions artérielles pulmonaires chez les sujets avec COPD.

Il y eut des différences significatives entre les valeurs moyennes pour la pression partielles de l'oxygène artériel ( $\text{PaO}_2$ ), le dioxyde de carbone artériel ( $\text{PaCO}_2$ ) et le dioxyde de carbone veineux mélangé ( $\text{PvCO}_2$ ) chez les sujets normaux et les sujets COPD.

Les valeurs moyennes et les déviations standards pour les déterminations des gaz du sang et le statut acide-base chez les chevaux pur-sang à l'entraînement furent les suivantes:  $\text{PaO}_2 = 77,4 \pm 4,3$  mm Hg;  $\text{PvO}_2$  (pression partielle d'oxygène veineux mélangé) =  $36,2 \pm 4,1$  mm Hg;  $\text{PaCO}_2 = 40,9 \pm 5,8$  mm Hg;  $\text{PvCO}_2 = 49,4 \pm 5,0$  mm Hg;  $\text{pHa}$  (artériel) =  $7,358 \pm 0,051$ ;  $\text{pHv}$  (veineux) =  $7,343 \pm 0,027$ ; bicarbonate standard =  $22,7$  mM/l.

Le  $\text{PaO}_2$ , le  $\text{PaCO}_2$  et le pH artériel furent en corrélation significative avec la fréquence respiratoire chez les sujets COPD.

$\text{PaCO}_2$  fut en corrélation hautement significative avec  $\text{PaO}_2$  et  $\text{pHa}$  chez les sujets COPD.

Les corrélations de pression diastolique pulmonaire de  $\text{PaO}_2$  et de  $\text{pHa}$  furent de signification probable ( $P < 0,05$ ) chez les sujets COPD.

### INTRODUCTION

External respiration is the process of gas exchange between alveolar gases and pulmonary capillary blood. The measurement of the oxygen and carbon dioxide partial pressures ( $\text{PO}_2$  and  $\text{PCO}_2$ ) in blood and alveolar gas as a means of assessing the severity of respiratory dysfunction is based upon the demonstration by Haldane & Priestley (1905) that the composition of alveolar gas (and consequently  $\text{PO}_2$  and  $\text{PCO}_2$  of pulmonary venous blood) remains within remarkably narrow limits in normal individuals. Thus a diminished  $\text{PaO}_2$  (arterial  $\text{PO}_2$ ) in the absence of upper respiratory tract obstruction, skeletal or neural restriction of breathing or cardiac disease is indicative of pulmonary dysfunction (Comroe, Forster, Dubois, Briscoe & Carlsen, 1962). However, prior to the development of accurate electrode systems (Severinghaus & Bradley, 1958), determinations of blood  $\text{PO}_2$  and  $\text{PCO}_2$  values were time-consuming and technically demanding, and it is only within the last decade that

blood gas measurements became routine practice in human and animal clinics.

The amount of oxygen which diffuses across the pulmonary membrane is determined by the patency of the airways, the  $\text{O}_2$  gradient across the membrane, and the properties and thickness of the membrane itself (Comroe *et al.*, 1962; Nunn, 1969; Kao, 1972). Pathological changes in the terminal airways or in the pulmonary membrane therefore affect adversely the transfer of  $\text{O}_2$  from the alveoli into the blood. The basic mechanisms contributing to chronic respiratory failure, according to Comroe *et al.* (1962), Berglund (1968) and Hugh-Jones (1958), are: (a) impaired ventilation leading to (b) alveolar hypoventilation, (c) diffusion limitation and (d) uneven ventilation in relation to blood flow. The end-result is hypoxaemia.

*The partial pressure of oxygen in arterial and venous blood ( $\text{PaO}_2$  and  $\text{PvO}_2$ )*

In normal man at sea level, the  $\text{PaO}_2$  and  $\text{PvO}_2$  values are considered to be 95 mm Hg and 40 mm Hg respectively (Kao, 1972). Similar values have been

reported for horses (Littlejohn, 1969; Sasse, 1971; Bergsten, 1974; Bisgard, Orr & Will, 1975).

Increasing altitude diminishes the  $\text{PaO}_2$ , since the  $\text{PO}_2$  of inspired air diminishes by approximately 10 mm Hg per 500 m increase in altitude (Guyton, 1971). At an altitude of 1 300 m, which is the altitude of the site of these studies, the mean  $\text{PaO}_2$  of 5 adult mares was  $75.5 \pm 3.5$  mm Hg (Littlejohn & Van Heerden, 1975; Littlejohn, 1975).

In horses with chronic pulmonary obstructive disease (COPD) near to sea level,  $\text{PaO}_2$  values of between 40 and 115 mm Hg were recorded by Sasse (1971). However, the mean value of 86 mm Hg in horses with COPD was highly significantly lower than the mean value for the clinically normal subjects of Sasse's (1971) study. Similar results were recorded by Gillespie, Tyler & Eberly (1964); Macpherson & Lawson (1974); Bergsten (1974) and Dixon (1978).

Sasse (1971) concluded from his studies that the determination of  $\text{PaO}_2$  in horses with suspected COPD could be an important clinical aid to diagnosis.

Bergsten (1974) found the mean  $\text{P}\bar{\text{V}}\text{O}_2$  of clinically normal horses to be  $33 \pm 6$  mm Hg, whereas that of horses with COPD was  $29 \pm 3$  mm Hg. There was no significant difference between the 2 mean values.

#### Arterial and venous $\text{PCO}_2$

Dripps & Severinghaus (1955) were the first to point out that the partial pressure of  $\text{CO}_2$  in arterial blood is a constant and sensitive measurement of the efficiency of ventilation. According to Comroe *et al.* (1962), the  $\text{PaCO}_2$  is invariably increased if hypoventilation occurs for any reason, including obstructive pulmonary disease. Impaired diffusion on the other hand seldom results in an increased  $\text{PaCO}_2$  because there is no hindrance to outward diffusion of  $\text{CO}_2$ , even in severe cases of alveolar-capillary block (Comroe *et al.*, 1962). This is due to the high solubility of  $\text{CO}_2$  in body fluids and tissues, including the pulmonary membrane.

Gillespie *et al.* (1964), Sasse (1971), Reinhard & Hurtienne (1972) and Bergsten (1974) found no significant increase in the mean  $\text{PaCO}_2$  of horses with COPD. However, 4 out of the 9 cases of COPD which were investigated by Gillespie *et al.* (1964), and 7 out of the 38 cases of COPD confirmed at autopsy by Sasse (1971) had  $\text{PaCO}_2$  values in excess of 50 mm Hg, i.e. in excess of one standard deviation above the mean value for  $\text{PaCO}_2$  in clinically normal horses. Neither Gillespie *et al.* (1964) nor Sasse (1971) were able to account for the high  $\text{PaCO}_2$  values which were found in some cases.

Comroe *et al.* (1962) stated that human patients with pulmonary emphysema may have a diminished response to carbon dioxide retention for a variety of reasons apart from mechanical limitation of ventilation. Such reasons were considered to include depression of the respiratory centre by anoxaemia or carbon dioxide retention itself, and a high buffer base value due to increased haemoglobin or bicarbonate concentrations. According to Davenport (1969) however, depression of  $\text{PaO}_2$  values below normal may increase the sensitivity of the respiratory regulatory mechanism to changes in  $\text{PCO}_2$ . There are thus conflicting views on the effects of hypoxaemia on the respiratory regulatory mechanisms in man. However, there is some information regarding horses in this respect; Muir, Moore & Hamlin (1975) found that the ventilatory response to hypoxia was considerably more sensitive in horses than in man.

#### Acid-base values

The series of 9 horses with COPD examined by Gillespie *et al.* (1964) showed no significant differences in pH or total  $\text{CO}_2$  values of arterial blood from those of normal horses. Similar findings were reported by Sasse (1971), who determined standard bicarbonate and base excess values in addition to  $\text{PaCO}_2$ . Neither Reinhard & Hurtienne (1972) nor Bergsten (1974) were able to demonstrate significant deviations from the normal in horses with COPD.

Deviations from normal acid-base values would be expected if respiratory acidosis was a constant finding in cases of COPD. However, since significant increases in  $\text{PaCO}_2$  have not been noted other than in a few individual cases, no such differences in mean values for pH or bicarbonate have been recorded. Since at the altitude of Onderstepoort (1 300 m) there was no information regarding blood gas and acid-base values of COPD or normal horses, the objects of the present studies were as follows:

1. To compare the mean blood gas and acid-base values of normal and COPD horses.
2. To determine the means and standard deviations of blood gas and acid-base values of Thoroughbred horses in training.
3. To investigate the relationships between clinical data, blood gas values, intracardiac and pulmonary arterial pressures in COPD subjects.

## MATERIALS AND METHODS

#### Nomenclature

The use of respiratory symbols and abbreviations follows that described by Comroe *et al.* (1962).

#### Subjects

The subjects were 38 normal and 20 COPD horses and ponies, as described in the first paper of this series (Littlejohn, 1980a). In addition, samples were obtained from 43 clinically normal Thoroughbred horses in training.

#### Collection of blood samples

Arterial and mixed venous blood samples were collected simultaneously. This was accomplished as follows: (a) The left carotid artery of the subject was palpated with the fingertips pressed into the jugular groove in the lower third of the left side of the neck. One to 2 ml of lignocaine hydrochloride was infiltrated subcutaneously and deeply over the carotid artery itself. (b) The right ventricle was cannulated as described by Littlejohn (1980b) and a 3-way plastic stopcock attached to the cannula was flushed with heparinized 0.9% saline solution and closed.

With the fingertip of the middle finger palpating the left carotid artery, a 4 cm 18-gauge needle was introduced through the skin and subcutaneous tissue adjacent to the trachea and into the carotid artery. A 10 ml sterile disposable plastic syringe was then attached to the needle and blood was slowly sucked into the syringe, the dead space of which was filled with heparin solution. As soon as blood began filling the syringe, an assistant attached a 10 ml syringe to the 3-way stopcock of the cannula, aspirated the heparinized saline solution from the cannula until only blood flowed from the cannula, then attached a heparinized syringe and collected 10 ml of mixed venous blood.

Aseptic technique was routinely followed for all blood collections. Sterile disposable needles and syringes were used on all occasions. The skin sites for needle puncture or cannulation were shaved and disinfected with 90% solution of ethyl alcohol in water.

The operation was carried out while subjects were at rest in a loose box at approximately the same time of day in each case, i.e. after feeding and before exercise, and the blood-filled syringes were placed in ice-water immediately after collection. Storage of samples in ice-water at 0–2 °C prevented changes occurring in the pH and in the partial pressures of O<sub>2</sub> and CO<sub>2</sub> of horse blood (Sasse, 1971).

The above technique, used for collecting arterial blood samples throughout these studies, was found to be more acceptable to owners or other onlookers than that described by Fisher (1959). The formation of a haematoma at the carotid site was prevented by digital pressure for 2 minutes.

#### *Partial pressures of oxygen (PO<sub>2</sub>)*

Individual variations in PaO<sub>2</sub> were investigated by Sasse (1971) who showed that the variations in PaO<sub>2</sub> in individual horses were within a range of ±10 mm Hg about the mean, i.e. within the normal range for the species. There are additional sources of error which may arise in the collection, storage and analysis of samples.

The numerous symposia, publications and textbooks on human and instrumental errors in blood-gas analysis (Payne & Hill, 1966; Lübbers, 1966; Severinghaus & Bradley, 1971) are evidence of the great attention being given to the subject, and illustrate the many pitfalls awaiting users of oxygen electrodes. However, despite the difficulties of measuring the true PO<sub>2</sub> of blood samples accurately, technical errors can be reduced by following exactly the same routine for each sample.

In this study all analyses of PO<sub>2</sub> in blood and gas samples were carried out with Radiometer Blood Micro-system 2\* in conjunction with Radiometer PO<sub>2</sub> electrode G5204, using exactly the same routine for each and every manipulation of the blood sample and of the equipment. Calibration with air was carried out before each blood sample was introduced into the electrode. The liquid-to-gas ratio of electrode output with water and air of the same PO<sub>2</sub> was carried out regularly and the requisite correction was applied at calibration as recommended by Severinghaus & Bradley (1971). This correction was necessary when blood samples, but not gas samples, were analysed.

The standard deviation of a series of 8 PO<sub>2</sub> determinations on the same sample of arterial horse blood maintained in ice water for 1 hour was ±0,74 mm Hg, a reproducibility close to that of ±1% claimed by the makers of the equipment. For venous blood the SD was ±0,58 mm Hg.

#### *Blood pH and the partial pressure of carbon dioxide (PCO<sub>2</sub>)*

Determinations of pH and PCO<sub>2</sub> in blood samples were carried out with Radiometer BMS 2\*, using the techniques originally described by Andersen, Engel, Jorgensen & Astrup (1960) and detailed in the instruc-

tion manuals distributed by the makers of the apparatus. Blood pH was determined with the glass electrode incorporated in BMS 2. Blood PCO<sub>2</sub> was determined by the technique of Andersen *et al.* (1960), known generally as the Astrup technique. This method for determining the PCO<sub>2</sub> of blood is based on the fact, demonstrated by Peters (1923), Brewin, Gould, Nashat & Neil, (1955) and Astrup (1956), that the pH/log PCO<sub>2</sub> curve of human blood *in vitro* at constant temperature is a straight line within the physiological range. The method is also valid for horse blood (Littlejohn, 1979).

Our procedure was as follows:

The pH of the blood sample was determined with the Radiometer pH electrode G198. Two 0,05 ml samples were then placed in the microtonometer and equilibrated for 3 minutes with a CO<sub>2</sub>/O<sub>2</sub> gas mixture containing 4% CO<sub>2</sub> (microtonometer cup 1) and 8% CO<sub>2</sub> (microtonometer cup 3). The pH of each of the 2 samples was then measured. Since the PCO<sub>2</sub> of each of the 2 samples was known, being equal to that of the equilibrating gas PCO<sub>2</sub>, 2 points were plotted on the pH/log PCO<sub>2</sub> graph. A straight line was drawn through these two points. This line represented the CO<sub>2</sub> buffer curve of the blood. The actual pH of the blood sample was entered on this buffer line, and the actual PCO<sub>2</sub> of the blood sample was then derived from the log PCO<sub>2</sub> scale on the ordinate. Determinations were carried out on fully oxygenated blood samples as demanded by the tonometry technique. A correction factor for venous blood was therefore used. This factor 'C' is necessary, because the CO<sub>2</sub> buffer line of blood shifts to the left, i.e. it becomes more acid, as desaturated blood becomes oxygenated. The shift to the left is due to the H<sup>+</sup> ion displacing effect of oxygenation on the haemoglobin molecule and is therefore also dependent on the concentration of haemoglobin in the blood sample (Davenport, 1969; Kao, 1972). The correction factor 'C' was calculated using the equation:

$$C = 0,3 \times \text{Hb (in g.}\%) \times \frac{100 - \text{oxygen saturation \%}}{100}$$

It is relevant to stress several points which were found to be important for accurate pH and PCO<sub>2</sub> determinations.

(a) Samples should be stored in ice-water immediately after collection.

(b) Thorough mixing of blood samples should be done before transferring samples to electrode chambers or equilibrating cups. Mixing is necessary because horse blood sediments rapidly (Archer, 1959) and erroneous Hb concentrations in samples give an incorrect CO<sub>2</sub> buffer line and consequently an inaccurate value for PCO<sub>2</sub>.

(c) The reproducibility of the pH electrode should be regularly checked. It was found that the reproducibility varied somewhat not only between different electrodes but also between tests of the same electrode at different times. A pH electrode was not used for these studies unless it gave a reproducibility of ±0,007 pH unit or better, when tested 8 consecutive times with Radiometer pH 7,381 buffer at 38 °C. The best performance recorded was ±0,003 pH unit.

(d) Theoretically, it is not possible to measure the true pH of a biological fluid with an accuracy greater than 0,02 pH unit (Semple, 1967). This is because the activity coefficient of the chloride ion at the reference electrode is not known. However, in a study such as this, where the main aim is discrimination between the

\* Radiometer, Copenhagen

pH,  $\text{PCO}_2$  or  $[\text{HCO}_3^-]$  values of 2 groups of subjects, such considerations may be left to the biomedical engineer, provided reproducibility is acceptable.

The 3rd decimal of pH unit displayed by the Radiometer Digital Analyser apparatus is therefore merely a figure on an electronic digital scale. Nevertheless we recorded it for statistical purposes, since the digital display of the Radiometer equipment did not round off to the nearest 2nd decimal place when the 3rd decimal was excluded from the circuit.

*Standard bicarbonate*

Standard bicarbonate is defined as the bicarbonate concentration in the plasma of a blood sample equilibrated with a mixture of oxygen and carbon dioxide at a  $\text{PCO}_2$  of 40 mm Hg (Andersen & Engel, 1960). A  $\text{PCO}_2$  of 40 mm Hg was selected by them because this figure established the ventilatory rate at which the  $\text{CO}_2$  eliminated from a human patient equalled the amount of  $\text{CO}_2$  produced—a logical baseline, as stressed by Severinghaus (1959). It must be pointed out, however, that this baseline referred to man at sea level (Mellemgard & Astrup, 1960). A value of 40 mm Hg for normal resting  $\text{PCO}_2$  cannot be accepted for all species, nor can it be accepted as valid at any altitude.

The  $\text{PaCO}_2$  for a given species should therefore be established for the altitude at which acid-base studies are carried out. In the 38 normal subjects studied, a  $\text{PaCO}_2$  of 38,3 mm Hg was the mean value. The figure of 38,5 mm Hg was therefore accepted as the figure from which standard bicarbonate was determined. Apart from this correction, the method used was identical with that described by Andersen & Engel (1960) and was as follows:

The  $\text{CO}_2$  buffer line of a sample of blood was constructed on the nomogram of Andersen *et al.* (1960). The pH on the buffer line corresponding to a  $\text{PCO}_2$  of 38,5 mm Hg was determined. The standard bicarbonate was then calculated by means of the Henderson-Hasselbalch equation, using the calculator designed by Severinghaus (1966) and distributed by Radiometer, Copenhagen. For venous samples, correction of the buffer line position was made according to the method of Andersen & Engel (1960), since reduced haemoglobin is more basic than oxyhaemoglobin. The magnitude of the effect is the same for equine blood as it is for human blood (Van Slyke, Wu & McLean, 1923; Henderson, 1928), and the same correction factor was used as for human blood.

*Pulmonary arterial pressure*

Pulmonary arterial pressures were obtained using the techniques described by Littlejohn (1980b).

*Clinical signs*

Clinical measurements were carried out as described by Littlejohn (1980a).

RESULTS

(1) *Blood gas and acid-base values of normal and COPD horses*

The mean values and standard deviations of the blood gas measurements of normal and COPD subjects are tabulated in Table 1. There was a highly significant difference between the mean  $\text{PaO}_2$  values of the normal and COPD subjects. The mean  $\text{PaCO}_2$  and

the mean  $\text{P}\bar{\text{V}}\text{CO}_2$  of the 2 groups of horses were also significantly different (Table 1).

Mean acid-base values (pH and Standard Bicarbonate) of the normal and COPD subjects were not significantly different (Table 2).

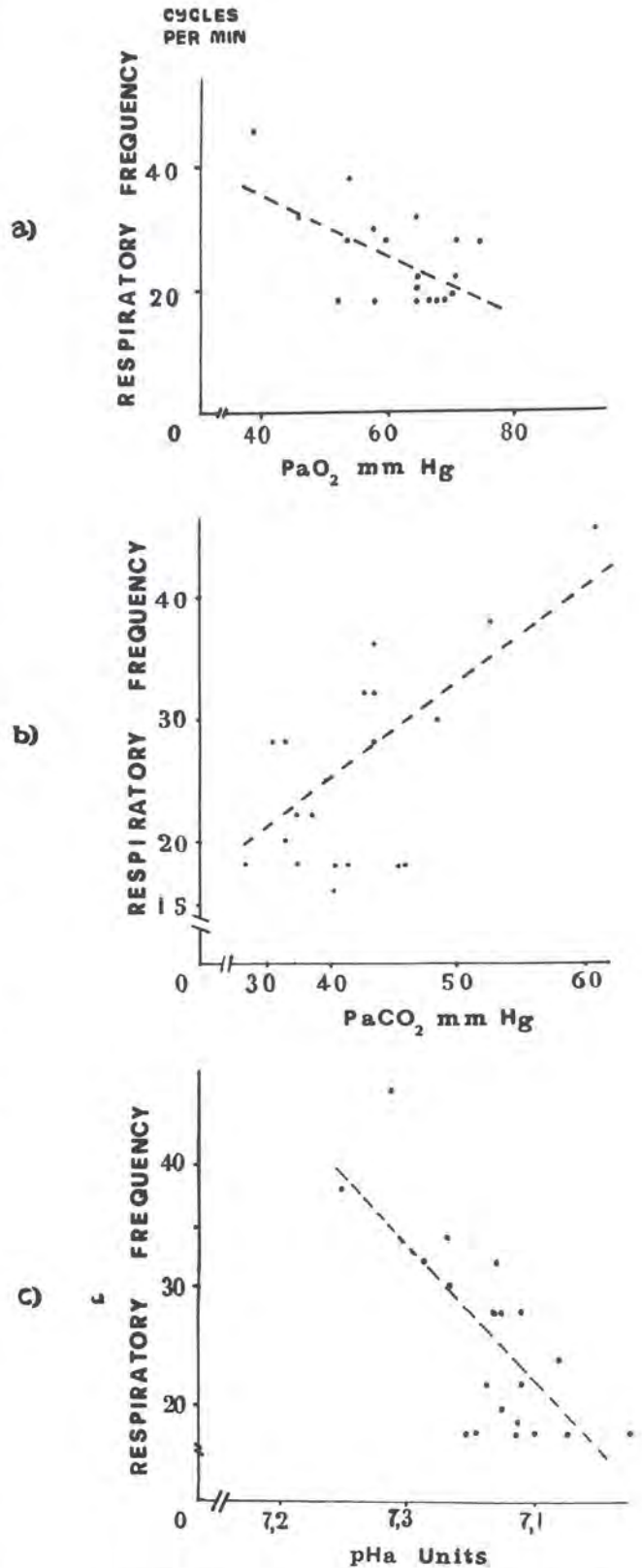


FIG. 1 Respiratory frequency of COPD subjects plotted against (a)  $\text{PaO}_2$ , (b)  $\text{PaCO}_2$  and (c) pHa. Dotted lines indicate regression of respiratory frequency on  $\text{PaO}_2$ ,  $\text{PaCO}_2$  and pHa

TABLE 1 The partial pressures of oxygen and carbon dioxide in the blood of normal and COPD horses

		Normal	n	COPD	n	Difference of means	Significance
PaO <sub>2</sub> .....	Mean.....	77,1	38	60,8	20	16,3	t=8,17 P<0,0001
	SD±.....	5,6		9,1			
PvO <sub>2</sub> .....	Mean.....	35,2	38	34,0	20	1,2	t=0,8 NS
	SD±.....	5,4		3,65			
PaCO <sub>2</sub> .....	Mean.....	38,3	38	42,3	19	4,0	t=2,90 P<0,01
mm Hg.....	SD±.....	±3,17		±7,13			
PvCO <sub>2</sub> .....	Mean.....	45,6	28	51,3	16	5,7	t=3,27 P<0,01
mm Hg.....	SD±.....	±4,7		±6,7			

TABLE 2 Arterial and venous pH and standard bicarbonate of normal and COPD subjects

		Normal n=38	COPD n=20	Difference of means	Significance
pH.....	Mean.....	7,384	7,369	0,015	t=1,26 NS
(Arterial).....	SD.....	±0,034	±0,052		
pH.....	Mean.....	7,362	7,343	0,019	t=1,41 NS
(Mixed venous).....	SD.....	±0,042	±0,05		
Standard.....	Mean.....	22,2	23,0	1,2	t=1,05 NS
Bicarbonate.....	SD.....	±2,76	±2,47		

TABLE 3 Blood gas and acid-base values of Thoroughbred horses in training

	mm Hg PaO <sub>2</sub>	mm Hg PvO <sub>2</sub>	mm Hg PaCO <sub>2</sub>	mm Hg PvCO <sub>2</sub>	Units pH (arterial)	Units pH (venous)	mM/l Standard HCO <sub>3</sub> <sup>-</sup>
n.....	14	43	14	43	14	43	43
Mean.....	77,4	36,2	40,9	49,4	7,358	7,343	22,7
SD±.....	4,3	4,1	5,8	5,0	0,051	0,027	3,5

TABLE 4 Linear regression analyses of the respiratory frequency with physiological variables in COPD subjects

Correlation	n	r	m	b	Significance
f / PaCO <sub>2</sub> .....	19	0,69	0,87	-11,2	t=3,94 P<0,001
f / PvCO <sub>2</sub> .....	16	0,60	0,50	37,6	t=2,80 P<0,02
f / PaO <sub>2</sub> .....	20	-0,59	-0,49	55,9	t=3,09 P<0,01
f / PvO <sub>2</sub> .....	19	-0,48	-0,26	40	t=2,33 P<0,05
f / pHa.....	20	-0,69	-0,004	7,48	t=7,16 P<0,001

(2) Blood gas and acid-base values of Thoroughbred horses in training

The mean values and standard deviations of blood gas and acid-base measurements of Thoroughbreds are shown in Table 3. There was a significant difference between the mixed venous PvCO<sub>2</sub> values of the Thoroughbreds and those of the normal group (t=3,25; P<0,01).

(3) Significant correlations between clinical data, blood gas values and pulmonary arterial pressures of COPD subjects

Values obtained from an analysis of the clinical data have been reported (Littlejohn 1980a).

The respiratory frequency was the only clinical measurement found to be significantly correlated to blood gas values. The results of linear regression

analyses tabulated in Table 4 show conclusively that the blood gas values of COPD horses are correlated with the respiratory frequency to a statistically significant extent.

The coefficients of multiple correlation relating the 3 variables, respiratory frequency, PaCO<sub>2</sub> and PaO<sub>2</sub>, are shown in Table 5. All were significant or highly significant. Fig. 1 shows the values for the respiratory frequency plotted against the PaO<sub>2</sub>, PaCO<sub>2</sub> and pHa values.

TABLE 5 Multiple correlation coefficients of regression between 3 variables, namely, 1. Respiratory frequency 2. PaCO<sub>2</sub> and 3. PaCO<sub>2</sub> in COPD subjects

Multiple correlations tested	n-3	r	Significance
f/PaCO <sub>2</sub> and PaO <sub>2</sub> .....	16	0,69	t=3,83 P<0,01
PaCO <sub>2</sub> /f and PaO <sub>2</sub> .....	16	0,82	t=5,75 P<0,001
PaO <sub>2</sub> /f and PaCO <sub>2</sub> .....	16	0,77	t=4,827 P<0,001

The values for pulmonary arterial pressures of COPD horses have been reported (Littlejohn 1980b).

Data from linear regression analyses of pulmonary arterial pressure with blood gas values in COPD subjects are tabulated in Table 6. Pulmonary diastolic pressure was significantly correlated to both PaO<sub>2</sub> and pHa.

Correlations between different blood gas values in COPD subjects are tabulated in Table 7.

TABLE 6 Linear regression analyses of pulmonary arterial minimum diastolic pressure with blood gas values

Correlation	n	r	m	b	Significance
PA minimum diastolic pressure..... PaO <sub>2</sub>	9	-0,68	-1,56	108,1	t=2,45 P<0,05
Pa minimum diastolic pressure..... PaCO <sub>2</sub>	9	0,51	0,40	32,4	NS
PA minimum diastolic pressure..... pHa	9	-0,76	-0,01	7,79	t=3,09 P<0,02

TABLE 7 Linear regression analyses of PaO<sub>2</sub> and PaCO<sub>2</sub> with other blood gas values

Correlation	n	r	m	b	Significance
PaO <sub>2</sub> / PaCO <sub>2</sub> .....	19	-0,77	-0,52	75,2	t=5,0; P<0,001
PaO <sub>2</sub> / PvO <sub>2</sub> .....	20	0,56	0,21	19,8	t=2,65; P<0,02
PaO <sub>2</sub> / PvCO <sub>2</sub> .....	16	-0,52	-0,42	75,94	t=2,25; P<0,05
PaCO <sub>2</sub> / PvCO <sub>2</sub> .....	16	0,68	0,70	20,5	t=3,23; P<0,01
PaCO <sub>2</sub> / pHa.....	20	-0,70	-0,005	7,61	t=4,16; P<0,001

DISCUSSION

(1) Blood gas and acid-base values of normal and COPD horses

The mean value of 77,1 mm Hg for PaO<sub>2</sub> in clinically normal subjects was considerably lower than the values reported by Gillespie *et al.* (1964), Littlejohn (1969a), Sasse (1971), McPherson & Lawson (1974), Bisgard *et al.* (1975), Bergsten (1974) and Dixon (1978). However, it was close to the mean values of 75,5 mm Hg reported by Littlejohn & Van Heerden (1975) in 5 Percheron mares investigated at the same altitude as that of these studies. The mean value for adult human beings in the Pretoria area is approximately 80 mm Hg (J. de Beer, H. F. Verwoerd Hospital, Pretoria, personal communication, 1977). The PaO<sub>2</sub> in horses at 1 300 m altitude thus corresponds closely to that observed in man at 1 300 m.

The highly significantly lower PaO<sub>2</sub> in 20 COPD subjects confirmed the findings of Gillespie *et al.* (1964), Sasse (1971), Bergsten (1974) and Dixon (1978), all of whom reported a similar finding. Although the values for PaO<sub>2</sub> in both normal and COPD subjects of the present study were lower, the difference between the mean PaO<sub>2</sub> of normal and COPD horses of 16,3 mm Hg was similar to that reported by Sasse (1971) and Gillespie *et al.* (1974), whose mean difference between PaO<sub>2</sub> of normal and COPD subjects was 18 mm Hg and 16 mm Hg respectively. Bergsten (1974) found a difference at rest of 26 mm Hg between the mean values of 17 normal horses and 11 COPD horses; his figures for PaO<sub>2</sub> were 94 mm Hg and 68 mm Hg respectively.

The results of PaO<sub>2</sub> determinations in the present investigations are therefore in good agreement with those of other authors. Although the mean values were lower than other reported values owing to the higher altitude at which studies were carried out,

there is no doubt about the significance of  $\text{PaO}_2$  determination as a clinical aid to the diagnosis of pulmonary disease in horses in South Africa.

There was no significant difference between the mean values for  $\text{P}\bar{\text{V}}\text{O}_2$  of the 2 groups of subjects.

This result was not entirely in agreement with those of Bergsten (1974), whose 16 clinically normal and 11 COPD horses had mean  $\text{P}\bar{\text{V}}\text{O}_2$  values of  $33 \pm 6$  mm Hg and  $29 \pm 3$  mm Hg respectively, a difference which was of probable significance. Possibly a proportion of the present series of COPD subjects were milder cases than the event horses investigated by Bergsten (1974).

The significant difference between the mean  $\text{PaCO}_2$  values for normal and COPD horses was a result which confirmed statistically the trends recorded by Gillespie *et al.* (1964), Sasse (1971), Reinhard & Hurtienne (1972) and Bergsten (1974).

The normal mean values for  $\text{PaCO}_2$  recorded by Littlejohn (1969a) and Littlejohn & Mitchell (1969), were 44,7 mm Hg and 44,9 mm Hg respectively. The somewhat lower normal mean  $\text{PaCO}_2$  value recorded in the present study was probably associated with increased alveolar ventilation. In the present series of subjects the mean respiratory rate was 16,7 per min, whereas in the study of Littlejohn (1969) the mean respiratory rate was 15,1 per min. However, it is difficult to account for the higher respiratory rate and lower resting  $\text{PaCO}_2$  of normal subjects, unless one assumes that the diminished  $\text{PaO}_2$  at an altitude of 1 300 m exerts an effect on the respiratory centres via the chemoreceptor systems of the aorta and the carotid arteries. The interactions of  $\text{PaO}_2$  on the ventilatory response in normal horses were investigated by Muir, *et al.* (1975), who found that diminishing the  $\text{PaO}_2$  increased the respiratory frequency.

Kao (1972) suggested that the carotid bodies acted as "oxygen electrodes". While this may be so, there are several mechanisms which may be expected to exert an effect on ventilatory homeostasis of normal and COPD horses.

Firstly, the respiratory centres and the peripheral chemoreceptors of man and experimental animals are extremely sensitive to changes in  $\text{PaCO}_2$ , since an increase of only 4 mm Hg  $\text{PaCO}_2$  is sufficient to cause a significant (50%) increase in ventilation (Fink, Hanks, Ngai & Papper, 1963).

Secondly, the ventilatory response is enhanced by hypoxia, and the ventilation is increased even more by a comparable increase in  $\text{PaCO}_2$  when the  $\text{PaO}_2$  is lowered (Fink *et al.* 1963).

Thirdly, man adapts to chronic hypercapnic states, the  $\text{CO}_2$  tolerance curve being shifted to the right and its slope reduced (Schaefer, 1963).

Fourthly, the experiments of Muir *et al.* (1975) showed that the respiratory frequencies in hypoxic horses were greater than those in hypercapnic horses and that the horse responds to  $\text{O}_2$  concentrations of approximately 16% by increasing both minute ventilation ( $V$ ) and respiratory frequency ( $f$ ). This is an earlier response than in man in whom increases in  $V$  do not occur until  $\text{O}_2$  concentrations decrease to 10% or less.

The findings with respect to  $\text{PaCO}_2$  and respiratory rates can thus be partly explained by postulating that all the mechanisms listed above were operating. Thus, in the normal subjects, the lowered  $\text{PaO}_2$  was detected by peripheral chemoreceptors which stimulated a slightly higher respiratory frequency and also a greater alveolar ventilation.

In the COPD subjects, the respiratory centres and/or peripheral chemoreceptors were sensitized by hypoxia so that the effect of small increases in  $\text{PaCO}_2$  was enhanced. Finally, with adaptation to a higher than normal  $\text{PaCO}_2$  level, stabilization of the respiratory frequency took place at a level which probably ensured that the work of breathing to maintain  $\text{PaCO}_2$  and arterial pH within physiological limits was minimal.

The mean partial pressure of  $\text{CO}_2$  ( $\text{P}\bar{\text{V}}\text{CO}_2$ ) in mixed venous samples from COPD subjects was significantly higher than that of normal subjects. No reports of comparisons between  $\text{P}\bar{\text{V}}\text{CO}_2$  values of normal and diseased horses were found in the literature. However, the work of Sasse (1971) showed that the work of breathing was greatly increased by COPD. Since  $\text{CO}_2$  is the major end-product of muscle metabolism in the body, it is not surprising that COPD subjects were found to have a higher  $\text{P}\bar{\text{V}}\text{CO}_2$  than that of normal subjects.

There was no significant difference between the mean pHa of the normal and COPD subjects. This result accords with those of Gillespie *et al.* (1964) and Sasse (1971), who found no significant difference between the pHa of normal and COPD horses. However, in both of the above investigations, the mean pHa of the COPD group was 0,02 pH units lower than that of the control group. These results and the results of the present studies, which also demonstrated a lower pHa value in the case of COPD subjects, reflect the fact that, as pointed out by Davenport (1969), compensation for respiratory acidosis is normally accomplished by means of increased alveolar ventilation, by blood and other body fluid buffers, and by renal secretion of acid and reabsorption of bicarbonate. However, compensation is seldom 100% effective and in the case of respiratory acidosis the pH stabilizes at a level close to but below the normal value (Comroe *et al.* 1962). These results are explicable on the basis of incomplete compensation and/or adaptation of the respiratory centres.

Bolz & Bieniek (1961) determined the venous pH and  $\text{PCO}_2$  of 12 horses with chronic pulmonary emphysema and obtained mean values of 7,349 pH unit and 49,88 mm Hg. The above results of Bolz & Bieniek (1961) are close to the mean values of 7,343 pH unit and 51,3 mm Hg recorded in the present study and lend support to the concept of  $\text{CO}_2$  retention and of possible increased  $\text{CO}_2$  production in COPD horses.

At the altitude of the present studies, therefore, horses with COPD may be expected to have a higher than normal  $\text{PaCO}_2$ ,  $\text{P}\bar{\text{V}}\text{CO}_2$  and respiratory frequency, and a lower than normal  $\text{PaO}_2$ .

## (2) Blood and acid base values of Thoroughbred horses in training

The somewhat higher arterial  $\text{PCO}_2$  of the Thoroughbreds and their significantly higher mixed venous  $\text{PCO}_2$  compared with normal horses not in training suggest that:

- (a) Thoroughbreds in training may have a faster basal rate of metabolism than horses not in training, and also that;
- (b) Respiratory homeostatic mechanisms in Thoroughbreds in training appear to "set" ventilation at a different level from that of horses not in training.

The mean respiratory frequency of the Thoroughbreds in training was slightly lower (at 14.5 per minute) than that of horses not in training (Table 7). The result of training would therefore appear to be an increased production of  $\text{CO}_2$ , resulting in a raised  $\text{PvCO}_2$  and  $\text{PaCO}_2$ , and a diminished respiratory frequency. However, although the  $\text{PaO}_2$  is not affected, the resultant Bohr effect in the blood would make more oxygen available to the tissues.

(3) *Correlations between clinical data, blood gas values and pulmonary arterial pressures of COPD subjects*

The significant correlations between the respiratory frequency and blood gases over the relatively narrow ranges encountered in this study illustrate the sensitivity of the chemoreceptor respiratory regulatory systems in horses. The role of the peripheral chemoreceptors in ventilatory acclimatization of ponies at altitude was studied by Forster, Bisgard, Rasmussen, Orr, Buss & Manohar (1976). They concluded that the peripheral chemoreceptors were essential in ponies for normal acclimatization to altitude. The present studies suggest that they are similarly essential for adaptation to chronic lung conditions which result in hypoxaemia. Of interest was the highly significant correlation between  $\text{pHa}$  and respiratory frequency, a phenomenon which had not previously been noted in horses. Such a response could be initiated by central chemoreceptors in the medulla and/or peripheral chemoreceptors in the aortic arch and carotid arteries. A third possibility suggested by Orr & Busija (1979) is the action of chemoreceptors in the airways sensitive to changes in  $\text{CO}_2$  and thus, by association, sensitive to changes in  $\text{pHa}$ .

Whatever the relative significance of different receptor mechanisms in the regulation of ventilation in horses, there is no doubt that the respiratory frequency in the resting, quiet horse is an important diagnostic indicator of pulmonary disease in horses at the altitude of 1 300 m. This is not the case at sea level (Sasse, 1971), where increased respiratory frequency was not a significant feature of COPD.

The more sensitive response of the horse to decreasing  $\text{O}_2$  concentrations of ambient air (compared to that of man) was demonstrated by Muir *et al.* (1975). This explains the significance of the respiratory frequency as a clinical sign at our altitude of 1 300 m.

The relationship of  $\text{PaO}_2$  to pulmonary arterial pressure was investigated in depth by Dixon (1978) who found a highly significant correlation between the 2 in horses and ponies with COPD. The results of the present study support his findings.

The genesis of the pulmonary hypoxic pressor response is unknown, as are the exact site and mode of action of the hypoxic stimulus (Fishman, Fritts & Cournand, 1960). The hypothesis of Weir (1978) that hypoxia inhibits normal bradykinin production in the lung and thus leads to pulmonary hypertension is one which accords closely with the known experimental findings regarding the phenomenon. Studies carried out at this institution with the bradykinase inhibitor SQ 14225 (Captopril) in horses with COPD suggest that oxygen and bradykinin play an important role in both the maintenance of normoxic pulmonary circulatory pressure and in the pathogenesis of pulmonary hypertension (Littlejohn, 1980, in preparation). Further studies of the role of bradykinin in pulmonary haemodynamics are indicated.

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