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RESPIRATORY AND CARDIOVASCULAR ADJUSTMENTS DURING EXERCISE OF INCREASING INTENSITY AND DURING RECOVERY IN THOROUGHBRED RACEHORSES

P. J. BUTLER[1], A. J. WOAKES[1], K. SMALE[1,2], C. A. ROBERTS[2], C. J. HILLIDGE[2], D. H. SNOW[2,*] and D. J. MARLIN[2]

[1]*School of Biological Sciences, The University of Birmingham, Edgbaston, Birmingham B15 2TT, UK* and [2]*Department of Comparative Physiology, Animal Health Trust, Newmarket, CB8 7DW, UK*

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Summary

A new design of flowmeter is described and used in a comprehensive study of the respiratory and cardiovascular adjustments that occur during a standardised exercise test in Thoroughbred horses. The flowmeter system and associated lightweight, fibreglass mask (total mass, 0.7kg) have a maximum dead space of 500ml and negligible resistance to airflow. They have no systematic effect on blood gases and, together with a rapidly responding mass spectrometer, enable an accurate computation of gas exchange to be performed together with breath-by-breath determination of other respiratory variables.

At the highest level of exercise ($12\,m\,s^{-1}$ on a 3° incline), the rate of oxygen uptake ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) increased to 29.4 times and 36.8 times their resting values, respectively. Respiratory minute volume ($\dot{V}_E$) increased to 27.0 times its resting value, with respiratory frequency (f_R) making the major contribution at the walk and trot. However, with increasing cantering speeds, f_R changed little as it was locked in a 1:1 fashion to stride frequency, and tidal volume (V_T) then made the major contribution to the increase in $\dot{V}_E$. The ratio of ventilatory dead space (V_D) to V_T in resting horses was lower than that previously reported in the literature and this could be the result of the different respiratory recording systems that were used. There was a close relationship between V_T and stride length at increasing cantering speeds.

Despite the fact that alveolar ventilation ($\dot{V}_A$) was well matched to $\dot{V}_{O_2}$, there was a significant reduction in arterial P_{O_2} (Pa_{O_2}) when the horses cantered at $8\,m\,s^{-1}$ and this eventually fell to 34% below the resting value. The present data tend to support the idea that $\dot{V}_A/\dot{V}b$ (where $\dot{V}b$ is cardiac output) inequalities are important in causing this hypoxaemia. However, the reduction in Pa_{O_2} was more than compensated for by an increase in haemoglobin concentration, [Hb], so the concentration of oxygen in the arterial blood (Ca_{O_2}) was significantly above the resting value at all levels of exercise. Both lactate concentration and Pa_{CO_2} increased during exercise, causing substantial reductions in pH of both arterial and mixed venous blood. This would have inevitably shifted the oxygen equilibrium curve of the Hb to the right, desaturating the arterial blood

*Present address: EquiSci International, 45 Campbell Drive, Wahroonga, NSW 2076, Australia.

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and thus exacerbating the effect of the hypoxaemia, as would the almost 4°C rise in blood temperature. The tight respiratory/locomotor linkage might prevent the acidosis and hyperthermia having the stimulatory effects on $\dot{V}_E$ that they have in humans at high work loads.

Cardiac output and heart rate ($\dot{V}b$) were 5 and 6 times their resting values, respectively, at the highest level of exercise, while cardiac stroke volume did not change significantly. There was both systemic and pulmonary hypertension, with mean pulmonary blood pressure reaching 2.6 times its resting value at the highest level of exercise. It is concluded that the pulmonary hypertension does not result from the hypoxaemia and that it could cause pulmonary oedema, which could contribute to the postulated diffusion limitation of the lungs during exercise.

Although this report confirms some results obtained by other workers, some discrepancies have been found. It is probable that the flowmeter and mask used here have a significantly reduced effect on the measured variables compared with methods used in many of the previous studies.

Introduction

Thoroughbred horses, together with dogs, foxes and pronghorned antelope, are considered to be elite athletes amongst terrestrial mammals (Taylor *et al.* 1981, 1987; Longworth *et al.* 1989; Lindstedt *et al.* 1991) in as much as their maximum oxygen uptake ($\dot{V}_{O_2max}$) is several times greater than that predicted for similar-sized terrestrial mammals. There is, therefore, much interest in the physiological mechanisms involved in the demand for and delivery of such high rates of oxygen. Clearly, the important variables in the process of oxygen delivery (and CO_2 removal) are those associated with ventilation and gas exchange itself. It is not easy to determine these variables in any animal during exercise, let alone in large animals such as horses. Masks incorporating one-way valves have been used and these enable expired gas to be collected, thus allowing the determination of tidal volume and respiratory minute volume, as well as gas exchange (Bayly *et al.* 1987*b*; Evans and Rose, 1987; 1988*a*). However, such a system has long lengths (2–3m) of wide-diameter (10cm) tubing attached to it and a relatively large (1.5l) dead space. It would be surprising if such a system did not affect the ventilatory characteristics of the horse in some way; it certainly causes a significant reduction in Pa_{O_2} and a significant increase in Pa_{CO_2} at all levels of exercise (Bayly *et al.* 1987*c*). The magnitude of these changes can be up to approximately 3kPa. A flow-through system using flow rates in excess of $6000\,l\,min^{-1}$, as used by Jones *et al.* (1989*a*), has no effect on blood gases (Bayly *et al.* 1987*c*), but does not allow the determination of tidal volume or respiratory minute volume. Neither of these systems allows the determination of respiratory flow rates or breath-by-breath analyses of respiratory variables.

The traditional method for obtaining such data from air-breathing vertebrates is to use a Fleisch pneumotachograph, which has to be connected to a differential pressure transducer. Such a system has been used with exercising ponies (Art and Lekeux, 1988), but two different pneumotachographs had to be used, one for the animals at rest and the other when they were exercising. With the latter, the total dead space was 700ml and the mask, plus the resistance to flow of the transducers, had a significant depressive effect on

respiratory frequency during exercise. It is likely, therefore, that tidal volume was elevated to compensate for this or that respiratory minute volume was suppressed. In their studies, Art and Lekeux (1988) did not exercise the ponies above a fast trot (3–5 $m s^{-1}$). Presumably, when cantering and galloping, the adverse effects of the mask on ventilation would have been greater as respiratory frequency and possibly tidal volume are linked to stride frequency and stride length, respectively (Bramble and Carrier, 1983). A flow-through system incorporating a Fleisch pneumotachograph has been used by Landgren *et al.* (1991). Flows of up to 10000 $l min^{-1}$ had to be generated and, again, long lengths of tubing were used. Although there appeared, from the data presented, to be little influence of this system on blood CO_2 levels, the authors do concede that the substantial loads imposed by the system 'probably influence breathing'. It must also be remembered that any deposition on the resistive element of the pneumotachograph will affect its performance (Art and Lekeux, 1988).

A different system has been developed in which airflow is determined by the force it exerts on a sensing flag that projects into the flow (Hörnicke *et al.* 1974; Kimmich and Spaan, 1980). The system is of complex mechanical design, requiring secondary sensors to compensate for the effects of gravity and acceleration and it will not cover the whole range of flows from rest to heavy exercise without a change in the internal diameter of the respiratory tube.

The problems and limitations associated with these various methods have been discussed by Rose and Evans (1987) who wrote: 'It is apparent that some effort needs to be put into the design and manufacture of a mask that is capable of handling the high respiratory flows that are generated at maximal exercise, without disturbing the respiratory pattern by increasing flow resistance. It is also important to minimize extra respiratory dead space and wasted ventilation due to the mask. This problem is particularly relevant in the horse during maximal exercise at the canter and gallop, when respiratory frequency is relatively high and tidal volume is relatively low'.

A new design of flowmeter has been developed which overcomes many of the problems mentioned above. This report details its use in a comprehensive study of the respiratory and cardiovascular adjustments that occur during a standardised incremental exercise test in Thoroughbred horses. This is the first time that such a range of variables has been measured directly in horses exercising at close to their $\dot{V}_{O_2max}$ with a system that has been demonstrated to have a minimal effect on levels of blood gases and no obvious effect on ventilation. The data obtained will be compared with those collected previously by the various methods described above. A brief report of a preliminary study has already been published elsewhere (Woakes *et al.* 1987), as have some of the present data (Anderson *et al.* 1989).

Materials and methods

Seven Thoroughbred horses (5 geldings, 2 fillies) were used in the present study; they had an average mass of 440±12kg and mean age of 5.3±1.3 years. They were all stabled under the same conditions and fed hunter cubes (Dodson and Horrell Ltd) and hay, with water provided *ad libitum*. A carotid artery was raised to a subcutaneous position in each

horse in order to aid its subsequent catheterization. The operation was performed under general anaesthesia in the Clinical Unit of the Animal Health Trust. Following this procedure, the animals were allowed a recovery period of at least 2 months before being exercised. They were then trained for 4–6 months to bring them to a level of racing fitness prior to the studies.

The studies were performed on a treadmill (Sato, Sweden) housed in an air-conditioned building maintained at a temperature of 20°C and a humidity of 60%. While on the treadmill, the horse wore a safety harness clipped onto a strap attached to a support frame above the treadmill. One air fan, placed in front of the horse, was used during the exercise tests to assist heat loss.

An incremental exercise test was used. This was preceded by a warm-up period consisting of a 6min walk at $1.6\,m\,s^{-1}$ followed by a 4min trot at $4\,m\,s^{-1}$ and a further 5 min walk at $1.6\,m\,s^{-1}$, all at 0° incline. The horses were then exercised for 2min at speeds of 6, 8, 10 and $12\,m\,s^{-1}$ (0° incline) and $12\,m\,s^{-1}$ (3° incline) with each speed separated by a 5min walk ($1.6\,m\,s^{-1}$). This was followed by a recovery period consisting of 15min of walking ($1.6\,m\,s^{-1}$, 0° incline) and 15min of standing. Exercising the horses for 2min enabled the present data to be compared with those obtained from previous studies (Bayly *et al.* 1987*c*; Evans and Rose, 1988*b*; Jones *et al.* 1989*a*; Wagner *et al.* 1989). Although there was a 5min walk between each exercise level, the data are presented graphically as exercise intensity increasing continuously.

The horses were fitted with a lightweight, fibreglass mask which housed two flow tubes (total mass 0.7kg), one for each nostril, leaving the mouth free (Woakes *et al.* 1987). The velocity of the airflow in the tubes was detected by phase shifts in beams of ultrasound transmitted alternately in one and then the other direction, passing diagonally across the airflow (Woakes and Butler, 1980). The dead space of the mask and flow tubes was, at most, 500ml. The resistance to airflow of the mask and tubes was determined at flow rates up to $20–30\,l\,s^{-1}$ and was found to be negligible. This is also the case for flow rates up to $60\,l\,s^{-1}$ (D. J. Marlin, in preparation). Instantaneous respiratory gas concentrations were monitored by a mass spectrometer (Airspec Ltd, MGA 2000) *via* a flexible capillary positioned in one of the flow tubes.

The efficacy of the face mask in monitoring all of the ventilatory gas flow and its effect on blood gases were determined. The mask was fitted to a horse, as usual, and an additional undertray, of the same material, was added to enclose the mouth and jaw. These two units were then completely sealed to the head by a flexible plastic film. A third flow tube was connected to the undertray so that any gas entering or leaving the horse's respiratory system, but bypassing the two tubes attached to the mask, could be monitored. This additional system was fitted to three horses, and flows were measured from rest to canter at $8\,m\,s^{-1}$. Peak airflows measured from the mouth chamber represented approximately 3.6% of the peak flows measured by the respiratory flowmeters. The flow profiles were dissimilar, and appeared to be related to the movement of the head within the additional mask structure. Leakage of respiratory airflow from around the mask seal was, therefore, considered not to represent an important source of error. The variation of flowmeter accuracy with gas concentration was determined through the use of a motor-driven piston pump (Boutellier *et al.* 1981) pushing a constant volume of gas of variable

composition through the flowmeter into a distendable but non-elastic bag. The change in gain of the flowmeters was as predicted by the theory of operation of such ultrasonic devices (inversely varying with the square of the velocity of sound), and appropriate correction factors were used to adjust the flow signal when determining exhalation variables. These corrections were less than 3% of the measured flow.

Two different assessments of the effect of the mask on levels of blood gases were made. In the first assessment, five horses completed the incremental exercise test described earlier both with and, at a later date, without the mask. In the second assessment, three horses performed another exercise test, in which the speed was raised in steps of $1ms^{-1}$ after 1min at each speed up to $11ms^{-1}$ (Butler *et al.* 1991), in the morning and afternoon of the same day, one with and the other without the face mask. Two-way analysis of variance on both sets of data indicated that the mask had a significant effect on partial pressure of CO_2 in arterial blood (Pa_{CO_2}) during the first assessment, but not during the second. Subsequent statistical analysis (Tukey multi-comparison test) of individual pairs of data points from the first assessments (Fig. 1) indicates that there was no systematic

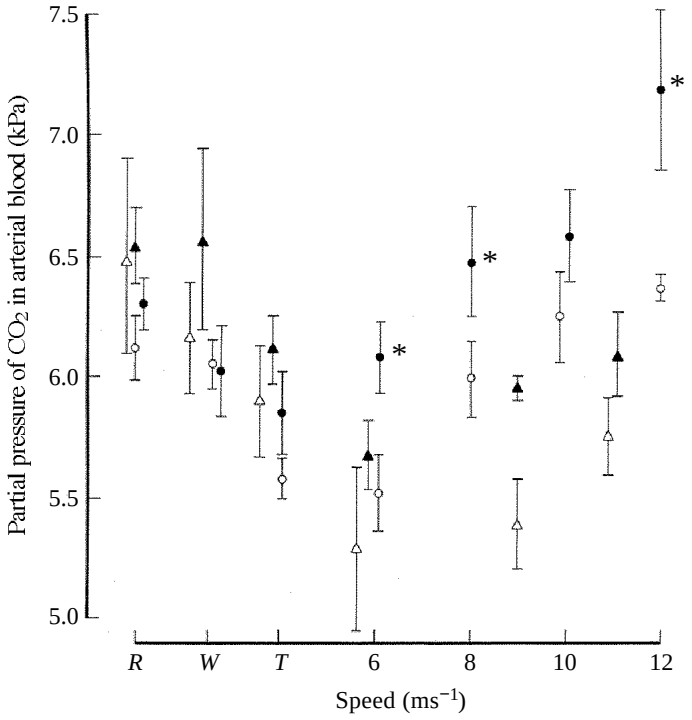

Fig. 1. The effect of the respiratory mask on P_{CO_2} of arterial blood in Thoroughbred horses at rest and during different levels of exercise. Mean values ± S.E.M. from five horses without (○) and with (●) the mask during an incremental exercise test with 2min of exercise at each speed and a 5min walk in between. Mean values ± S.E.M. from three horses without (△) and with (▲) the mask during an incremental exercise test with 1min of exercise at each speed and no rest in between. * indicates a significant difference between two pairs of values (Tukey multiple-comparison test after two-way ANOVA). *R*, rest; *W*, walk; *T*, trot.

effect of the mask on $P_{a_{CO_2}}$ during this test (i.e. there were significant effects of the mask at speeds of 6, 8 and 12ms^{-1}, but not at 10ms^{-1}).

Instantaneous respiratory airflow through both tubes and respiratory O_2 and CO_2 concentrations (Fig. 2) were recorded on a six-channel recorder with rectilinear coordinates (Gould, USA). The following variables were determined as described by Woakes *et al.* (1987) and Butler *et al.* (1991): peak inspiratory and expiratory flows, end tidal (ET) O_2 and CO_2 partial pressures, respiratory frequency (f_R), tidal volume (V_T), respiratory minute volume ($\dot{V}_E$), rate of oxygen consumption ($\dot{V}_{O_2}$), rate of CO_2 production ($\dot{V}_{CO_2}$). Values for V_T and $\dot{V}_E$ were corrected to BTPS and those for $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are at STPD. Alveolar ventilation ($\dot{V}_A$) was calculated from the formula:

$$\dot{V}_A = (\dot{V}_{CO_2}/P_{a_{CO_2}}) \times 863,$$

and physiological dead space ratio (V_D/V_T) from the formula:

$$V_D/V_T = \{[V_T(P_{a_{CO_2}} - P_{E_{CO_2}})/P_{a_{CO_2}})] - \text{mask dead space}\}/V_T$$

(Jones *et al.* 1966), where E refers to expired gas.

The accuracy of the basic measurement system and associated computer analyses for determining $\dot{V}_{O_2}$ was tested in humans (N=6) against an open-flow system in which air was drawn through a T-piece, mounted on the end of an ultrasonic flowmeter held in the mouth, at a known rate which always exceeded the peak inspired and expired flow rates. This flowmeter was a more sensitive version of the system used on horses. The oxygen contents of air entering and leaving the T-piece were determined by a paramagnetic oxygen analyser (Servomex Ltd). The subjects exercised on a bicycle ergometer, and measurements were made over a range of exercise levels, from rest to the maximum of which each subject was capable. When the values of $\dot{V}_{O_2}$ obtained simultaneously by the two methods were plotted against one another, the intercept (-0.046 ± 0.078) was not significantly different from zero and the slope (0.954 ± 0.047, N=29) was not significantly different from unity.

Heart rate (f_H) in exercising horses was recorded telemetrically (PEH 100 Hippocard, Switzerland) from electrodes on the mid and lateral thorax and synchronised with the other recording systems. The accuracy of this system has already been tested (Physick-Sheard *et al.* 1987). A veterinary surgeon monitored the performance of the horse and, by pressing a button on a recording watch, marked the recording so that specific times during the exercise could be recognised.

Four catheters were inserted into the left jugular vein and the raised carotid artery while the horse was standing in stocks. The areas of insertion were clipped and scrubbed with povidone–iodine (Pevidine, BK Veterinary Products Ltd, UK) and surgical spirit. After the area had been shaved, local anaesthetic (lignocaine hydrochloride, 2% aqueous solution without adrenaline; Astra Pharmaceuticals, UK) was administered before incisions were made in the skin. A polyethylene catheter (i.d. 1.67mm, o.d. 2.42 mm, Portex, UK) for sampling mixed venous blood and a transducer-tipped catheter (6F, Millar Mikro-tip, Millar Instruments, USA) were inserted into the left jugular vein. The former was inserted until its tip lay in the right atrium and the latter was advanced until it recorded pulmonary artery pressure. A catheter for temperature

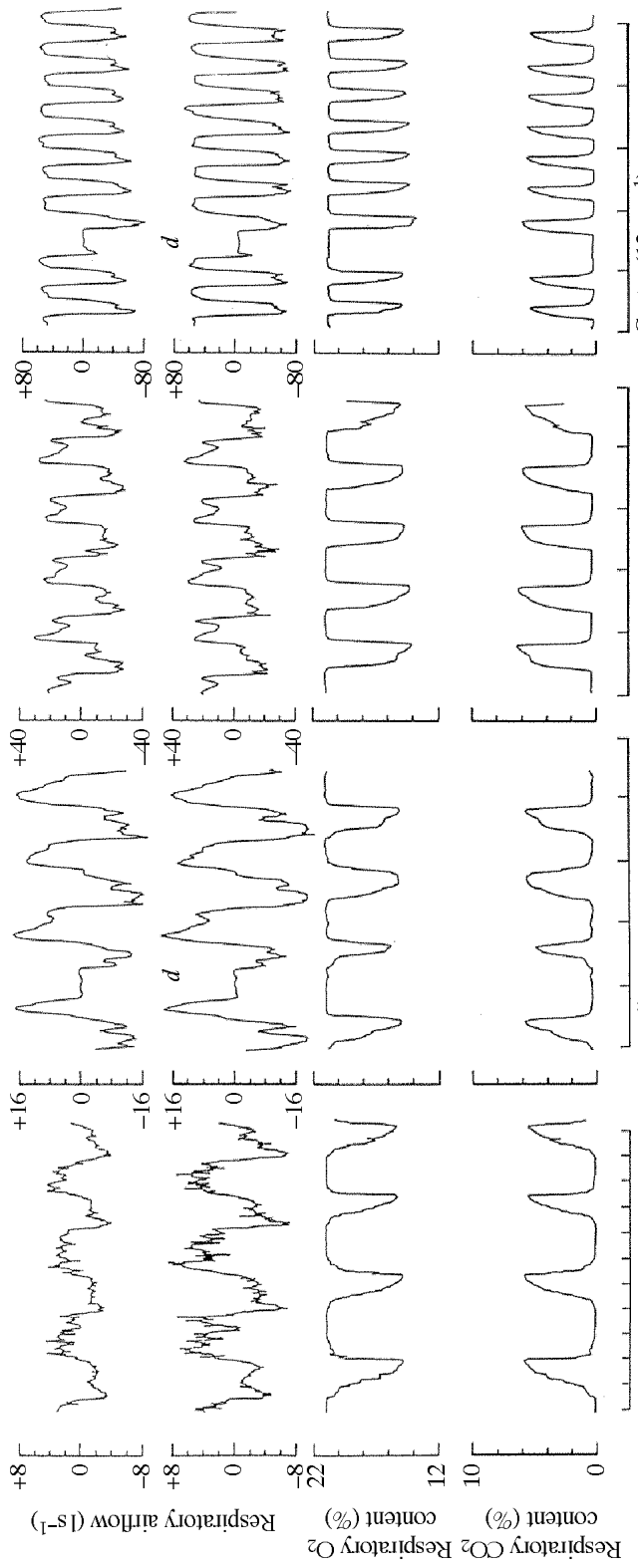

Fig. 2. Traces of respiratory airflow through both nostrils and respiratory gas concentrations from a Thoroughbred racehorse at rest, during walking and trotting and while cantering at a speed of 12 m s^{-1}. Zero flow is obtained when the horse spontaneously swallows (*d*).

measurement and blood sampling (7F, Criticath, Spectramed Inc., USA) and a transducer-tipped catheter (6F Millar Mikro-tip) were inserted into the raised carotid artery. The catheters were flushed regularly with heparinized saline (10–20 i.u.ml^{-1}) to keep them patent. Preliminary tests on two horses at different levels of exercise indicated that blood taken from the right atrium was representative of mixed venous blood as there was no significant difference in the P_{O_2} measured at this site and in samples taken simultaneously from the right pulmonary artery. Pulsatile and mean blood pressures from the pulmonary and carotid arteries were displayed on a six-channel recorder (Gould, USA).

Arterial (a) and mixed venous ($\bar{v}$) blood samples were taken, in plastic syringes, during the last 10s of each work level for the analysis of pH, partial pressures and contents of oxygen and carbon dioxide (P_{O_2}, P_{CO_2}, C_{O_2}, C_{CO_2}), and lactate and haemoglobin, [Hb], concentrations. After withdrawal of the blood sample, the syringe was placed on crushed ice and water for storage (<1h) before analysis. Before a sample was analysed for blood gases and pH, it was mixed thoroughly and the blood in the tip of the syringe discarded. Storage on ice had no significant effect on pH (+0.003±0.004) or P_{CO_2} (+0.04±0.02kPa) for periods up to 2.5h. There was, however, a significant increase in P_{O_2} (+0.46±0.11kPa) (see Mahoney *et al.* 1991).

Blood gas measurements and pH were performed on a blood gas analyser (ABL 330, Radiometer, Denmark) with an automatic temperature correction facility based on the characteristics of human blood. These temperature corrections are also accurate for horse blood (Butler *et al.* 1991). Since the blood gas and pH measurements were corrected to the temperature recorded in the arterial blood of the animal at the time they were taken, they are correct (see Jones *et al.* 1989*b*). Oxygen content was measured by the method of Tucker (1967). Cardiac output ($\dot{V}$b) was calculated from $\dot{V}_{O_2}$ and ($Ca_{O_2} - C\bar{v}_{O_2}$) using the Fick equation. Packed cell volume (PCV) was measured by a microhaematocrit (Hawksley, UK), and [Hb] was determined by a haemoglobinometer (Coulter Electronics Ltd, UK). Blood lactate concentration was determined from venous blood by an enzymatic assay (Hohorst, 1963).

All variables are calculated as means ± S.E.M. A repeated-measures analysis of variance (ANOVA) test was used to determine the effect of exercise on each variable. If significant (P<0.05), pairwise comparisons were made between the resting values and those at different levels of exercise using Fisher's PLSD test.

Results

Exercise

The mean resting values for the measured variables are given in Table 1. Both $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ increased significantly above the resting value during the walk. There was a linear relationship between the different levels of exercise and exchange of the two respiratory gases (Fig. 3) and, although the horses were exercising strenuously at 12 m s^{-1}+3°, they did not appear to reach their maximum rate of oxygen uptake ($\dot{V}_{O_2\ max}$), despite attaining 29.4 times the resting value at 60.6±3.6 l min^{-1} (137.1±10.3ml min^{-1} kg^{-1}), with $\dot{V}_{CO_2}$ at 36.8 times the resting value (75.1±4.3 l min^{-1}). There was a significant increase in the

respiratory exchange ratio (RE) at the walk; it then decreased to levels similar to that at rest until the horse was galloping at 12 m s^{-1}, when it was again significantly above the resting value. $\dot{V}_E$ also increased significantly at the walk and at the highest levels of gas exchange it was 27.0 times the resting level, although it can be seen from Fig. 4 that this variable is essentially the same at the two highest levels of $\dot{V}_{O_2}$. When the horses were

Table 1. *Mean (±S.E.M.) resting values of respiratory and cardiovascular variables measured in seven fit Thoroughbred horses of mean mass 440±12kg*

Variable	Units	
Oxygen consumption ($\dot{V}_{O_2}$)	$l\,min^{-1}$ STPD	2.1±0.3
	$ml\,min^{-1}\,kg^{-1}$ STPD	4.7±0.6
CO_2 production ($\dot{V}_{CO_2}$)	$l\,min^{-1}$ STPD	2.0±0.3
	$ml\,min^{-1}\,kg^{-1}$ STPD	4.5±0.6
Respiratory exchange ratio (RE)		0.99±0.3
Respiratory frequency (f_R)	$breaths\,min^{-1}$	13.5±1.5
Tidal volume (V_T)	ml BTPS	5.1±0.5
Respiratory minute volume ($\dot{V}_E$)	$l\,min^{-1}$ BTPS	67.0±8.1
Alveolar ventilation ($\dot{V}_A$)	$l\,min^{-1}$ BTPS	37.5±5.3
V_D/V_T		0.41±0.05
Peak inspiratory flow	$l\,s^{-1}$ ATPS	3.4±04
Peak expiratory flow	$l\,s^{-1}$ ATPS	5.7±0.5
Partial pressure of end-tidal oxygen (P_{ETO_2})	kPa	13.8±0.2
Partial pressure of oxygen in arterial blood (Pa_{O_2})	kPa	13.1±0.2
Partial pressure of oxygen in mixed venous blood ($P\bar{v}_{O_2}$)	kPa	5.2±0.2
Partial pressure of end-tidal CO_2 (P_{ETCO_2})	kPa	6.6±0.2
Partial pressure of CO_2 in arterial blood (Pa_{CO_2})	kPa	6.3±0.1
Partial pressure of CO_2 in mixed venous blood ($P\bar{v}_{CO_2}$)	kPa	6.9±0.2
pH of arterial blood (pHa)		7.414±0.006
pH of mixed venous blood ($pH\bar{v}$)		7.389±0.007
Blood lactate concentration	$mmol\,l^{-1}$	0.5±0.1
Temperature of arterial blood (T_a)	°C	37.2±0.3
Packed cell volume (PCV)	%	41.1±1.9
Haemoglobin concentration ([Hb])	$g\,dl^{-1}$	15.1±0.4
Oxygen content of arterial blood (Ca_{O_2})	$ml\,dl^{-1}$	20.8±0.6
Oxygen content of venous blood ($C\bar{v}_{O_2}$)	$ml\,dl^{-1}$	16.5±0.8
Heart rate (f_H)	$beats\,min^{-1}$	33.8±2.2
Cardiac output ($\dot{V}b$)	$l\,min^{-1}$	50.4±8.8
	$ml\,min^{-1}kg^{-1}$	115.0±20.7
$\dot{V}_A/\dot{V}b$		0.78±0.05
Mean pressure in the carotid artery	kPa	14.1±0.7
Mean pressure in the pulmonary artery	kPa	4.3±0.3

ATPS, air temperature pressure saturated.

running at 12 m s^{-1} on the flat, $\dot{V}_{O_2}$ was 25.7 times the resting value, $\dot{V}_{CO_2}$ was 28.9 times the resting value and $\dot{V}_E$ was 26.6 times the resting value.

The relative contributions of V_T and f_R to the changes in $\dot{V}_E$ are related to the gait of the horse. f_R increased significantly at the walk, whereas there was no significant increase in V_T above the resting value until the trot (Fig. 4). From rest to canter at 6 m s^{-1}, $\dot{V}_E$ increased to 13.3 times the resting value, with f_R increasing to 8.2 times the resting value

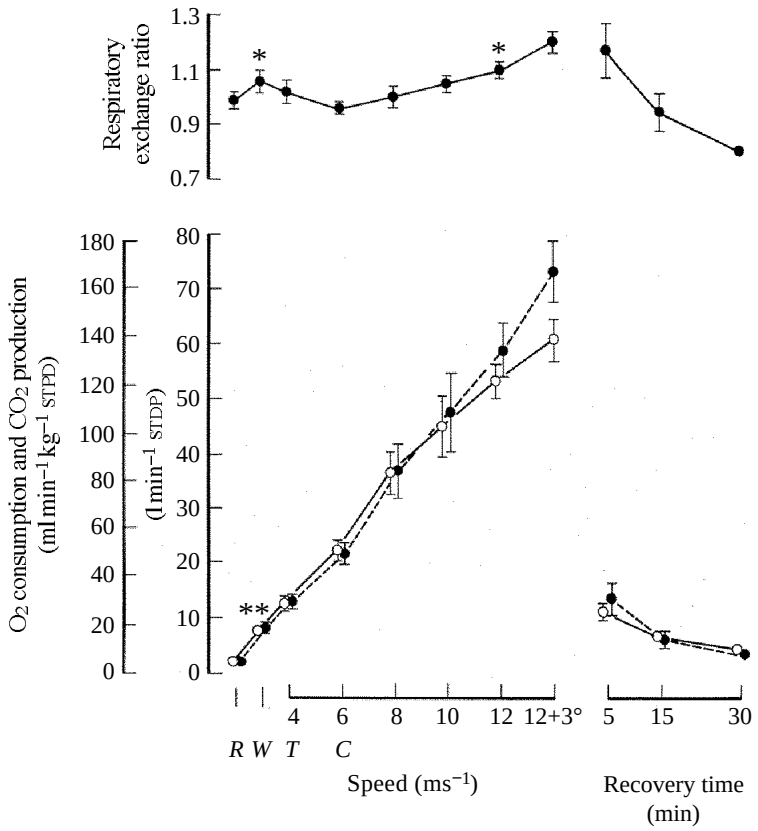

Fig. 3. Mean values ± S.E.M. of respiratory exchange ratio (RE), oxygen consumption (○) and carbon dioxide production (●) from seven Thoroughbred horses at rest (*R*), while walking (*W*), trotting (*T*) and cantering (*C*) at increasing speeds and during recovery. The horses walked on the level for 6min, trotted for 4min, walked for 5min, cantered at 6 m s^{-1} for 2 min, walked for 5min and so on at increasing speed in 2 m s^{-1} steps up to 12 m s^{-1}. After the next 5min walk, the treadmill was inclined to 3° and the horses ran for a further 2min at 12 m s^{-1}. Only data from the last 10–15s at each increasing work rate are given. This was followed by a recovery period of 15min walking and 15min standing. The first time a variable was significantly different from the resting value is indicated by an asterisk. In the case of RE, the difference then became non-significant, so the second asterisk indicates when it became significantly different from the resting value again. The relationship between oxygen consumption ($\dot{V}_{O_2}$) and speed (U) is given by the equation: $\dot{V}_{O_2}=5.2U-3.21$, $P<0.001$, $r=0.99$. For carbon dioxide production ($\dot{V}_{CO_2}$), the equation is: $\dot{V}_{CO_2}=4.4U-0.75$, $P<0.001$, $r=0.99$.

and V_T to only 1.6 times the resting value. Between walking and cantering at 6m s^{-1}, there was no significant change in V_T. As exercise intensity increased, there was only a slight (8%) further change in f_R, as it was then locked in a 1:1 fashion with stride frequency (f_S), which also changed little. The further (twofold) increase in $\dot{V}_E$ up to $12\,m\,s^{-1}$ was largely the result of a similar (1.8-fold) increase in V_T. Thus, the relationship between V_T and $\dot{V}_{O_2}$ was biphasic, with an abrupt change in slope once f_R stopped increasing, i.e. from $6\,m\,s^{-1}$ to $8\,m\,s^{-1}$ (Fig. 4). $\dot{V}_A$ increased significantly at the

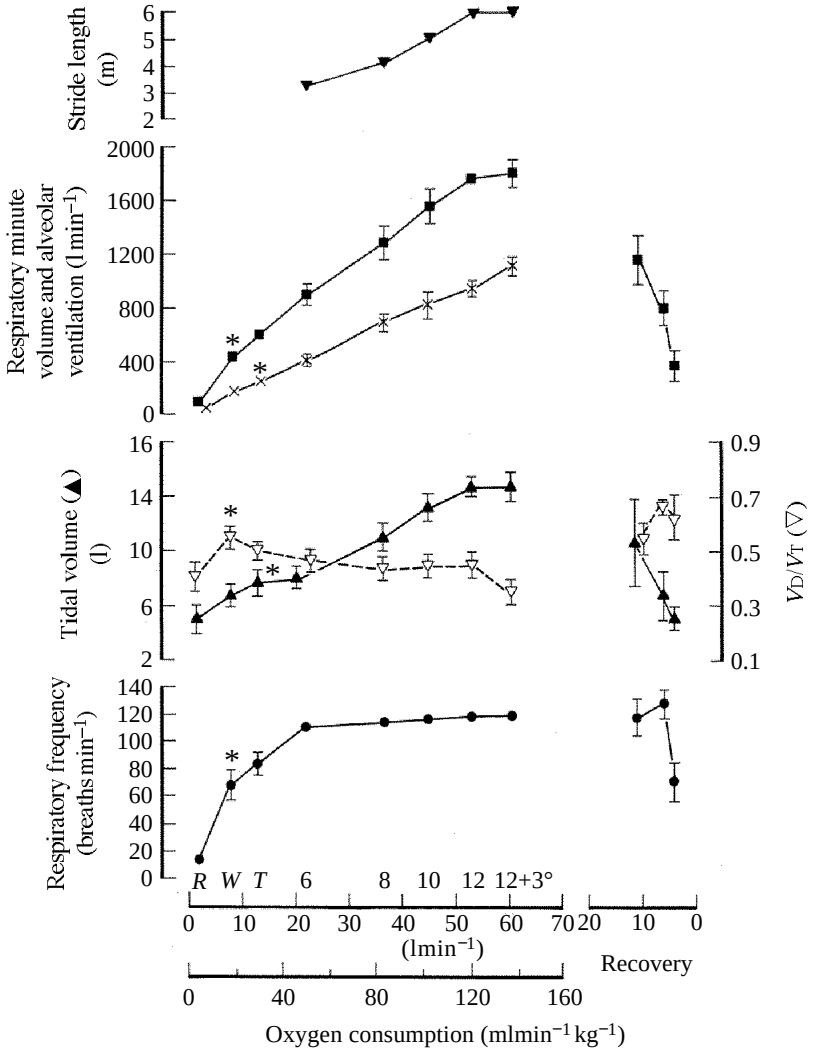

Fig. 4. Mean values ± S.E.M. of stride length (only mean values given), respiratory minute volume (■), alveolar ventilation (×), tidal volume (▲), V_D/V_T, (▽) and respiratory frequency from seven Thoroughbred horses at rest and at different levels of oxygen uptake during increasing exercise levels and recovery. The different speeds at which each mean value was obtained are given along the abscissa. For further details, see caption to Fig. 3.

trot and then rose linearly with $\dot{V}_{O_2}$ to 1121±91 l min^{-1} at 12 m s^{-1}+3°, an increase of almost 30-fold. V_D/V_T increased significantly at the walk and then decreased to values similar to that at rest.

Another interesting feature shown in Fig. 4 is the close relationship during cantering between V_T and stride length (S_L). Both variables increased by a similar proportion (approximately 2 times) between 6 and 12ms^{-1}, but neither changed at 12ms^{-1}+3°. The constant V_T at 12ms^{-1} and 12ms^{-1}+3° is compensated by a decrease in the partial pressure of end-tidal oxygen ($P_{ET_{O_2}}$). Although there is no significant difference between the values of $P_{ET_{O_2}}$ at 10ms^{-1} and 12ms^{-1} (14.1±0.2 kPa and 13.9±0.2kPa), the value at 12 m s^{-1}+3° (13.5±0.2kPa) is significantly lower than that at 10ms^{-1} (see Fig. 6). Peak airflow was significantly greater during expiration than during inspiration at the trot and the difference between them became progressively greater at higher levels of exercise (Fig. 5).

Despite the fact that $\dot{V}_A$ was well matched to $\dot{V}_{O_2}$, there was a progressive reduction in $P_{a_{O_2}}$ below the resting value as soon as the horses began to canter (Fig. 6). $P_{a_{O_2}}$ was significantly below the resting value at 8 m s^{-1} and eventually reached a value some 34% below the resting value. $P_{ET_{O_2}}$, after an initial significant increase, was not significantly below the resting value even at the highest level of exercise, so the difference between $P_{ET_{O_2}}$ and $P_{a_{O_2}}$ became progressively greater (Fig. 6). $P_{ET_{CO_2}}$ and $P_{a_{CO_2}}$ followed each other closely and it was not until the highest levels of exercise (12 m s^{-1} and 12 m s^{-1}+3°) that these two variables were significantly above the resting levels (at

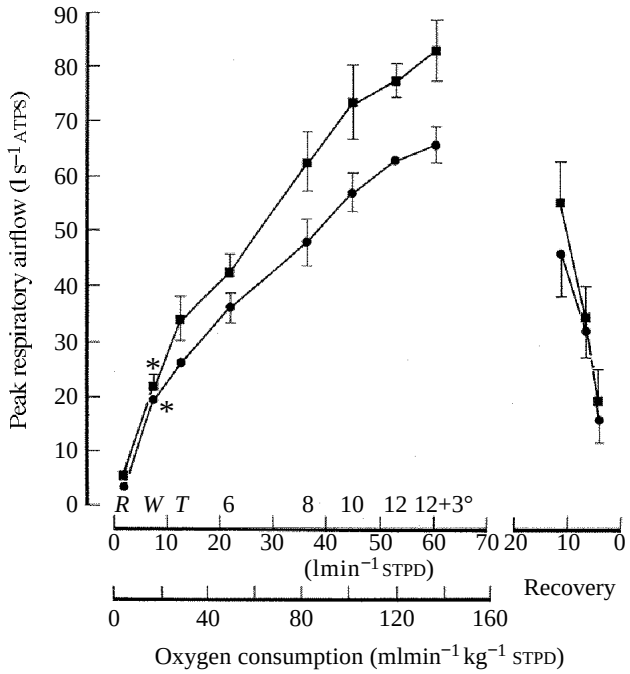

Fig. 5. Mean values ± S.E.M. of peak inspiratory (●) and peak expiratory (■) airflows from seven Thoroughbred horses at rest and at different levels of oxygen uptake during increasing exercise levels and recovery. For further details, see captions to Figs 3 and 4.

$12\,ms^{-1}+3^{\circ}$, they were both approximately 26% above the resting value). At the lower levels of exercise, $P_{ET_{CO_2}}$ mirrored $P_{ET_{O_2}}$ in that it was significantly below the resting value at walk, trot and $6\,ms^{-1}$. From walking onwards, $P\bar{v}_{CO_2}$ showed an exponential increase. It was significantly above the resting value at $8\,ms^{-1}$ and at the highest level of exercise was 2.4 times the resting value.

Lactate concentration in the venous blood increased exponentially and was significantly above the resting value at $10\,ms^{-1}$. At $12\,ms^{-1}+3^{\circ}$ it was 28 times the resting value (Fig. 7). Mixed venous blood became significantly acidotic at $8\,ms^{-1}$, whereas arterial blood became acidotic at $10\,ms^{-1}$. The pH of both then declined at approximately the same rate, being, on average, 0.38 and 0.30 of a pH unit, respectively, below their resting values at $12\,ms^{-1}+3^{\circ}$. Fig. 7 also illustrates the large (almost $4^{\circ}C$) increase in arterial temperature that occurred during the exercise test. It had increased significantly above the resting value at the trot.

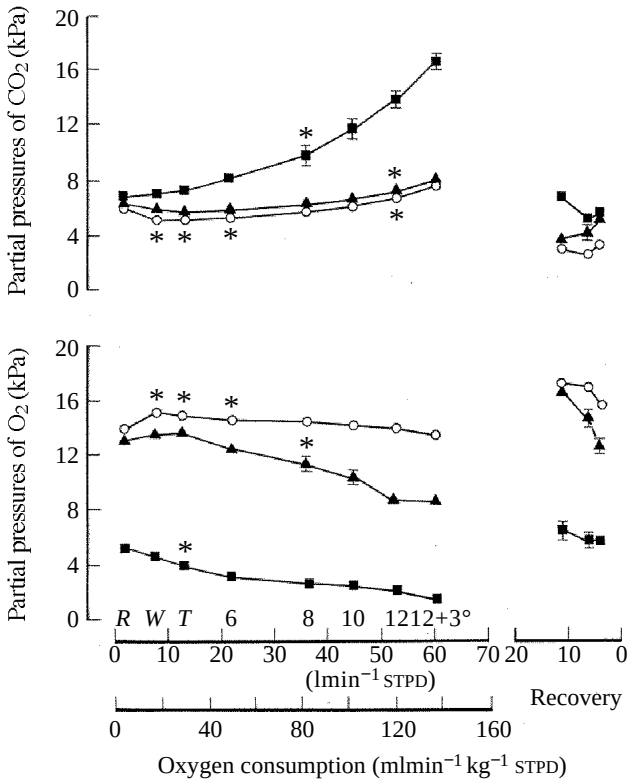

Fig. 6. Mean values ± S.E.M. of P_{CO_2} and P_{O_2} of mixed venous blood (■), of arterial blood (▲) and of end-tidal gases (○) from seven Thoroughbred horses at rest and at different levels of oxygen uptake during increasing exercise levels and recovery. For further details, see captions to Figs 3 and 4. In the cases of $P_{ET_{CO_2}}$ and $P_{ET_{O_2}}$ the values remained significantly different from rest for a number of measuring times (*) but the difference then became non-significant. In the case of the former, it became significantly different once more, and this is indicated by a further *.

As soon as the horses began to exercise, i.e. to walk, there were significant increases in PCV and [Hb] (Fig. 8). Approximately half of the eventual increases in these two variables occurred while the horses were trotting. Thus, although there was no significant decrease in Pa_{O_2} until the horses were galloping at $8\,ms^{-1}$ (Fig. 6), there was a significant increase in Ca_{O_2} while they were walking (Fig. 8). Ca_{O_2} peaked at $8–10\,ms^{-1}$ and then began to decline. At $12\,ms^{-1}+3^{\circ}$ it was significantly below the value at $8\,ms^{-1}$. However, because the rise in [Hb] more than compensated for the fall in Pa_{O_2}, Ca_{O_2} was 24% above the resting value at $12\,ms^{-1}+3^{\circ}$. The decline in $P\bar{v}_{O_2}$ was matched by an equally dramatic fall in $C\bar{v}_{O_2}$, eventually to 13% of its resting value. $C\bar{v}_{O_2}$ was significantly below the resting value at $6\,ms^{-1}$. The difference between Ca_{O_2} and $C\bar{v}_{O_2}$ increased from $4.3\pm0.27 ml dl^{-1}$ at rest to $23.7\pm0.6 ml dl^{-1}$ at $12\,ms^{-1}+3^{\circ}$, the latter value being 14% greater than Ca_{O_2} in the horses at rest.

Heart rate and cardiac output had both increased significantly at the walk and at

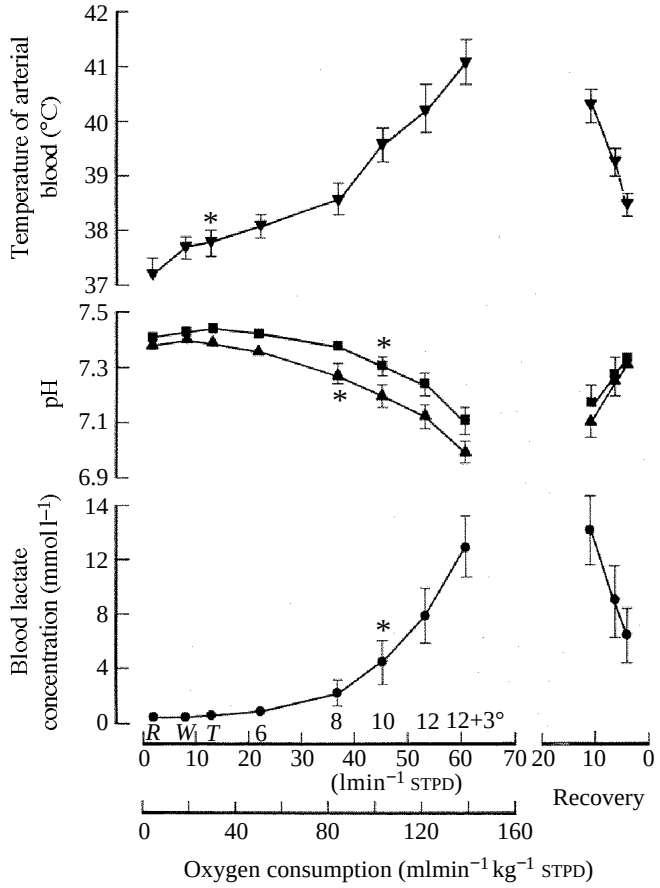

Fig. 7. Mean values ± S.E.M. of arterial blood temperature, pH of arterial blood (■), pH of mixed venous blood (▲) and blood lactate concentration from seven Thoroughbred horses at rest and at different levels of oxygen uptake during increasing exercise levels and recovery. For further details, see captions to Figs 3 and 4.

12 m s^{-1}+3° were 6 and 5 times their resting values, respectively (Fig. 9), while cardiac stroke volume did not change significantly (1.1l at rest, 1.0l at 12 m s^{-1}+3°). $\dot{V}_A/\dot{V}b$ increased to 4.33±0.14 at 12 m s^{-1}+3°. Associated with the increase in $\dot{V}b$ were increases in mean pressures in both carotid and pulmonary arteries (Fig. 9). Although both peaked at 10–12 m s^{-1}, with the former being 1.6 times the resting value and the latter 2.6 times the resting value, pulmonary artery pressure was significantly above its resting value at the trot whereas carotid artery pressure was not significantly elevated above the resting value until 8 m s^{-1}. Resistances to flow in the two major circuits were 0.32±0.05kPa min l^{-1} in the systemic and 0.10±0.02kPamin l^{-1} in the pulmonary at rest. They fell to 0.10±0.01kPamin l^{-1} and 0.05±0.01kPamin l^{-1}, respectively, at 12 m s^{-1}.

Recovery

Even after 30min of recovery (15min of walking and 15min of standing), $\dot{V}_{O_2}$ was still

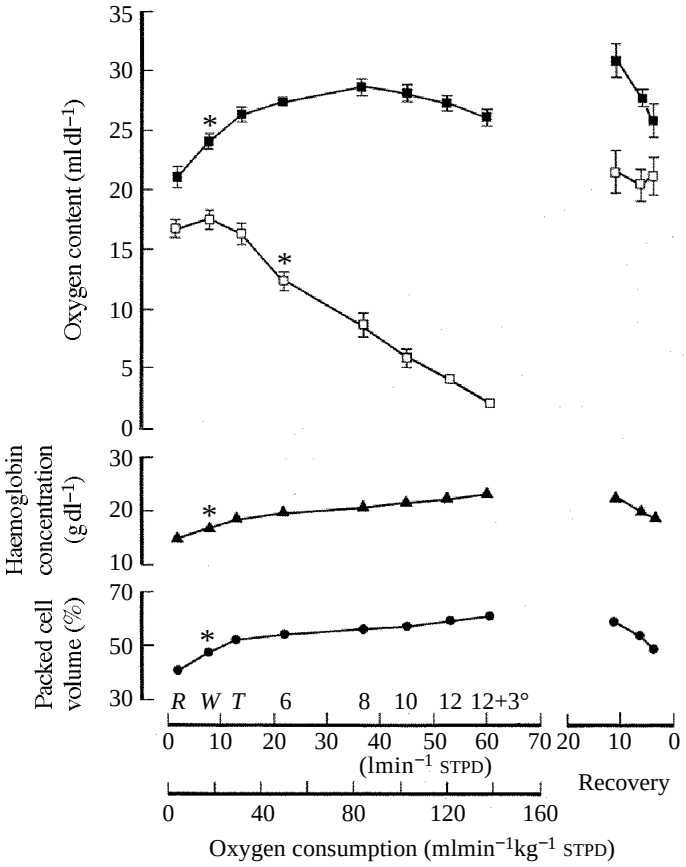

Fig. 8. Mean values ± S.E.M. of oxygen concentration in arterial (■) and mixed venous blood (□), haemoglobin concentration and packed cell volume from seven Thoroughbred horses at rest and at different levels of oxygen uptake during increasing exercise levels and recovery. For further details, see captions to Figs 3 and 4.

more than twice the pre-exercise resting value (Fig. 3). However, $\dot{V}_E$ and f_R were, at the same time, approximately 5.5 times their resting values, while V_T had returned to its resting value (Fig. 4). V_D/V_T was significantly above the resting value for the whole of the recovery period. Pa_{O_2} and Pa_{CO_2} were, respectively, above and below their resting values 5 min after the end of exercise (Fig. 6). The former had returned to its resting value but the latter was still significantly below the resting value 30min after the cessation of running. Lactate concentration and arterial temperature remained significantly above their resting values, and arterial pH significantly below, even after 30min of recovery (Fig. 7).

Mean systemic arterial blood pressure had returned to its resting value by 15min of

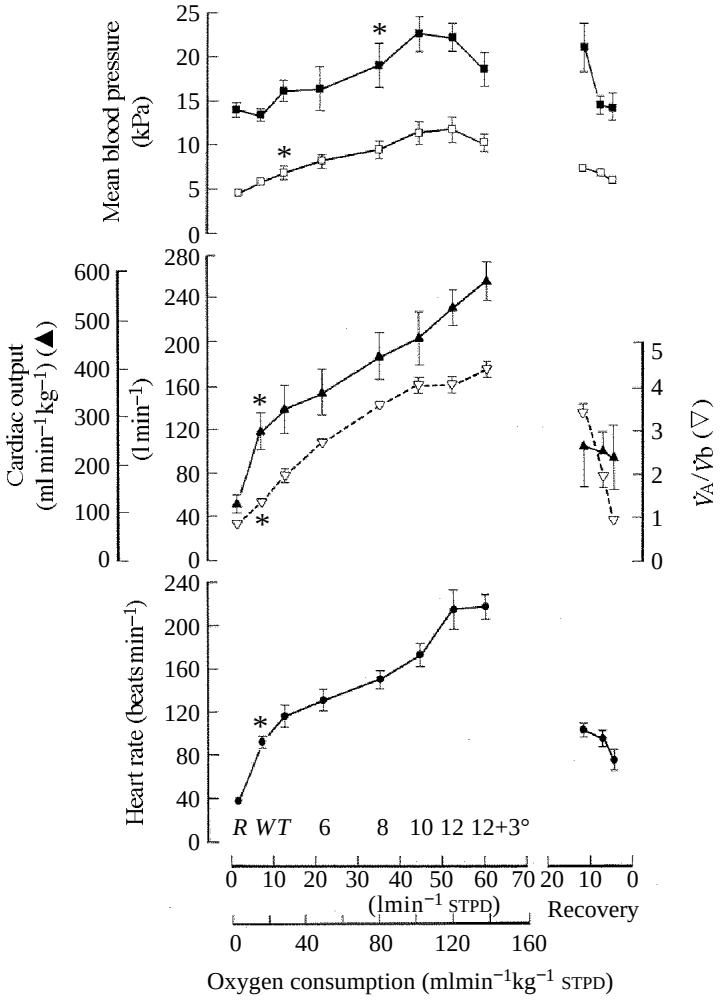

Fig. 9. Mean values ± S.E.M. of carotid (■) and pulmonary (□) blood pressures, cardiac output (▲), ventilation/perfusion ratio, $\dot{V}_A/\dot{V}b$ (▽) and heart rate from seven Thoroughbred horses at rest and at different levels of oxygen uptake during increasing exercise levels and recovery. For further details, see captions to Figs 3 and 4.

recovery, although mean pulmonary arterial pressure was still significantly above the resting value. After 30min of recovery, the latter had returned to its resting value (Fig. 9). f_H and $\dot{V}b$ were approximately twice their resting values 30min after cessation of running. However, after the 15min of recovery walking, both of these variables were at the same levels as those recorded during the initial period of walking before the exercise test began.

Discussion

The mask and airflow system used in the current experiments copes with the full range of respiratory airflows and gas exchange of Thoroughbred horses from rest to high levels of exercise and it has no systematic effect on the partial pressures of blood gases. The airflow profiles were indicative of those from healthy horses without chronic obstructive pulmonary diseases (Pollman and Hörnicke, 1987). It is in this context that the present data must be compared with those obtained using different mask and airflow systems.

Although it appears that $\dot{V}_{O_2max}$ was not reached in the present study, the highest mean value of $\dot{V}_{O_2}$ is very similar to some reported values of $\dot{V}_{O_2max}$ for Thoroughbreds (Rose *et al.* 1988; Evans and Rose, 1988*c*), but somewhat less than those reported for fully fit Thoroughbred horses (Butler *et al.* 1991; Knight *et al.* 1991). As previously described, respiratory minute ventilation increased linearly with the increase in $\dot{V}_{O_2}$, although it was higher in the present study than in that of Evans and Rose (1987) at similar levels of $\dot{V}_{O_2}$ (1807 *versus* 1250 $l\,min^{-1}$ at a $\dot{V}_{O_2}$ of 140 $mlkg^{-1}\,min^{-1}$). It is, perhaps, strange that minute ventilation does not show a disproportionate increase at high work loads, when metabolic acidosis and hyperthermia are apparent, as is the case in man (Wasserman *et al.* 1967). This may be because the mechanical coupling that exists between limb movements and ventilation in cantering and galloping horses (Bramble and Carrier, 1983; Young *et al.* 1992) actually restrains lung ventilation and prevents increases in both frequency and volume beyond those related to the mechanical factors. At lower speeds (up to and including 6 ms^{-1}) the large increase in f_R is the major contributor to the increase in pulmonary ventilation, whereas above that speed f_R changes very little and V_T increases substantially. As predicted by Bramble (1989), tidal volume is proportional to stride length, at least at speeds at and above 6 ms^{-1}, even at 12 ms^{-1} and 12 ms^{-1}+3°, despite the increased work load associated with the latter compared with the former. This relationship is not apparent from the data of Bayly *et al.* (1987*b*), who found that V_T was constant between speeds of 5 and 10 ms^{-1} (on a 10° incline). Since $\dot{V}_E$ increased significantly (see Fig. 3 of Bayly *et al.* 1987*b*), there must have been an equally significant increase in f_R, which is strange in view of the fact that, as already mentioned, f_R normally changes little above a speed of 6 ms^{-1}. Maximum peak inspiratory and expiratory airflows from the present study were similar to those reported by Hörnicke *et al.* (1987).

Few determinations of V_D/V_T have been made in horses. Three studies report a value at rest of around 0.6 (Rose and Evans, 1987; Bayly *et al.* 1987*b*; Pelletier *et al.* 1987). The value from the present study is lower than this, but similar to that measured in man (Jones *et al.* 1966). The latter authors also show that this ratio declines to around 0.15 at high work loads. Bayly *et al.* (1987*b*) report a drop to a similar level in Thoroughbred horses

running at $10\,m\,s^{-1}$ on a 10° incline, whereas in the present study there was no such decline below the resting value.The reasons for these differences in the values of V_D/V_T and in the response of this ratio to exercise in the Thoroughbred horse may be related to the different respiratory equipment used. Bayly *et al.* (1987*b*) and Pelletier *et al.* (1987) obtained their values of V_T, $\dot{V}_A$ and V_D/V_T using a valved mask system. This has a relatively large dead space (1.2–1.5l) and is known to cause large increases in $P_{a_{CO_2}}$, particularly at high levels of exercise (Bayly *et al.* 1987*c*), which would influence the calculation of V_D/V_T (see Materials and methods). If these authors did not take into account the (large) dead space of the mask system they used when calculating V_D/V_T, and there is no indication that they did, this would explain why they obtained such high resting values. Also, the value of minute ventilation that Bayly *et al.* (1987*b*) obtained at an oxygen uptake of approximately $140 ml\,kg^{-1}\,min^{-1}$ was, at $1200\,l\,min^{-1}$ BTPS, substantially lower than the $1800\,l\,min^{-1}$ obtained in the present study and in that of Hörnicke *et al.* (1983: approximately $1600\,l\,min^{-1}$ STPD) at a similar oxygen uptake.

Thus, unlike the situation in man (Jones *et al.* 1966; Wasserman *et al.* 1967), there may not be a reduction in V_D/V_T below the resting value in exercising Thoroughbred horses and this may, at least partly, explain why these animals become hypercapnic and hypoxaemic at higher levels of exercise. It has been demonstrated, using the multiple inert gas elimination technique, that the major cause of the arterial hypoxaemia in exercising horses is diffusion limitation, but that relative hypoventilation and mild $\dot{V}_A/\dot{V}b$ mismatch also contribute (Wagner *et al.* 1989). A morphometric and physiological study (Constantinopol *et al.* 1989) however, indicates the opposite, with relative hypoventilation and $\dot{V}_A/\dot{V}b$ inhomogeneities being the most likely cause of the hypoxaemia. The relative hypoventilation during exercise may not result entirely from an inadequate increase in minute ventilation, but may be aggravated by an unusually high V_D/V_T ratio. It seems that in the present study the physiological dead space increases approximately in proportion to the increase in V_T. The increase in physiological dead space is thought to be due to an increase in the anatomical, or airway, component (Jones *et al.* 1966), and this could be exaggerated in horses if areas of the lung are ventilated but remain relatively underperfused during exercise (Wasserman *et al.* 1967). A contributory factor to this condition could well be the anomalous blood supply of large portions of the lung (up to 22% of total lung volume) by bronchial arteries in horses with exercise-induced pulmonary haemorrhage (O'Callaghan *et al.* 1987). Also, the hypoxaemia that develops during higher levels of exercise could exacerbate any local tendencies towards hypoxia in the lung and this could lead to reduced alveolar perfusion (Kuriyama *et al.* 1984).

Mean pulmonary blood pressure increased to a similar level to that reported by Erickson *et al.* (1990). As there was a significant increase in this variable (at the trot) before there was a significant fall in $P_{a_{O_2}}$ (at $8\,m\,s^{-1}$), the latter cannot be the cause of the former, at least at relatively low levels of exercise. The study of Pelletier and Leith (1991) suggests that pulmonary hypoxia is not an important mechanism in pulmonary hypertension, even at high levels of exercise. As both cardiac output and packed cell volume increase significantly, even when the horses are walking, these are the most likely causes of pulmonary hypertension (Erickson *et al.* 1990). It is interesting to speculate that

the high pulmonary pressure could cause oedema (cf. Younes *et al.* 1987), which would contribute to the diffusion limitation of the lungs during exercise (but see West *et al.* 1991).

The significance of the large increases in heart rate and in Ca_{O_2}, despite the arterial hypoxaemia in the cardiovascular response to exercise in the horse, has been noted previously (Evans and Rose 1988*c*; Jones *et al.* 1989*a*). The values of cardiac output, cardiac stroke volume and $Ca_{O_2}-C\bar{v}_{O_2}$ in the present study are similar to those determined by the above groups. $\dot{V}_A/\dot{V}b$ is similar to that reported by Wagner *et al.* (1989).

Changes in arterial and mixed venous pH values were similar to those reported by Bayly *et al.* (1989) and the increase in blood lactate concentration was similar to that reported by Seeherman and Morris (1990) for adult Thoroughbred horses. However, high-level exercise to exhaustion in the laboratory (Rose *et al.* 1988) and when racing in the field (Bayly *et al.* 1987*a*) causes lactate concentrations that are twice as great as those determined in the present study. There is evidence that, as might be expected, blood pH is lower at the higher values of lactate concentration (Bayly *et al.* 1983). Presumably, such low pH values tend to shift the oxygen equilibrium curve (OEC) to the right and desaturate the arterial blood, thus exacerbating the effect of the hypoxaemia.

Another factor that is almost bound to influence the OEC is the large rise in arterial temperature that occurs during intense exercise. It is clear from the study of Jones *et al.* (1989*b*) that deep rectal temperature gives a false impression of good thermoregulation during heavy exercise in horses since it is approximately 2.5˚C lower than arterial blood temperature after 6min of exercise at $\dot{V}_{O_2max}$. An increase in blood temperature of 4˚C would be expected to be a powerful stimulus to ventilation and, as discussed earlier, the mechanical linkage of ventilation with limb movements may prevent thermal and metabolic stimuli from exerting their influence on ventilation during exercise. Certainly, during the post-exercise recovery period, respiratory minute volume remains significantly elevated above the resting value, mainly as a result of the high respiratory frequency, despite a dramatic reduction in gas exchange. Arterial and mixed venous pH return almost to the resting values, but blood temperature is still over 1˚C above the resting value. It is deduced, therefore, that immediately upon cessation of intense exercise, minute ventilation is high because of thermal and metabolic stimuli. As the recovery period continues, the latter exerts proportionately less influence. A strong thermal stimulus may also explain the high value of V_D/V_T during recovery.

Does the temperature in the arterial blood reflect that in the brain? Certainly during cooling in the cat it does (Kiley *et al.* 1984). However, in a number of mammals it has been demonstrated that, during heat load, brain temperature may be lower than the temperature of the arterial blood (e.g. Baker and Hayward, 1968; Baker, 1972; Johnson *et al.* 1987) and this is also true during exercise-induced hyperthermia (Taylor and Lyman, 1972; Caputa *et al.* 1983; Chesy *et al*. 1985). Whether this exists in the horse remains to be seen, but it could be that an elevated brain temperature has an inhibiting effect on exercise and, therefore, contributes to the phenomenon of fatigue (see Caputa *et al.* 1986). It is also evident from the data presented here that limitations of the respiratory system may well restrict the maximum performance of the Thoroughbred horse.

This report confirms some results obtained by other workers, but discrepancies have been found between previous work and the current study. It is probable that the new technique of measuring respiratory variables used here (the ultrasonic flowmeter) significantly reduces the effect of the measuring system on the measured variables, an effect very evident in many of the previously reported studies.

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References

ANDERSON, L. S., BUTLER, P. J., ROBERTS, C., SMALE, K., SNOW, D. H. AND WOAKES, A. J. (1989). Partial pressures of arterial and end-tidal gases during graded exercise in Thoroughbred racehorses. *J. Physiol., Lond.* **418**, 131P.

ART, T. AND LEKEUX, P. (1988). A critical assessment of pulmonary function testing in exercising ponies. *Vet. Res. Comm.* **12**, 25–39.

BAKER, M. A. (1972). Influence of the carotid rete on brain temperature in cats exposed to hot environments. *J. Physiol., Lond.* **220**, 711–728.

BAKER, M. A. AND HAYWARD, J. N. (1968). The influence of the nasal mucosa and the carotid rete upon hypothalamic temperature in sheep. *J. Physiol., Lond.* **198**, 561–579.

BAYLY, W. M., GRANT, B. D., BREEZE, R. G. AND KRAMER, J. W.(1983). The effects of maximal exercise on acid–base balance and arterial blood gas tension in Thoroughbred horses. In *Equine Exercise Physiology* (ed. D. H. Snow., S. G. B. Persson and R. J. Rose), pp. 401–407. Cambridge: Granta Editions.

BAYLY, W. M., GRANT, B. D. AND PEARSON, R. C. (1987*a*). Lactate concentrations in Thoroughbred horses following maximal exercise under field conditions. In *Equine Exercise Physiology 2* (ed. J. R. Gillespie and N. E. Robinson), pp. 426–437. Davis, CA: ICEEP Publications.

BAYLY, W. M., HODGSON, D. R., SCHULZ, D. A., DEMPSEY, J. A. AND GOLLNICK, P. D. (1989). Exercise-induced hypercapnia in the horse. *J. appl. Physiol.* **67**, 1958–1966.

BAYLY, W. M., SCHULZ, D. A., HODGSON, D. R. AND GOLLNICK, P. D. (1987*b*). Ventilatory response to exercise in horses with exercise-induced hypoxemia. In *Equine Exercise Physiology 2* (ed. J. R. Gillespie and N. E. Robinson), pp. 172–182. Davis, CA: ICEEP Publications.

BAYLY, W. M., SCHULZ, D. A., HODGSON, D. R. AND GOLLNICK, P. D.(1987*c*). Ventilatory responses of the horse to exercise: effect of gas collection systems. *J. appl. Physiol.* **63**, 1210–1217.

BOUTELLIER, U., GOMEZ, U. AND MÄDER, G. (1981). A piston pump for respiration simulation. *J. appl. Physiol.* **50**, 663–664.

BRAMBLE, D. M. (1989). Axial–appendicular dynamics and the integration of breathing and gait in mammals. *Am. Zool.* **29**, 171–186.

BRAMBLE, D. M. AND CARRIER, D. R. (1983). Running and breathing in mammals. *Science* **219**, 251–256.

BUTLER, P. J., WOAKES, A. J., ANDERSON, L. S., SMALE, K., ROBERTS, C. A. AND SNOW, D. H.(1991). The effect of cessation of training on cardiorespiratory variables during exercise. In *Equine Exercise Physiology 3* (ed. S. G. B. Persson, A. Lindholm and L. G. Jeffcott), pp. 71–76. Davis, CA: ICEEP Publications.

CAPUTA, M., FEISTKORN, G. AND JESSEN, C. (1986). Effects of brain and trunk temperatures on exercise performance in goats. *Pflügers Arch.* **406**, 184–189.

CAPUTA, M., KADZIELA, W. AND NAREBSKI, J. (1983). Cerebral temperature regulation in resting and running guinea-pigs (*Cavia porcellus*). *J. therm. Biol.* **8**, 265–272.

CHESY, G., CAPUTA, M., KADZIELA, W., KOZAK, W. AND LACHOWSKI, A. (1985). Selective brain cooling in the ox (*Box taurus*) during heavy exercise. *J. therm. Biol.* **10**, 57–61.

CONSTANTINOPOL, M., JONES, J. H., WEIBEL, E. R., TAYLOR, C. R., LINDHOLM, A. AND KARAS, R. H.

(1989). Oxygen transport during exercise in large mammals. II. Oxygen uptake by the pulmonary gas exchanger. *J. appl. Physiol.* **62**, 871–878.

ERICKSON, B. K., ERICKSON, H. H. AND COFFMAN, J. R.(1990). Pulmonary artery, aortic and oesophageal pressure changes during high intensity treadmill exercise in the horse: a possible relation to exercise-induced pulmonary haemorrhage. In *Exercise Physiology* (ed. D. H. Leach, R. J. Rose and D. H. Snow). *Equine vet. J. Supplement* **9**, 47–52.

EVANS, D. L. AND ROSE, R. J. (1987). Maximum oxygen uptake in racehorses: changes with training state and prediction from submaximal cardiorespiratory measurements. In *Equine Exercise Physiology 2* (ed. J. R. Gillespie and N. E. Robinson), pp. 52–67. Davis, CA: ICEEP Publications.

EVANS, D. L. AND ROSE, R. J.(1988*a*). Cardiovascular and respiratory responses to submaximal exercise training in the Thoroughbred horse. *Pflügers Arch.* **411**, 316–321.

EVANS, D. L. AND ROSE, R. J. (1988*b*). Dynamics of cardiorespiratory function in standardbred horses during different intensities of constant-load exercise. *J. comp. Physiol.* B **157**, 791–799.

EVANS, D. L. AND ROSE, R. J.(1988*c*). Cardiovascular and respiratory responses in Thoroughbred horses during treadmill exercise. *J. exp. Biol.* **134**, 397–408.

HOHORST, H. S. (1963). L-(+)-lactate. In *Methods for Enzymatic Analysis* (ed. H. U. Bergmeyer), pp. 266–270. New York and London: Academic Press.

HÖRNICKE, H., EHRLEIN, H.-J., TOLKMITT, G., HUSCH, H., NAGEL, M., DECKER, D., EPPLE, E., KIMMICH, H. P. AND KREUZER, F. (1974). Respiratory telemetry in exercising horses. In *Biotelemetry II* (ed. P. A. Neukomm), pp. 146–148. Basel: Karger.

HÖRNICKE, H., MEIXNER, R. and POLLMANN, U. (1983). Respiration in exercising horses. In *Equine Exercise Physiology* (ed. D. H. Snow, S. G. B. Persson and R. J. Rose), pp. 7–16. Cambridge: Granta Editions.

HÖRNICKE, H., WEBER, M. and SCHWEIKER, W. (1987). Pulmonary ventilation in Thoroughbred horses at maximum performance. In *Equine Exercise Physiology 2* (ed. J. R. Gillespie and N. E. Robinson), pp. 216–224. Davis, CA: ICEEP Publications.

JOHNSON, H. K., BLIX, A. S., MERCER, J. B. AND BOLZ, K.-D. (1987). Selective cooling of the brain in reindeer. *Am. J. Physiol.* **253**, 848–853.

JONES, J. H., LONGWORTH, K. E., LINDHOLM, A., CONLEY, K. E., KARAS, R. H., KAYAR, S. R. AND TAYLOR, C. R.(1989*a*). Oxygen transport during exercise in large mammals. I. Adaptive variation in oxygen demand. *J. appl. Physiol.* **67**, 862–870.

JONES, J. H., TAYLOR, C. R., LINDHOLM, A., STRAUB, R., LONGWORTH, K. E. AND KARAS, R. H. (1989*b*). Blood gas measurements during exercise: errors due to temperature correction. *J. appl. Physiol.* **67**, 879–884.

JONES, N. L., MCHARDY, G. J. R., NAIMARK, A. AND CAMPBELL, E. J. M. (1966). Physiological dead space and alveolar–arterial gas pressure differences during exercise. *Clin. Sci.* **31**, 19–29.

KILEY, J. P., ELDRIDGE, F. L. AND MILLHORN, D. E. (1984). Brain, blood and rectal temperature during whole body cooling. *Comp. Biochem. Physiol.* **79**A, 631–634.

KIMMICH, H. P. AND SPAAN, J. G. (1980). Combined flow and P_{O_2} sensors for telemetric assessment of oxygen uptake in horses. In *Biotelemetry V* (ed. G. Matsurmoto and H. P. Kimmich), pp. 165–168. Japan: Sapporo and Kyoto.

KNIGHT, P. K., SINHA, A. K. AND ROSE, R. J. (1991). Effects of training intensity on maximum oxygen uptake. In *Equine Exercise Physiology 3* (ed. S. G. B. Persson, A. Lindholm and L. B. Jeffcott), pp. 77–82. Davis, CA: ICEEP Publications.

KURIYAMA, T., LATHAM, L. P., HORWITZ, L. D., REEVES, J. T., WAGNER, W. W., JR (1984). Role of collateral ventilation in ventilation–perfusion balance. *J. appl. Physiol.* **56**, 1500–1506.

LANDGREN, G. L., GILLESPIE, J. R. AND LEITH, D. E. (1991). No ventilatory response to CO_2 in Thoroughbreds galloping at $14\,m\,s^{-1}$. In *Equine Exercise Physiology 3* (ed. S. G. B. Persson, A. Lindholm and L. B. Jeffcott), pp. 59–65. Davis, CA: ICEEP Publications.

LINDSTEDT, S. L., HOKANSON, J. F., WELLS, D. J., SWAIN S. D., HOPPELER, H. AND NAVARRO, V. (1991). Running energetics in the pronghorn antelope. *Nature* **353**, 748–750.

LONGWORTH, K. E., JONES, J. H., BICUDO, J. E. P., TAYLOR, C. R. AND WEIBEL, E. R.(1989). High rate of O_2 consumption in exercising foxes: large P_{O_2} difference drives diffusion across the lung. *Respir. Physiol.* **77**, 263–276.

MAHONEY, J. J., HARVEY, J. A., WONG, R. J. AND VAN KESSEL, A. L. (1991). Changes in oxygen measurements when whole blood is stored in iced plastic or glass syringes. *Clin. Chem.* **37**, 1244–1248.

O'CALLAGHAN, M. W., PASCOE, J. R., TYLER, W. S. AND MASON, D. K. (1987). Vascular changes in the lungs of horses with exercise-induced pulmonary hemorrhage. In *Equine Exercise Physiology 2* (ed. J. R. Gillespie and N. E. Robinson), pp. 728–737. Davis, CA: ICEEP Publications.

PELLETIER, N., BLAIS, D., VRINS, A. AND ROBINSON, N. E. (1987). Effect of sub-maximal exercise and training on dead space ventilation in the horse. In *Equine Exercise Physiology 2* (ed. J. R. Gillespie and N. E. Robinson), pp. 225–234. Davis, CA: ICEEP Publications.

PELLETIER, N. AND LEITH, D. E. (1991). Hypoxia does not contribute to high pulmonary artery pressure in exercising horses. In *Equine Exercise Physiology 3* (ed. S. G. B. Persson., A. Lindholm and L. B. Jeffcott), pp. 30–36. Davis, CA: ICEEP Publications.

PHYSICK-SHEARD, P. W., HARMAN, J. C., SNOW, D. H. AND WOAKES, A. J. (1987). Evaluation of factors influencing the performance of four equine heart rate meters. In *Equine Exercise Physiology 2* (ed. J. R. Gillespie and N. E. Robinson), pp. 102–116. Davis, CA: ICEEP Publications.

POLLMANN, J. and HÖRNICKE, H. (1987). Characteristics of respiratory airflow during exercise in horses with reduced performance due to pulmonary emphysema or bronchitis. In *Equine Exercise Physiology 2* (ed. J. R. Gillespie and N. E. Robinson), pp. 760–771. Davis, CA: ICEEP Publications.

ROSE, R. J. AND EVANS, D. L. (1987). Cardiovascular and respiratory function in the athletic horse. In *Equine Exercise Physiology 2* (ed. J. R. Gillespie and N.E. Robinson), pp. 1–24. Davis, CA: ICEEP Publications.

ROSE, R. J., HODGSON, D. R., KELSO, T. B., MCCUTCHEON, L. J., REID, T.-A., BAYLY, W. M. AND BOLLNICK, P. D. (1988). Maximum O_2 uptake, O_2 debt and deficit and muscle metabolites in Thoroughbred horses. *J. appl. Physiol.* **64**, 781–788.

SEEHERMAN, H. J. AND MORRIS, E. A. (1991). Comparison of yearling, two-year-old and adult Thoroughbreds using a standardised exercise test. *Equine vet. J.* **23**, 175–184.

TAYLOR, C. R., KARAS, R. H., WEIBEL, E. R. AND HOPPELER, H. (1987). Adaptive variation in the mammalian respiratory system in relation to energetic demand. II. Reaching the limits to oxygen flow. *Respir. Physiol.* **69**, 7–26.

TAYLOR, C. R. AND LYMAN, C. P. (1972). Heat storage in running antelopes: independence of brain and body temperatures. *Am. J. Physiol.* **222**, 114–117.

TAYLOR, C. R., MALOIY, G. M. O., WEIBEL, W. R., LANGMAN, V. A., KAMAU, J. M. Z., SEEHERMAN, H. J. AND HEGLUND, N. C. (1981). Design of the mammalian respiratory system. III. Scaling maximum aerobic capacity to body mass: wild and domestic mammals. *Respir. Physiol.* **44**, 25–37.

TUCKER, V. A.(1967). Method for oxygen content and dissociation curves on microliter blood samples. *J. appl. Physiol.* **23**, 410–413.

WAGNER, P. D., GILLESPIE, J. R., LANDGREN, G. L., FEDDE, M. R., JONES, B. W., DEBOWES, R. M., PIESCHL, R. L. AND ERICKSON, H. H. (1989). Mechanism of exercise-induced hypoxemia in horses. *J. appl. Physiol.* **66**, 1227–1233.

WASSERMAN, K., VAN KESSEL, A. L. AND BURTON, G. G. (1967). Interaction of physiological mechanisms during exercise. *J. appl. Physiol.* **22**, 71–85.

WEST, J. B., TSUKIMOTO, K., MATHIEU-COSTELLO, O. AND PREDILETTO, R. (1991). Stress failure in pulmonary capillaries. *J. appl. Physiol.* **70**, 1731–1742.

WOAKES, A. J. AND BUTLER, P. J. (1980). An implantable transducer for the measurement of respiratory airflow. In *Handbook on Biotelemetry and Radiotracking* (ed. C. J. Amlaner and D. W. Macdonald), pp. 287–292. Oxford: Pergamon Press.

WOAKES, A. J., BUTLER, P. J. AND SNOW, D. H. (1987). The measurement of respiratory airflow in exercising horses. In *Equine Exercise Physiology 2* (ed. J. R. Gillespie and N. E. Robinson), pp. 194–205. Davis, CA: ICEEP Publications.

YOUNES, M., BSHOUTY, Z. AND ALI, J. (1987). Longitudinal distribution of pulmonary vascular resistance with very high pulmonary blood flow. *J. appl. Physiol.* **62**, 344–358.

YOUNG, I. S., ALEXANDER, R. MCN., WOAKES, A. J., BUTLER, P. J. AND ANDERSON, L. (1992). The synchronization of ventilation and locomotion in horses (*Equus caballus*). *J. exp. Biol.* **166**, 19–31.