Effect of prolonged heavy exercise on pulmonary gas exchange in horses

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[1]*Division of Physiology, Department of Medicine, University of California San Diego, La Jolla, California 92093-0623;* [2]*Department of Veterinary Clinical Sciences, Washington State University, Pullman, Washington 99164; and* [3]*School of Veterinary Science, University of Melbourne, Werribee, Victoria 3030, Australia*

Hopkins, S. R., W. M. Bayly, R. F. Slocombe, H. Wagner, and P. D. Wagner. Effect of prolonged heavy exercise on pulmonary gas exchange in horses. *J. Appl. Physiol.* 84(5): 1723–1730, 1998.—During short-term maximal exercise, horses have impaired pulmonary gas exchange, manifested by diffusion limitation and arterial hypoxemia, without marked ventilation-perfusion ($\dot{V}_A/\dot{Q}$) inequality. Whether gas exchange deteriorates progressively during prolonged submaximal exercise has not been investigated. Six thoroughbred horses performed treadmill exercise at ~60% of maximal oxygen uptake until exhaustion (28–39 min). Multiple inert gas, blood-gas, hemodynamic, metabolic rate, and ventilatory data were obtained at rest and 5-min intervals during exercise. Oxygen uptake, cardiac output, and alveolar-arterial P_{O_2} gradient were unchanged after the first 5 min of exercise. Alveolar ventilation increased progressively during exercise, from increased tidal volume and respiratory frequency, resulting in an increase in arterial P_{O_2} and decrease in arterial P_{CO_2}. At rest there was minimal $\dot{V}_A/\dot{Q}$ inequality, log SD of the perfusion distribution (log $SD_{\dot{Q}}$) = 0.20. This doubled by 5 min of exercise (log $SD_{\dot{Q}}$ = 0.40) but did not increase further. There was no evidence of alveolar-end-capillary diffusion limitation during exercise. However, there was evidence for gas-phase diffusion limitation at all time points, and enflurane was preferentially overretained. Horses maintain excellent pulmonary gas exchange during exhaustive, submaximal exercise. Although $\dot{V}_A/\dot{Q}$ inequality is greater than at rest, it is less than observed in most mammals and the effect on gas exchange is minimal.

ventilation-perfusion inequality; pulmonary mechanics; intrapulmonary gas mixing

PULMONARY LIMITATIONS to exercise are well documented in horses (2, 18) and include an increase in the alveolar-arterial P_{O_2} gradient [$(A\text{-}a)P_{O_2}$] and arterial hypoxemia during heavy exercise (2, 29). In these animals, the hypoxemia is associated with an increase in both $(A\text{-}a)P_{O_2}$ and arterial P_{CO_2} (Pa_{CO_2}) (2). Thus there is both a deterioration of pulmonary gas exchange and insufficient compensatory hyperventilation to avoid hypercapnia. During maximal exercise, ~70% of the $(A\text{-}a)P_{O_2}$ can be attributed to pulmonary diffusion limitation (30). Although ventilation at rest is closely matched to perfusion, [log SD of the perfusion distribution (log $SD_{\dot{Q}}$) is ~0.3], and there is little deterioration in ventilation-perfusion relationships ($\dot{V}_A/\dot{Q}$) from rest to maximal levels of exercise, the balance of the increased $(A\text{-}a)P_{O_2}$ is related to the mild degree of $\dot{V}_A/\dot{Q}$ inequality in these animals (30).

The cause of worsening $\dot{V}_A/\dot{Q}$ relationships with exercise is unknown. In humans, there are also pulmonary limitations to exercise (3, 5, 9, 25), and $\dot{V}_A/\dot{Q}$ relationships worsen to a greater extent than in horses during short-term maximal exercise (9). In athletes the log $SD_{\dot{Q}}$ may approach 0.7, contributing to at least 60% of the $(A\text{-}a)P_{O_2}$ (9). The increase in the log $SD_{\dot{Q}}$ persists well into recovery from heavy exercise and several minutes after ventilation and cardiac output have returned to normal (21). Subjects who have previously suffered from high-altitude pulmonary edema have both higher pulmonary arterial pressures and greater $\dot{V}_A/\dot{Q}$ inequality during sea-level exercise than do control subjects (15). Additionally, pig lungs show an increase in perivascular edema on histological examination (20) in exercised animals compared with resting controls. Combined, this information argues for subclinical interstitial pulmonary edema secondary to high pulmonary arterial pressures as a cause of exercise-induced increases in $\dot{V}_A/\dot{Q}$ inequality.

Recently, the effects of prolonged submaximal exercise on the ventilatory response of horses to exercise have been reported (1) and show a progressive hypocapnia secondary to an increase in tidal volume and thus alveolar hyperventilation. However, there were no significant changes in arterial P_{O_2} (Pa_{O_2}) over the course of the exercise, despite the increase in alveolar ventilation, suggesting an increase in the $(A\text{-}a)P_{O_2}$ and worsening of pulmonary gas exchange. We hypothesized that, in horses, submaximal exercise with prolonged exposure of the pulmonary vascular bed to high pulmonary arterial pressure would result in greater $\dot{V}_A/\dot{Q}$ inequality in prolonged submaximal exercise than has been previously observed with ~5 min of maximal exercise and help to explain these previous findings. We therefore sought to investigate the effects of prolonged submaximal exercise on pulmonary gas exchange in horses by using the multiple inert gas elimination technique.

METHODS

This experiment was approved by the Institutional Animal Care and Use Committee of Washington State University. Six adult thoroughbred horses of either sex, with weights ranging from 430 to 559 kg, were trained to run on an equine treadmill (Sato I, Uppsala, Sweden). During the 2 wk before the experiment, maximal O_2 uptake ($\dot{V}_{O_{2max}}$) of each animal was determined by using previously described methods (19). In the week before the experiment, the animal underwent treadmill exercise to select a workload that elicited ~60% of $\dot{V}_{O_{2max}}$ and that the animal could sustain for at least 25 min. The selected treadmill speeds ranged from 4.3 to 4.8 m/s at a 10% grade. On the day of the experiment, a previously translocated subcutaneous left carotid artery was cannulated with an 18-gauge catheter for arterial blood-gas sampling,

and the left jugular vein was cannulated for infusion of an inert gas solution (see *Multiple inert gas measurements*). A no. 7-F Swan-Ganz catheter was inserted into the right external jugular vein and advanced into the pulmonary artery by using direct pressure monitoring for sampling of mixed venous blood and measurement of pulmonary arterial pressure and blood temperature.

The experiments took place in a temperature-controlled ventilated room (21–23°C), and the animal was cooled during the study by using large electric fans. Data were collected at rest and at 5-min intervals during exercise until the animal was fatigued, as judged by an inability of the exercising animal to keep up with the treadmill. Each set of measurements included ventilation; respiratory frequency; tidal volume; transpulmonary pressure; pulmonary arterial pressure measurements and sampling of pulmonary mixed venous blood; arterial blood and mixed expired gases for the multiple inert gas analyses; blood gases; cardiac output calculations; and metabolic rate measurements. Duplicate sets of blood-gas and inert gas measurements were made at rest, and the results were averaged. Single measurements were made thereafter.

Ventilation, pulmonary mechanics, and metabolic rate measurements. The animal had a face mask strapped to its head, and room air was drawn in through bias flow entry ports at a rate of 1,500 l/min at rest and 6,000 l/min during exercise. This face mask design has been previously described (2). Briefly, the system consisted of a shutter-type bias flow entry port on either side of the mask, which was briefly shut (for 5 breaths maximum) for measurement of ventilatory mechanics. With the closure of these ports, airflow was drawn through two identical 160-mm-diameter pneumotachs (Mercury Electronics, Glasgow, UK) and the pressure drop across the pneumotach was measured by using differential pressure transducers (Validyne, DP45–26, Northridge, CA) at a time when the bias flow had ceased. Transpulmonary pressures (Validyne, DP-45–34) were measured as previously described (1, 23). Pressures were measured in the mask just cranial to the nares and from an esophageal balloon catheter. Total pulmonary resistance and work of breathing were calculated. Mixed expired O_2 and CO_2 concentrations were measured from a large (1,500-liter) Tissot spirometer from a sampling of the bias flow output.

Multiple inert gas measurements. The multiple inert gas technique was applied in the usual manner, modified for horses, as has been previously described (30). The inert gas solution was prepared in 5% dextrose and infused for ~20 min before collection of the resting samples. Because of the relatively long duration of the study (~30 min) and the high infusate flow rate (175–250 ml/min) required to match the high respiratory bias flow during exercise, the inert gas infusion was turned on 2 min before the collection of the exercise sample (see paragraph below) and turned off immediately afterward to minimize the fluid load to the animal. Because the pulmonary blood flow and ventilation are both extremely high even during submaximal exercise in horses, 2 min of infusion are sufficient to ensure steady-state conditions (see DISCUSSION). The total volume of fluid infused during the course of the study was 5 ± 1 liters, which is hemodynamically insignificant in these animals.

At rest, quadruplicate 15-ml samples of mixed expired gas were obtained from the bias flow stream. Duplicate 6-ml samples of pulmonary and systemic arterial blood were obtained in gastight syringes from animals at rest for measurement of the steady-state concentrations of the six inert gases (sulfur hexafluoride, ethane, cyclopropane, enflurane, ether, and acetone) by using a gas chromatograph (Hewlett-Packard 5890A, Wilmington, DE) (31). During exercise, duplicate mixed expired gas and single pulmonary mixed venous and arterial blood samples were obtained. $\dot{V}_A/\dot{Q}$ distributions were obtained by using the multiple inert gas elimination technique in the usual fashion. Solubilities, retentions (R = ratio of arterial to mixed venous partial pressure), and excretions (E = ratio of mixed expired to mixed venous partial pressure) for the inert gases were determined, corrected for body temperature, and $\dot{V}_A/\dot{Q}$ distributions were calculated from the inert gas data (6, 32). The second moment of the perfusion distribution, exclusive of intrapulmonary shunt ($\log SD_{\dot{Q}}$), and the second moment of the ventilation distribution, exclusive of dead space ($\log SD_{\dot{V}}$), are used as indicators of the degree of $\dot{V}_A/\dot{Q}$ inequality (i.e., the greater the $\log SD_{\dot{Q}}$ or the $\log SD_{\dot{V}}$, the greater the $\dot{V}_A/\dot{Q}$ inequality). The residual sum of squares (RSS) was used as an indicator of the adequacy of fit of the data to the 50-compartment model of the lung (32).

Hemodynamic measurements. The pressure transducer (Transpac II, Abbott Laboratories, Salt Lake City, UT) was zeroed to the level of the right atrium, and pulmonary arterial pressures were recorded immediately before each set of inert gas measurements. Cardiac output was calculated from the mixed venous, arterial, and mixed expired inert gas concentrations by using the Fick principle.

Blood-gas measurements. Two-milliliter arterial and mixed venous samples were collected immediately after each inert gas arterial and mixed venous blood sample and maintained on ice (average time 1.5 h) until analyzed for P_{O_2}, P_{CO_2}, and pH by using an AVL995 (Radiometer America, Westlake, OH) blood-gas analyzer. Each sample had hemoglobin and O_2 saturation measured by using an IL 282 CO-oximeter (Instrumentation Laboratories, Lexington, MA), and hematocrit was determined. The blood gases were corrected to pulmonary arterial blood temperature.

Statistical analyses. Repeated-measures analysis of variance (SuperANOVA 1.11, Abacus Concepts, Berkeley CA) was used to statistically test changes in the dependent variables from rest and over the duration of exercise. Preplanned contrasts (means comparisons) were used to compare the changes from rest to exercise. Significance was accepted at $P < 0.05$, two tailed. All results are reported as means ± SE.

RESULTS

General data. Barometric pressure averaged 700 Torr during the study. The animals ran at a treadmill speed of between 4.3 and 4.8 m/s at a 10% grade. At this speed and grade, four of the animals trotted exclusively, whereas two animals had brief periods (~2–3 min) of cantering early in the test. All animals tolerated the study well, and all six were able to complete at least 28 min of exercise by using this protocol. Four animals ran between 28 and 33 min in total, and two others completed between 38 and 39 min of exercise. Mean running time was 33 ± 5 min.

Metabolic rate and hemodynamic data. Metabolic and hemodynamic data are given in Fig. 1. Only data to 30 min are included so that all horses contributed to all data points. Animals reached a steady state of cardiac output and O_2 consumption within 5 min, and there were no changes in those two variables over time. O_2 consumption averaged 44 l/min, which was 57% of $\dot{V}O_{2max}$. Each animal had a progressive lactic acidosis, and mean blood lactate averaged 5.4 mmol at the end of exercise. Pulmonary arterial pressures averaged 28 ± 4

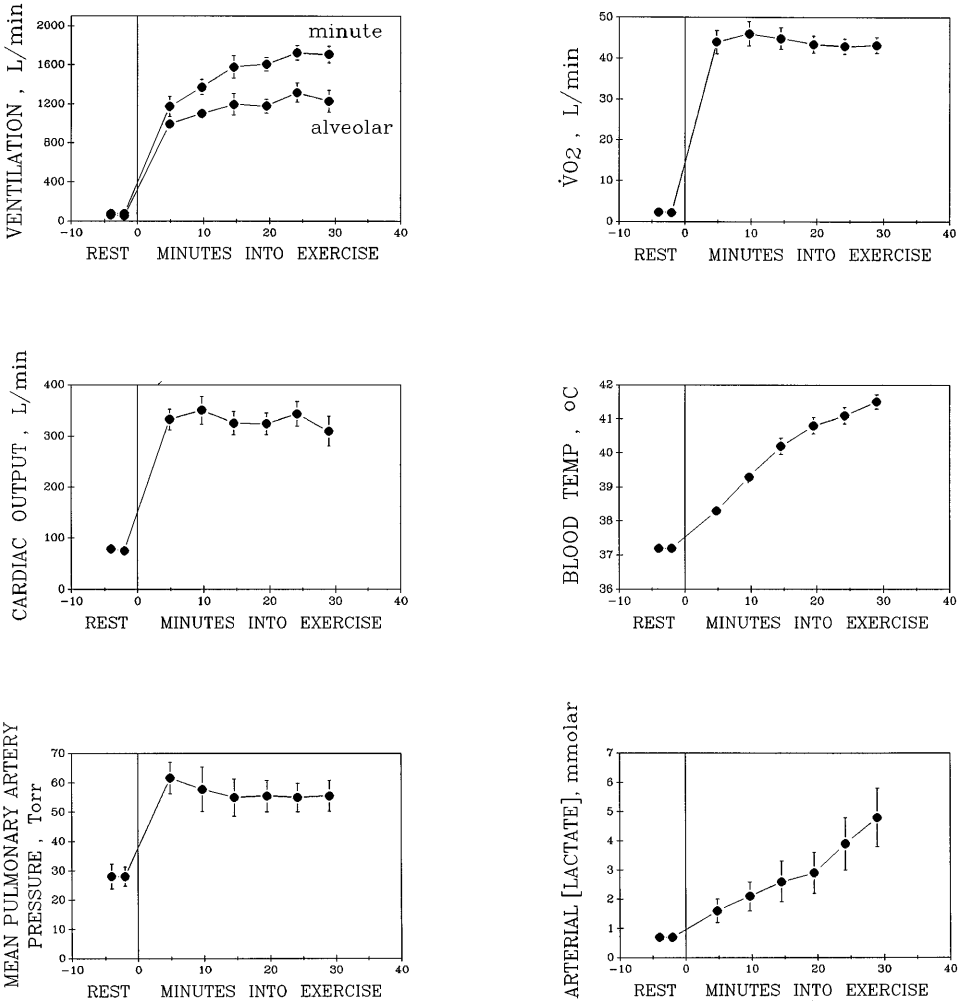

Fig. 1. Metabolic and hemodynamic data from horses at rest and during exhaustive submaximal exercise at 60% of maximal O_2 uptake ($\dot{V}O_2$). Values are means ± SE. Brackets indicate concentration.

mmHg at rest and increased rapidly within 5 min to 62 ± 5 mmHg. Pulmonary arterial pressure decreased an average of 7 ± 5 mmHg over the course of the exercise period, but this was not statistically significant ($P < 0.06$).

Pulmonary mechanics and ventilation. The ventilatory response of the animals consisted of a significant increase over the course of the exercise period in total ventilation ($P < 0.0001$) and alveolar ventilation ($P < 0.0001$), with a resultant fall in Pa_{CO_2} ($P < 0.0001$). This was accomplished by significant increases in respiratory frequency ($P < 0.0002$) and tidal volume ($P < 0.0001$). Breathing frequency increased with exercise duration, and the values for 15, 20, and 25 min were greater than those after 5 and 10 min (see Table 1). Toward the end of the exercise period, there was a trend toward a decline in breathing frequency, and the value after 25 min was less than at the exercising time points from 10 to 25 min. Horses that exercised longer than 30 min had lower breathing frequencies (97 ± 1 breaths/min at 35 min and 93 ± 1 breaths/min at 40 min) than those recorded for the same group as 20 and 25 min (109 ± 7 and 105 ± 6 breaths/min, respectively).

Total pulmonary resistance increased from rest to the first 5 min of exercise ($P < 0.05$) but did not increase further with increasing exercise duration. There were also progressive increases in maximum transpulmonary pressure ($P < 0.0001$) and peak inspiratory ($P < 0.0001$) and expiratory ($P < 0.0001$) airflow rates. The work of breathing increased significantly from rest to the first 5 min of exercise ($P < 0.0001$) and continued to increase progressively during the exercise test ($P < 0.0001$) as minute ventilation rose.

Pulmonary gas exchange (Fig. 2, Table 2). Pa_{O_2} averaged 84 Torr at rest and increased significantly ($P < 0.005$) with exercise, averaging 89 Torr throughout the exercise test. Note that the barometric pressure averaged 700 Torr; thus the expected resting alveolar P_{O_2} is 87 Torr.

Pa_{CO_2} fell between rest and the first 5 min of exercise ($P < 0.0005$) and continued to fall progressively as exercise continued ($P < 0.0001$). The (A-a)P_{O_2} decreased from 8 ± 3 to 4 ± 1 Torr between rest and the first 5 min of exercise ($P < 0.05$) but was not different from rest throughout the remainder of the exercise test and averaged 9 ± 3 Torr.

Inert-gas data for each of the experimental conditions are given in Table 2 and Fig. 2. Averaged over all the data sets, the mean RSS was 50, which is greater

Table 1. *Selected respiratory variables in horses at rest and after 5 and 28–30 min of exercise*

Variable	Rest	Exercise, min 5	Exercise, min 28–30
Respiratory frequency, breaths/min	12.5 ± 2.6	86.7 ± 6.1*	105.2 ± 4.0†
Tidal volume, liters	6.1 ± 0.4	12.4 ± 1.0*	14.5 ± 0.9†
Minute ventilation, l/min, BTPS	80.8 ± 17.0	1,162.8 ± 103.8*	1,685.0 ± 74.3†
Maximum transpulmonary pressure change, cmH_2O	7.8 ± 2.8	45.3 ± 1.9*	61.4 ± 1.8†
Peak inspiratory airflow, l/s	8.1 ± 0.8	64.6 ± 2.7*	78.0 ± 3.2†
Peak expiratory airflow, l/s	3.8 ± 0.9	55.8 ± 5.5*	71.5 ± 4.9†
Resistance, $cm \cdot s \cdot l^{-1}$	0.27 ± 0.04	0.35 ± 0.02*	0.38 ± 0.03
Work of breathing, kg·cm	11.6 ± 2.7	349.4 ± 37.2*	635.8 ± 64.3†

Values are means ± SE; $n = 6$ horses. *Significant change from rest, $P < 0.05$. †Significant change across exercise, $P < 0.05$.

than the 99th-percentile expectation of the sum of squares for $n = 6$ gases. The reason for this high sum of squares is not random error, because enflurane was in all cases overretained, contributing substantially to the RSS. When enflurane and sulfur hexafluoride were eliminated (these are the gases of the highest molecular weight and are therefore the most vulnerable to gas-phase diffusion limitation) and only four gases were used, averaged over all data sets the RSS was reduced to 15; only 11 of the 54 data sets exceeded the 99th-percentile expectation and over one-half were <5, corresponding roughly to the 50th percentile. The majority of the data sets with high RSS were at rest.

The error (ϵ) between the measured retention and best-fit retention for enflurane, the heaviest gas, and cyclopropane, one of the lightest gases, is expected to be positive with gas-phase diffusion limitation (incomplete intrapulmonary gas mixing, stratified inhomogeneity). Gas-phase diffusion limitation will cause enflurane, the gas of the highest molecular weight, to be preferentially overretained relative to cyclopropane. Because random errors for enflurane and cyclopropane may in theory be directional, such that their mean is not zero, we randomly perturbed a homogeneous data set by using a Monte Carlo approach and generated 100 new data sets. This allowed us to calculate ϵ on the basis of random experimental error. In this analysis, ϵ was positive 53% of the time and averaged 0.13 ± 1.15. This is, in fact, not significantly different from zero and indicated that random errors in these gases should have produced a zero mean error over all data sets. However, in our experimental data, ϵ was always positive and significantly greater than predicted by the Monte Carlo simulation at rest and during all exercising time points, a result showing that high-molecular-weight inert gases are not eliminated as efficiently as low-molecular-weight gases. This is compatible with gas-phase diffusion limitation in the lung (7). ϵ did not change systematically with exercise. Note that the molecular weight of enflurane is 185 compared with O_2 (molecular weight = 32) and CO_2 (molecular weight = 44), and thus the small amount of gas-phase diffusion limitation detected here will not affect the behavior of physiological gases.

The log $SD_{\dot{Q}}$ increased from rest to exercise, roughly doubling, but did not change systematically over the exercise period. Even with the increase associated with exercise, the log $SD_{\dot{Q}}$ was within normal limits for

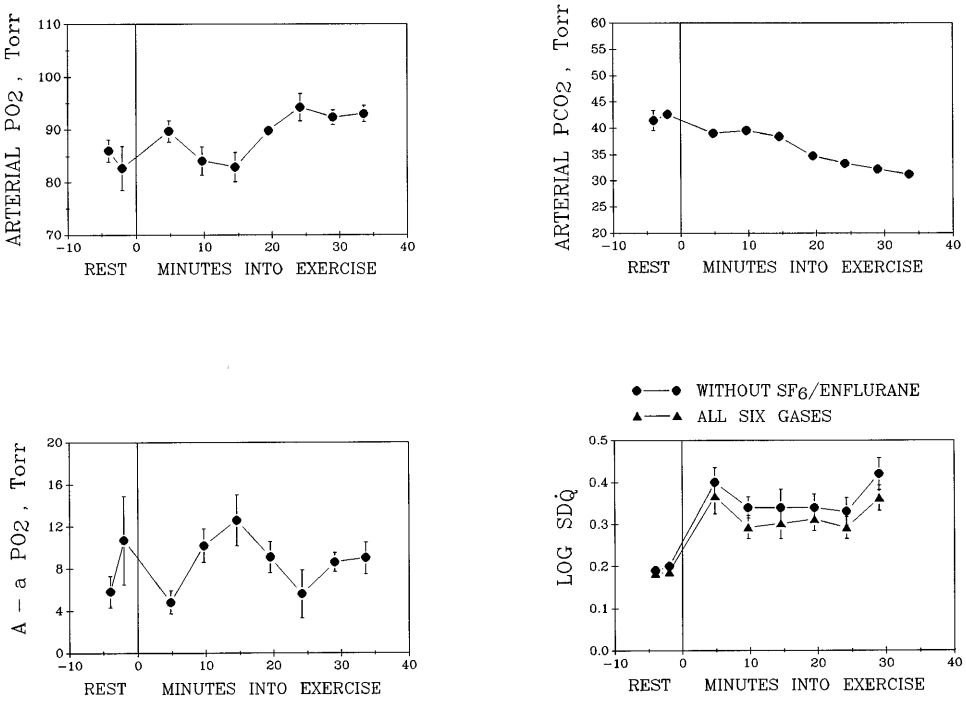

Fig. 2. Pulmonary gas exchange in horses at rest and during exhaustive submaximal exercise at 60% of $\dot{V}O_{2max}$. Values are means ± SE. (A-a)P_{O_2}, alveolar-arterial P_{O_2} gradient; SF_6, sulfur hexafluoride. There is a significant increase in log SD of perfusion distribution (log $SD_{\dot{Q}}$) from rest to exercise, indicating increased ventilation perfusion inequality. Log $SD_{\dot{Q}}$ does not increase with increasing exercise duration.

Table 2. *Principal cardiopulmonary variables in horses at rest and during exercise*

Variable	Rest	Exercise, min					
		5	10	15	20	25	28–30
$\dot{V}_{O_2}$, l/min	2.3 ± 0.3	44.0 ± 2.8*	46.0 ± 3.0	44.7 ± 2.6	43.4 ± 2.1	42.9 ± 1.9	43.2 ± 1.9
$\dot{Q}_T$, l/min	40 ± 2	332 ± 20*	350 ± 27	325 ± 22	324 ± 21	343 ± 24	309 ± 29
(A-a)P_{O_2}, Torr	8 ± 3	4 ± 1*	10 ± 2	12 ± 2	9 ± 2	5 ± 2	8 ± 1
(A-a)$P_{O_{2pred}}$, Torr	1 ± 1	6 ± 5	13 ± 2	8 ± 2	11 ± 2	9 ± 2	10 ± 2
Pa_{O_2}, Torr	84 ± 3	90 ± 2	84 ± 3	83 ± 3	90 ± 1	94 ± 3	93 ± 1
Pa_{CO_2}, Torr	42.3 ± 1.2	39.0 ± 0.9*	39.5 ± 0.6	38.4 ± 0.9	34.7 ± 1.2	33.3 ± 1.1	33.9 ± 1.5†
RSS (6 gases)	72 ± 11	6 ± 15	32 ± 5	47 ± 8	66 ± 16	48 ± 8	51 ± 8
RSS (4 gases)	62 ± 7	3 ± 1*	6 ± 2	21 ± 17	7 ± 3	7 ± 3	3 ± 1
Log $SD_{\dot{Q}}$	0.20 ± 0.01	0.40 ± 0.04*	0.34 ± 0.03	0.34 ± 0.04	0.34 ± 0.03	0.33 ± 0.03	0.42 ± 0.04
Log $SD_{\dot{V}}$	0.20 ± 0.01	0.39 ± 0.03*	0.33 ± 0.02	0.33 ± 0.04	0.34 ± 0.03	0.33 ± 0.03	0.41 ± 0.04

Values are means ± SE; n = 6 horses. $\dot{V}_{O_2}$, O_2 consumption; $\dot{Q}_T$, total cardiac output; (A-a)P_{O_2}, alveolar-arterial pressure difference for O_2; (A-a)$P_{O_{2pred}}$, (A-a)P_{O_2} predicted from inert gases; Pa_{O_2}, arterial partial pressure of O_2; Pa_{CO_2}, arterial partial pressure of CO_2; RSS, residual sum of squares; high RSS for 4 gases at 15-min time point was due to data from 1 animal; RSS without this animal was 4 ± 2; log $SD_{\dot{Q}}$, log SD of perfusion distribution; Log $SD_{\dot{V}}$, log SD of ventilation distribution. *Significant change from rest, $P < 0.05$. †Significant change across exercise, $P < 0.05$.

resting humans. The recovered distributions were in almost all cases unimodal, and there were no areas of low $\dot{V}_A/\dot{Q}$ ratio in any animal at any time point. Also, there were no areas of intrapulmonary shunting at rest or at any point during exercise.

The multiple inert gas elimination technique allows an analysis of alveolar-end-capillary diffusion limitation by computing the Pa_{O_2} and (A-a)P_{O_2} that would be expected from the recovered $\dot{V}_A/\dot{Q}$ distribution, assuming end-capillary diffusion equilibrium, and comparing it with measured values of Pa_{O_2} or (A-a)P_{O_2} (25). Because the inert gases are essentially invulnerable to alveolar-end-capillary diffusion limitation, when a measured Pa_{O_2} value is less than that predicted from the inert gas exchange [or the (A-a)P_{O_2} (2) is greater], this suggests alveolar-end-capillary diffusion limitation. There were no significant differences between the measured and predicted values for Pa_{O_2} at either rest or during exercise, indicating absence of alveolar-end-capillary diffusion limitation for O_2 at this exercise intensity throughout the protocol.

DISCUSSION

In contrast to the previous study (1), which found a decrease in Pa_{CO_2} while Pa_{O_2} was unchanged, suggesting a progressive impairment of pulmonary gas exchange, the results of this study confirm a mild increase in $\dot{V}_A/\dot{Q}$ inequality after 5 min of submaximal exercise in horses but do not demonstrate any further time-dependent changes with more prolonged exercise. Although $\dot{V}_A/\dot{Q}$ inequality is increased from rest to exercise, the (A-a)P_{O_2} was unchanged because the overall $\dot{V}_A/\dot{Q}$ ratio was shifted to the right with exercise. Unlike previous observations during short-term maximal exercise in horses (30), we found no evidence of alveolar-end-capillary diffusion limitation or inadequate alveolar ventilation. Rather, ventilation steadily rose and Pa_{CO_2} fell in concert with an increase in blood lactate concentrations. There was evidence to suggest gas-phase diffusion limitation for the high-molecular-weight inert gases.

Pulmonary mechanics. The effects of exercise on ventilatory mechanics are similar to those reported previously in submaximally exercising horses (1), and there was a gradual increase in ventilatory efforts over time, despite constant speed. It is noteworthy that large differences existed in breathing frequency despite the relatively small range in speeds at which the animals worked. This is likely because trotting horses have a much greater ability to regulate or vary their breathing frequency compared with the tight coupling of stride and breathing that occurs while galloping. The increase in ventilation over time may play an important thermoregulatory role and increase respiratory heat loss with increasing ventilation (1).

Total pulmonary resistance increased significantly over time. A previous study of horses exercising at a lower intensity (~40% of $\dot{V}_{O_{2max}}$) for a longer duration (up to 60 min) showed that total pulmonary resistance increased markedly in the latter part of the exercise test (1). A similar trend was beginning to develop in the present study at the time most of the horses ceased exercising. Although the reason for any increase in resistance is not clear, the increase could possibly reflect effects of increased turbulence in association with increased inspiratory flow rates. The impact of these increases in flow is not clear, although if disruption of laminar flow becomes great enough, it may possibly lead to greater disturbances in $\dot{V}_A/\dot{Q}$ inequality. If such changes occur, they may help explain the widening of the (A-a)P_{O_2} that had previously been reported in horses exercising for 1 h at a constant speed (1) but that was not seen in the present study.

$\dot{V}_A/\dot{Q}$ relationships. There was an increase in the log $SD_{\dot{Q}}$ between rest and the first 5 min of exercise but no further increase over the course of exercise. Despite this mild deterioration in gas exchange, there was no worsening of (A-a)P_{O_2}. This is because of the overall rightward shift of the mean $\dot{V}_A/\dot{Q}$ ratio of the lung (i.e., with exercise, the increase in ventilation is greater than the increase in blood flow) such that the effects of inequality on gas exchange are minimized.

The cause of increased $\dot{V}_A/\dot{Q}$ inequality with exercise is unknown. Possible mechanisms include heterogeneity of hypoxic pulmonary vasoconstriction (11), reduc-

tion of gas mixing in large airways (27), heterogeneity due to increased ventilation alone, or interstitial pulmonary edema (21). Interstitial pulmonary edema, resulting from rapid transcapillary fluid flux in excess of the lymphatic drainage capacity of the lung, is the most likely causative factor for the following reasons: *1*) the relationship of $\dot{V}_A/\dot{Q}$ inequality to hypoxia (5) and exaggeration in extreme hypobaric hypoxia (33); *2*) the improvement in $\dot{V}_A/\dot{Q}$ inequality with 100% O_2 breathing (6), which would be expected to reduce pulmonary arterial pressure and reduce driving pressure for fluid flux; and *3*) the lack of evidence for bronchoconstriction, despite moderately severe $\dot{V}_A/\dot{Q}$ inequality.

It would be expected that if interstitial pulmonary edema were the cause of the increased $\dot{V}_A/\dot{Q}$ inequality with exercise, prolonged exercise might exacerbate the $\dot{V}_A/\dot{Q}$ inequality by increasing the duration of the exposure of the pulmonary vascular bed to increased pulmonary arterial pressures. This could lead to increased filtration of fluid across the capillary endothelium in excess of the capacity of lymphatic drainage, resulting in interstitial pulmonary edema. Because we did not find increased $\dot{V}_A/\dot{Q}$ inequality after the first 5 min of exercise, we can only speculate as to the lack of an increase with the prolonged duration of exercise in this study. It is possible that increases in pulmonary arterial pressure and increased transcapillary fluid flux are not important causes of exercise-induced $\dot{V}_A/\dot{Q}$ inequality in this species. However, horses are remarkable for their very tight matching of ventilation and perfusion compared with humans, and possibly the majority of the pulmonary capillaries are not exposed to particularly high pressures. Although there is convincing evidence that in horses the dorsal caudal region of the lung develops bleeding and rupture of pulmonary capillaries, secondary to high exercising pulmonary transmural pressures (34), the exercise intensity of the present study was well below the levels of exercise at which such bleeding is known to occur. There was no clinical evidence of pulmonary bleeding in the present study.

As mentioned earlier, another possible cause of $\dot{V}_A/\dot{Q}$ inequality during exercise is heterogeneity due to increased ventilation per se because small resting variations in resistance among small airways may translate into significant time constant inequality for gas mixing within the lung during exercise. This possibility has not been investigated in the past. Although we did not examine this directly in the present study, there was a progressive increase in ventilation with increasing duration of exercise. The change in ventilation represented an ~50% increase between the end of the first 5 min of exercise and exhaustion, yet there was no accompanying increase in the log $SD_{\dot{Q}}$. Therefore, we feel that the results of this study provide evidence against this mechanism of increased $\dot{V}_A/\dot{Q}$ inequality with exercise.

Alveolar-end-capillary diffusion limitation. Inert gases have extremely rapid rates of equilibration between alveolar tissue and blood and are therefore less vulnerable to alveolar-end-capillary diffusion limitation than the physiological gases. Alveolar-end-capillary diffusion limitation is detected by the inert gases as a discrepancy between the measured Pa_{O_2} or $(A\text{-}a)P_{O_2}$ and the Pa_{O_2} value predicted from the inert gases by using the 50-compartment model (25). This gives a Pa_{O_2} or $(A\text{-}a)P_{O_2}$ that accounts for the effect of $\dot{V}_A/\dot{Q}$ inequality and intrapulmonary shunt. Any discrepancy between measured and predicted values is therefore due to pulmonary diffusion limitation (or extrapulmonary shunting, although this is likely very small particularly during exercise) (25). As discussed below, we did find gas-phase or molecular weight-dependent diffusion limitation for enflurane (molecular weight = 185). However, this would not likely affect O_2, which has a molecular weight of 32. Because gas-phase diffusion limitation may cause a slight distortion of the recovered distributions (in this case a reduction in the log $SD_{\dot{Q}}$ from 0.20 to 0.18), the predicted Pa_{O_2} and $(A\text{-}a)P_{O_2}$ values are derived from four-gas data and are therefore not influenced by the behavior of the high-molecular-weight gases.

We did not find evidence of alveolar-end-capillary diffusion limitation in horses during the prolonged submaximal exercise of the present study. Significant alveolar-end-capillary diffusion limitation in these animals occurs only during short-term exercise, at treadmill speeds >10 m/s on a 10% grade (30), representing a much greater exercise intensity (90% of $\dot{V}_{O_{2max}}$ compared with the 57% of $\dot{V}_{O_{2max}}$ that we studied). This is similar to the findings in human athletes exercising at sea level (6, 9), in which significant pulmonary diffusion limitation develops only at exercise intensities approaching $\dot{V}_{O_{2max}}$. From an evolutionary standpoint, it is not surprising that pulmonary diffusion limitation does not occur during moderate-intensity exercise. This would represent a failure of adaptation to a relatively commonplace situation, whereas exposure to maximal exercise would be relatively rare and of short duration.

The mean pulmonary arterial pressure fell by 7 mmHg between the first 5 min of exercise and exhaustion without a change in cardiac output. Pulmonary arterial pressure during exercise has been shown to be related to arterial hemoglobin concentration (29). Hematocrit fell significantly during the same interval, despite profuse sweating (balanced in part by ~70 ml/kg inert gas infusion), which would be expected to reduce plasma volume. Although it is unlikely that either the volume of 5% (5 ± 1 liters) dextrose infused or the volume of blood withdrawn (120 ml) was sufficient to reduce hemoglobin, it is possible that gut absorption of fluid may have contributed to the falling hemoglobin concentration and subsequently to a reduction in pulmonary arterial pressure. Splenic contraction in horses is mediated by action of norepinephrine on α-adrenergic receptors (14) and leads to a significant increase in both blood volume and hematocrit (14). During short-term maximal exercise, pulmonary arterial pressures reach a peak as the highest workload is reached and decrease as exercise duration increases (29). Therefore, in the present study it is also possible that excitement of the animal, and subsequent catecholamine release, led to an "overshoot" in both pulmonary arterial pressure and hematocrit, which abated as exercise progressed.

Gas-phase diffusion limitation. The RSS, the difference between the measured retention and the predicted retention for the $\dot{V}_A/\dot{Q}$ distribution is expected to be <5 for six gases 50% of the time, given random experimental error (17). The large RSS in the present study is unusual and has not been seen previously in prior work in horses (29, 30) or humans (15, 21, 25) but has been reported in pneumonectomized dogs (10). The high RSS can be a function of poor technique and/or faulty equipment, or it may be the failure of inert gas exchange to conform to the several assumptions of the multiple inert gas elimination technique. Experimental error as a cause is unlikely with this study for several reasons. The systematic nature of the errors (see RESULTS) argues against random technical problems, and the high RSS values were most marked only during rest and not during exercise. Also, we transported all the necessary equipment for the measurement of inert gases to the site of the study, and thus the experimental setup was identical to the one we have used before and after the study without such high values for the high RSS. The individuals making the inert gas measurements were the same as in previous studies.

In light of the high RSS of the data, it is important to address the issue of steady state during the study because this could, in theory, contribute to the high RSS. The ventilatory bias flow system requires high rates of infusion of the inert gas mixture to off set the dilution of the expired gases by the bias flow stream. Because we wanted to minimize the amount of infusion given to the animal and prevent any artificial pulmonary edema or other complications related to fluid overload, the inert gas infusion was run for 15 min before collection of the resting data and for 2 min before collection of each exercise sample. The time constant for attainment of pulmonary steady state is a function of the ratio of lung gas conductance ($\dot{V}_A + \lambda\dot{Q}_T$) to alveolar gas and tissue capacitance (FRC + Vti), where $\dot{V}_A$ is alveolar ventilation, λ is the blood-gas partition coefficient, $\dot{Q}_T$ is total blood flow, FRC is functional residual capacity, and Vti is lung tissue volume. By using blood flow and ventilation data from Fig. 1 and assuming an FRC of 22 liters (24) and Vti of ~6 liters, the time to 95% equilibrium is ~1 min at rest and ~4 s during exercise. Thus failure of equilibration is not likely to be a contributing factor to the overall high RSS. Also, the RSS was lower during exercise than at rest despite the longer infusion time at rest, which also argues against short infusion time as a cause of high RSS.

A contributing factor to the high RSS at rest is the small amount of $\dot{V}_A/\dot{Q}$ mismatch itself. As explained several years ago, a given amount of random experimental error produces a larger RSS when the lung is nearly homogeneous than when it is more heterogeneous (28). This does not explain the systematic nature of the error and cannot explain the very high RSS seen in the present study.

The most likely explanation for the high RSS in our study is gas-phase diffusion limitation (diffusion-dependent heterogeneity, otherwise called incomplete intraregional gas mixing). Gas-phase diffusion limitation for inert gases is suggested by high RSS that occurs as a result of nonrandom error. ϵ between the measured retention and best-fit retention for enflurane, the heaviest gas, and cyclopropane, the lightest gas, is expected to increase in the presence of gas-phase diffusion limitation because the retention of the heaviest gas will be increased in contrast to gases of low molecular weight (4). Such was the case in our study. The error between the retention of enflurane and cyclopropane was always positive and significantly greater than predicted by the Monte Carlo simulation at rest and during all exercising time points, suggesting gas-phase diffusion limitation in the lung. Note that this molecular weight-dependent behavior cannot be explained by diffusion of the gases across the blood-gas barrier because the rates of equilibration, even for high-molecular-weight gases, are sufficiently high that none should be diffusion limited. Also, because the rate of equilibration of O_2 exchange is much slower than that of the inert gases, diffusion limitation of O_2 would be expected, which was not the case.

Gas-phase diffusion limitation has not been previously reported in horses but has been observed in pneumonectomized dogs (10), anesthetized rats (26), resting varanid lizards (8), and anesthetized alligators (16). Bulk convective flow conveys fresh gas to regions of the gas-exchanging areas, and then molecular diffusion must provide the final transport of gas to and from the blood-gas barrier. The conducting airways act as a means of reducing the gas-phase diffusion distance. Several factors may predispose to the development of gas-phase diffusion limitation. First, low bulk convective flow associated with low respiratory rates, such as occurs in resting spontaneously breathing reptiles, may be an important determinant of the development of gas-phase diffusion limitation. Lung structures in which large diffusional distances are present, such as in the pneumonectomized dog, may also accentuate the problem. Mixing of gas may also be affected by collateral ventilation, which can provide a route for distribution of gas between parallel gas-exchanging units (13). In horses, the resistance to collateral gas transport is substantially higher than that reported for dogs (12) and may provide an additional contributory mechanism to the development of gas-phase diffusion limitation.

Although the RSS value was high, this is not likely to affect the results of our study because removing enflurane from the data sets greatly reduced the RSS but did not affect the physiological conclusions (see Fig. 2). When gas mixing is incomplete, axial gradients for resident gases will occur and will reduce the efficiency of gas exchange. Gas-phase diffusion limitation will distort the recovered $\dot{V}_A/\dot{Q}$ distributions and reduce log $SD_{\dot{Q}}$ in the main mode but may also apparently increase perfusion to areas of low and high $\dot{V}_A/\dot{Q}$ (7, 22). It should be noted that O_2 and CO_2, which have molecular weights almost an order of magnitude smaller than that of enflurane, are not likely be affected by a small,

albeit detectable, amount of gas-phase diffusion limitation (7).

In summary, we have shown a mild increase in $\dot{V}_A/\dot{Q}$ inequality with exercise in horses that does not worsen with increasing duration of exercise. There was a progressive increase in alveolar ventilation secondary to an increase in both respiratory frequency and tidal volume. Despite the small increase in $\dot{V}_A/\dot{Q}$ inequality, the $(A\text{-}a)P_{O_2}$ was unchanged, and pulmonary gas exchange was preserved. There was no evidence of pulmonary diffusion limitation during exercise. However, there were data suggestive of gas-phase diffusion limitation, both at rest and during exercise, in these animals.

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