

# Hemorheologic alterations induced by incremental treadmill exercise in Thoroughbreds

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## Summary

Hemorheologic alterations induced by incremental treadmill exercise were examined in 5 Thoroughbreds. Blood viscosity; PCV; RBC filterability, density gradient profile, and shape; serum and RBC electrolyte concentrations; and plasma total solids and lactate concentrations were measured before exercise, at treadmill speeds of 9 and 13 m/s, and 10 minutes after exercise. Exercise was associated with significant ( $P < 0.05$ ) increases in PCV, blood viscosity, and plasma total solids concentration. After adjustment of PCV to 40% by adding or removing each horse's own plasma, blood viscosity remained significantly greater in the sample obtained at 13 m/s, compared with that in samples taken at rest. Filterability of RBC was significantly decreased at 13 m/s, compared with values from other sampling times. During exercise, a significantly greater proportion of the RBC were less dense and were found in the upper layers of the RBC density gradient profile, compared with resting values. This change was associated with a significant increase in RBC mean cell volume. Rapid increases in serum sodium and potassium concentrations during exercise were accompanied by significant increases in RBC potassium and chloride concentrations. This study revealed a consistent pattern of hemorheologic alterations associated with exercise in Thoroughbreds, suggesting that multiple hemorheologic tests are needed to adequately define these complex alterations during exercise in horses.

increase in PCV has largely been attributed to catecholamine-induced splenic contraction, resulting in mobilization of splenic RBC into the circulating compartment.<sup>3,4</sup> The importance of altered blood flow properties in the pathogenesis of equine exercise-associated diseases, such as exercise-induced pulmonary hemorrhage and exertional myopathy, is unknown. Increased blood viscosity may contribute to exercise-induced pulmonary and systemic hypertension.<sup>5,6</sup> Further, chronic or repeated exposure to hyperviscosity may induce pathologic alterations in blood vessels.<sup>7,8</sup> In rabbits, high hydrostatic pressure in pulmonary capillaries has been reported to cause ultrastructural changes leading to edema and hemorrhage.<sup>9</sup>

Primary factors that determine flow properties of blood include PCV, shear rate, plasma viscosity, RBC aggregation, and RBC deformability.<sup>9-12</sup> In large vessels, bulk flow properties of blood predominate, and changes in viscosity are primarily determined by shear rate and PCV.<sup>13</sup> Methods for determining bulk flow properties, such as measurement of high-shear-rate blood viscosity, provide an estimate of flow properties in large vessels.<sup>13,14</sup> However, within the microvasculature, individual blood cells traverse capillaries that have an internal diameter smaller than the cell diameter. Resistance to flow in capillaries primarily depends on RBC deformability and aggregation.<sup>10,15</sup> Cell deformability is determined by cell shape, surface area-to-volume ratios, cytoplasmic viscosity, and biophysical properties of the cell membrane.<sup>10</sup> Methods for determining rheologic properties of individual blood cells, such as the ability of cells to pass through 3- to 5- $\mu\text{m}$ -diameter pores, ektacytometry, and micropipette aspiration, are relevant in assessing microvascular blood flow.<sup>10</sup>

The purpose of the study reported here was to investigate hemorheologic alterations induced by treadmill exercise in Thoroughbreds. To better understand blood flow changes in the microvasculature, as well as in large vessels, we selected tests for evaluation of bulk flow properties of blood (ie, blood viscosity) and tests for evaluation of RBC deformability (RBC filterability) and RBC density (RBC density gradient centrifugation).

## Materials and Methods

**Horses**—Five healthy Thoroughbreds (3 mares, 2 geldings), ranging in age from 3 to 5 years and weighing 405 to 485 kg (mean, 458 kg), were used

High-intensity running has been recognized to induce pronounced alterations in PCV and blood viscosity in horses. After racing in Thoroughbreds, RBC count and PCV increased by 50 to 60%, whereas blood viscosity increased two- to threefold.<sup>1,2</sup> The

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in this study. All horses had raced within the previous 12 months, and were without a history of exercise-induced pulmonary hemorrhage. The horses were dewormed and inoculated against eastern and western equine encephalomyelitis, tetanus, equine influenza, and equine herpesvirus infection. The horses were housed in box stalls with 2 to 3 hours of turn out per day. Diet consisted of a mixture of concentrate and grain, and grass hay. Water and salt were provided ad libitum. Prior to the study, all horses were trained on a high-speed treadmill<sup>a</sup> at a minimum of 4 times weekly for 8 weeks. Exercise training consisted of combinations of long slow distance and interval training, and a step test, so that all horses were able to complete the exercise protocol used in this study without difficulty.

**Experimental protocol**—A 14-gauge, 18-cm catheter<sup>b</sup> was placed aseptically in the left jugular vein and was secured in position by use of skin sutures. Blood samples were drawn for hematologic, biochemical, and rheologic tests at 5 time periods: 30 minutes and immediately before treadmill exercise, during the last 30 seconds of 2-minute increments at a treadmill speed of 9 and 13 m/s, and 10 minutes after completion of exercise. The catheter was flushed with approximately 15 ml of sterile 0.9% sodium chloride solution after collection of blood samples. Blood samples were placed in sterile evacuated clot tubes and in tubes containing tripotassium EDTA, lithium heparin, or sodium fluoride/potassium oxalate as anticoagulants. After thorough mixing, blood samples were placed on ice and were processed or frozen within 1 hour of collection.

Horses were exercised on the treadmill, with the incline fixed at 6°. The exercise protocol consisted of a 4-minute warm-up at a speed of 4 m/s; followed by 2 minutes each at speeds of 9, 11, and 13 m/s; and a 10-minute cool-down at a speed of 1.5 m/s. Treadmill speeds of 9, 11, and 13 m/s approximately correspond to 80, 90, and 100% of maximal oxygen consumption for trained Thoroughbreds.<sup>16</sup> Each horse completed 2 exercise tests, separated by a minimum of 5 days. Horses were weighed 30 minutes before and 10 minutes after completion of exercise. Heart rate was continuously monitored, using a heart rate meter.<sup>17,c</sup> Heart rates were recorded during the last 10 seconds of each increment of exercise.

**Analysis of PCV, and total plasma solids and plasma lactate concentration**—Packed cell volume was measured in duplicate by spinning EDTA-treated samples in a microhematocrit centrifuge (14,000 × g) for 5 minutes. Correction for trapped plasma was not made. Plasma total solids (TS) were estimated by refractometry.<sup>d</sup> Plasma TS were used, rather than adjusting the refractive index to estimate plasma protein concentration, because this adjustment requires assumptions about the relationship between the refractive index and the protein concentration of plasma

that have not been demonstrated to be valid in samples obtained during exercise. Plasma lactate concentration was determined after deproteinization of sodium fluoride/potassium oxalate-treated samples with 1M perchloric acid, using a commercial enzymatic spectrophotometric technique.<sup>18,e</sup>

**Serum and RBC electrolyte determinations**—Blood samples for determination of RBC potassium and chloride concentrations were collected in tubes with lithium heparin. Blood first was frozen and thawed 3 times to lyse RBC. Potassium and chloride concentrations in serum and blood then were measured in duplicate, using a flame photometer<sup>f</sup> and a chloride titrator,<sup>g</sup> respectively.

Blood and serum electrolyte concentrations were used to calculate RBC electrolyte concentrations, using the equation<sup>19</sup>:

$$[\text{electrolyte}]_{\text{RBC}} = \{ [\text{electrolyte}]_{\text{B}} - [\text{electrolyte}]_{\text{serum}} \times (1-h) \} \times h^{-1}$$

where  $[\text{electrolyte}]_{\text{RBC}}$  is the electrolyte concentration in the RBC;  $[\text{electrolyte}]_{\text{B}}$  is the measured blood electrolyte concentration;  $[\text{electrolyte}]_{\text{serum}}$  is the measured serum electrolyte concentration; and  $h$  is  $0.99 \times \text{PCV}$  of the sample, which corrects the PCV for trapped plasma. The RBC electrolyte concentrations were expressed per liter of RBC.

**Blood viscosity**—A cone-and-plate rotational viscosimeter<sup>h</sup> was used to determine blood viscosity for samples collected in tubes with EDTA. The viscosimeter was calibrated at 38 C, and all samples were assayed in triplicate. The viscosimeter allowed blood viscosity measurements at 5 shear rates of 11.25, 22.5, 45, 90, and 225 seconds<sup>-1</sup>. Viscosity was expressed in millipascal·seconds (mPa·s). Because PCV is a major determinant of blood viscosity, measurements were repeated on all samples after adjusting the PCV to 40% by adding or removing each horse's own plasma. In previous studies,<sup>1,20</sup> this method of viscosimetry provided repeatable blood viscosity values at various known PCV and shear rate in horses. However, blood viscosity measured at the lower shear rates (< 11.25 s<sup>-1</sup>) had marked instrument and horse-to-horse variation.<sup>20</sup> Other investigators have reported that the coefficient of variation of individual readings on different aliquots of the same sample varies from 2% at high shear rates (230 s<sup>-1</sup>) to > 15% at lower shear rates.<sup>21,22</sup> The minimal shear rate at which reliable readings can be obtained is probably 23 s<sup>-1</sup>, in which the coefficient of variation is approximately 8%.<sup>22</sup>

**Red blood cell filtration studies**—Seven-milliliter samples of EDTA-anticoagulated blood were centrifuged for 15 minutes at 800 × g. Plasma and buffy coat were removed and RBC were washed 3 times in

<sup>a</sup> Sato Inc, Uppsala, Sweden.

<sup>b</sup> Angiocath, Deseret Co, Sandy, Utah.

<sup>c</sup> Equisat, EQB Inc, Unionville, PA.

<sup>d</sup> T/S, American Optical Corp, Buffalo, NY.

<sup>e</sup> Lactate kit, Sigma Chemical Co, St. Louis, MO.

<sup>f</sup> Model 143 flame photometer, Instrumentation Laboratories Inc, Boston, Mass.

<sup>g</sup> Model 4-2008, Buchler-Cotlove, Fort Lee, NJ.

<sup>h</sup> Micro-Cone/Plate, Model LVT, Wells-Brookfield, Stoughton, Mass.

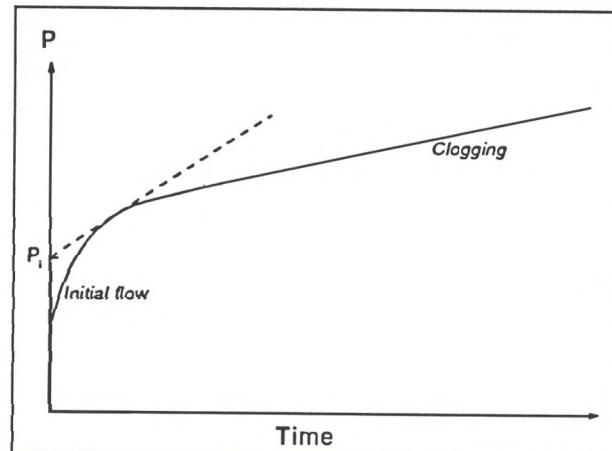


Figure 1—Schematic of pressure ( $P$ ) vs time for RBC filtration. Pressure curves were characterized by an initial sharp increase and a secondary, less rapid increase. The pressure at the initial plateau ( $P_i$ ) was determined. The initial sharp pressure increase reflects resistance of the cells to filtration, whereas the secondary pressure increase reflects plugging of pores in the filter.

phosphate-buffered saline solution (PBSS; pH 7.4; osmolality, 290 mOsm/kg of  $H_2O$ ) containing bovine serum albumin (BSA, 0.5 g/100 ml) and glucose (0.1 g/100 ml). The buffer solution was filtered before use, to remove contaminating particles. The RBC were resuspended in PBSS-BSA to a PCV of 10%. Temperature of the suspending fluid was 37 C. The RBC suspensions contained < 100 WBC/ $\mu$ l. Red blood cell filtration was performed as described.<sup>23</sup> In brief, the cell suspensions were placed in a 60-ml plastic syringe and were pumped<sup>i</sup> at a constant flow of 1.1 ml/min through 13- $\mu$ m polycarbonate filters<sup>j</sup> with mean pore size of  $2.7 \pm 0.3 \mu$ m and mean pore density of  $20.1 \pm 0.38 \times 10^3/mm^2$ . All filters were from the same lot and were used only once. The filtration pressure during constant flow was measured on the pump side of the filter with a pressure transducer<sup>k</sup> connected to a polygraph recorder.<sup>l</sup> A pressure curve was generated that was characterized by an initial sharp increase in pressure, which plateaued within the first 2 seconds, and a secondary, less-rapid, pressure increase (Fig 1). The pressure at the initial plateau ( $P_i$ ) was enumerated for each sample. The  $P_i$  has previously been shown to reflect the resistance of cells to filtration, whereas the secondary pressure increase reflects plugging of the pores in the filter.<sup>24</sup> All samples were analyzed in triplicate, and mean values were calculated. The coefficient of variation for repeated determinations of  $P_i$  was  $5.3 \pm 1.3\%$ .

**Density separation of RBC—Arabinogalactan<sup>m</sup>** solution was prepared as previously described.<sup>25</sup> The HEPES buffer solution ( $pK_a^1 = 7.5$ ), glucose, and sodium chloride were added to the stock solution to bring the final concentration of HEPES solution to 10 mM, and of glucose to 1 mg/ml, and the osmolality

<sup>i</sup> Model 975, Harvard Apparatus Co, Millis, Mass.

<sup>j</sup> Nucleopore Corp, Pleasanton, Calif.

<sup>k</sup> Model MP45-14, Validyne Engineering Corp, Northridge, Calif.

<sup>l</sup> Model 385, Linear Instruments Corp, Costa Mesa, Calif.

<sup>m</sup> Larex-LO, Consulting Associates Inc, Tacoma, Wash.

Table 1—Packed cell volume and plasma total solids (TS) and lactate concentrations for 5 Thoroughbreds before, during (9 and 13 m/s), and 10 minutes after treadmill exercise

Variable	Before exercise		Exercise			Recovery
	Rest (30 min before exercise)	Immediately before exercise	9 m/s	13 m/s		
PCV (%)	40 $\pm 0.5^a$	41 $\pm 0.4^a$	55.5 $\pm 0.6^b$	59 $\pm 0.7^c$	51 $\pm 0.7^d$	
TS (g/dl)	6.5 $\pm 0.1^a$	6.5 $\pm 0.1^a$	7.2 $\pm 0.1^b$	7.8 $\pm 0.1^c$	6.8 $\pm 0.1^b$	
Lactate (mmol/L)	0.68 $\pm 0.1^a$	0.74 $\pm 0.1^a$	4.0 $\pm 0.3^b$	17.9 $\pm 1.1^c$	16.6 $\pm 2.0^c$	

Values are mean  $\pm$  SEM. Means with different superscripts are significantly ( $P < 0.05$ ) different.

to 290 mOsm/kg of  $H_2O$ . Eight solutions with densities of 1.149, 1.1385, 1.130, 1.120, 1.108, 1.095, 1.082, and 1.073 were prepared from this stock and were designated layers 8 through 1, respectively. Osmolality was 290 mOsm/kg of  $H_2O$  and pH was 7.4 for each layer. To prepare the density gradient, 0.5 ml of stock solution was placed in the bottom of a 13-ml polycarbonate tube,<sup>n</sup> followed by 0.5 ml of layer 8, 2 ml each of layers 7 through 4, and 1 ml each of layers 3 through 1.

Blood samples were adjusted to a PCV of 30% with autologous plasma, and 1 ml of blood was layered on top of the gradient. The tubes were spun in a refrigerated centrifuge at  $20,000 \times g$  for 45 minutes at 4 C. After centrifugation, the density gradients were photographed. Each of the RBC layers was removed and washed in Dulbecco's phosphate-buffered saline solution containing 2.5 g of BSA/L (290 mOsm/kg). Then, each of the RBC fractions was fixed at 37 C in glutaraldehyde by sequential addition of 0.1% glutaraldehyde and 3.0% glutaraldehyde in White's saline solution.<sup>1,26</sup> After fixation, 1 aliquot of each cell layer was prepared for determination of mean cell volume (MCV) and RBC count, using an automated electronic cell counter.<sup>o</sup> Glutaraldehyde-fixed RBC were used for determination of MCV to prevent changes in cell volume associated with addition of cell-counting diluent. However, hardened RBC may displace a larger fluid volume, compared with that of deformable RBC, and therefore, values for MCV may be falsely increased by fixation.<sup>21</sup> The number of RBC in each layer was determined from the RBC count and the volume of the layer. The relative number of RBC in each fraction also was quantified by scanning densitometry<sup>p</sup> of the photographs.

Wet mounts of glutaraldehyde-fixed RBC from each layer were examined by light microscopy, and 300 cells were classified as normal discocytes, echinocytes, stomatocytes, or others.<sup>1,27</sup> Type-I echinocytes were defined as cells with irregular or angular edges, but lacking distinct spicules; type-II echinocytes were flat cells with multiple, regularly spaced, blunt spicules; and type-III echinocytes were small, round cells with multiple, regularly spaced, sharp spicules. Echinocyte numbers in the blood samples

<sup>n</sup> Ultra-Clear centrifuge tubes, Beckman Instruments Inc, Palo Alto, Calif.

<sup>o</sup> Model ZBI, Coulter Electronics Inc, Hialeah, Fla.

<sup>p</sup> CS-9000, Shimadzu Corp, Kyoto, Japan.

were not determined, and the effects of density gradient centrifugation on RBC shape were not defined.

**Analysis of data**—Data from the exercise tests were analyzed by ANOVA for repeated measures. Least-significant difference was used for pair-wise comparison of means.<sup>28</sup> Significance was reported at  $P < 0.05$ . Data are presented as mean  $\pm$  SEM.

## Results

All horses were able to complete the exercise tests without difficulty. Heart rate increased from  $40 \pm 2$  beats/min at rest to  $185 \pm 3$ ,  $205 \pm 4$ , and  $222 \pm 4$  beats/min at 9, 11, and 13 m/s, respectively. Mean values for body weight before and after exer-

cise were  $452 \pm 6$  and  $443 \pm 6$  kg, respectively. Fecal loss during exercise contributed to the observed decrease in body weight.

**Determination of PCV, and total plasma solids and plasma lactate concentration**—Values for PCV and plasma TS concentration increased with treadmill speed (Table 1). Peak values for PCV and plasma TS concentration were  $47.5 \pm 2.5$  and  $20.7 \pm 1.2\%$  greater, respectively, compared with preexercise values. Plasma lactate concentration (Table 1) increased from  $0.74 \pm 0.1$  mmol/L immediately before exercise to a peak of  $17.9 \pm 1.1$  mmol/L at 13 m/s.

**Blood viscosity**—Values for blood viscosity were significantly greater at all shear rates during and after exercise (Fig 2). The increase was greater at lower shear rates. Peak values were detected at the maximal exercise intensity (13 m/s); at shear rates of 11.25, 22.5, 45, 90, and  $225 \text{ s}^{-1}$ , the increase in viscosity

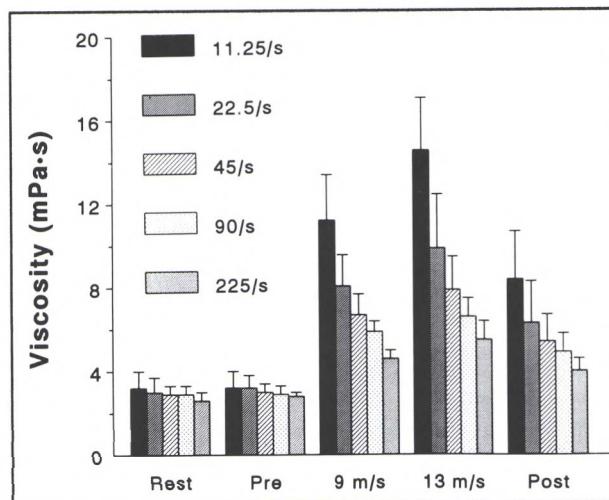


Figure 2—Blood viscosity at various shear rates for 5 Thoroughbreds 30 minutes (Rest) and immediately before (Pre) exercise, at 9 and 13 m/s increments of treadmill speed, and 10 minutes after exercise (Post). Values for blood viscosity at all shear rates during and after exercise were significantly ( $P < 0.001$ ) greater, compared with resting values. Values are mean  $\pm$  SEM.

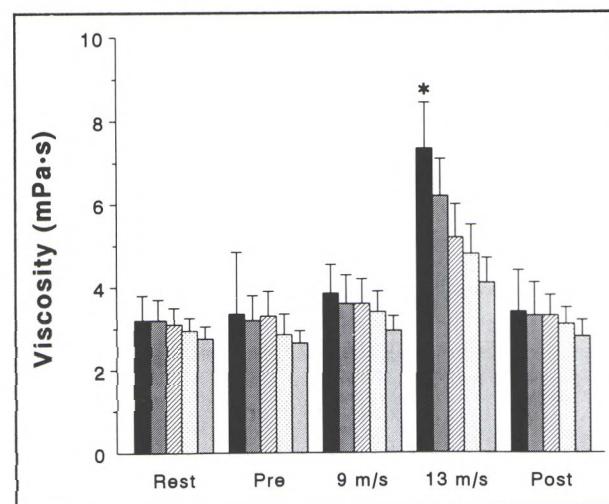


Figure 3—Blood viscosity at various shear rates after adjustment of PCV to 40% for 5 Thoroughbreds. Asterisk indicates significant ( $P < 0.05$ ) difference at all shear rates, compared with values at all other sampling periods. See Figure 2 for key. Values are mean  $\pm$  SEM.

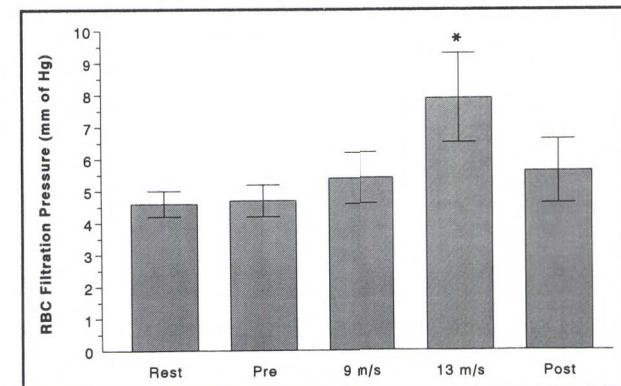


Figure 4—Effects of treadmill exercise in 5 Thoroughbreds on RBC filtration through 13- $\mu\text{m}$  polycarbonate filters, with a mean pore size of  $2.7 \pm 0.3 \mu\text{m}$ . Asterisk indicates significant ( $P < 0.001$ ) difference, compared with other values. See Figure 2 for key. Values are mean  $\pm$  SEM.

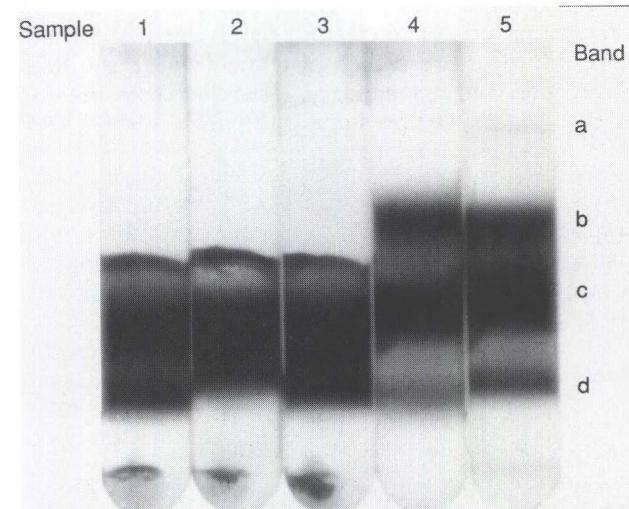


Figure 5—Example of density gradient RBC separation 30 minutes (1) and immediately before (2) exercise, at 9 (3) and 13 m/s (4) increments of treadmill speed, and 10 minutes after exercise (5). Notice the increased percentage of RBC in band b during exercise at 13 m/s, and the higher position of bands b and c (sample 4).

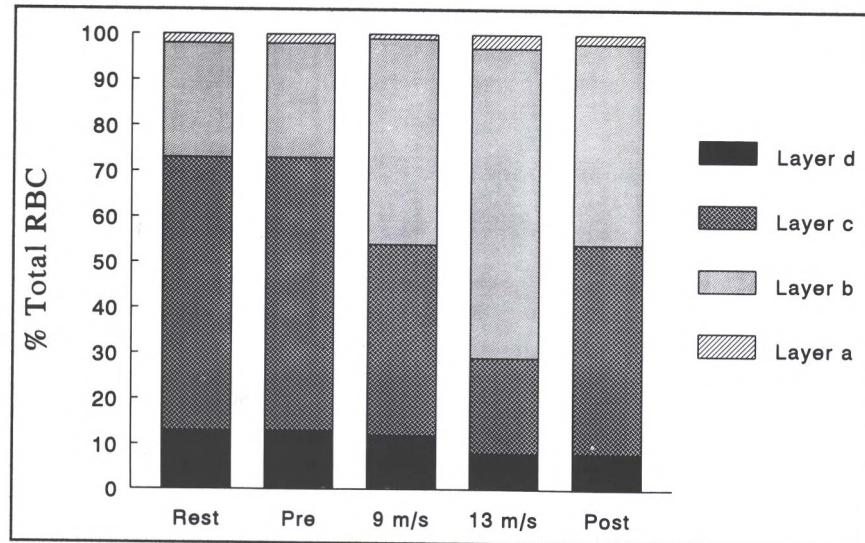


Figure 6—Effects of treadmill exercise on RBC density gradient profile in 5 Thoroughbreds. During and after exercise, there was a significant ( $P < 0.05$ ) increase in the size of layer b (less dense RBC), with a corresponding decrease in the size of layer c. See Figure 2 for key.

was 258, 231, 139, 122, and 104%, respectively, compared with preexercise values. After adjustment of PCV to 40%, values for blood viscosity at all shear rates were significantly greater, compared with preexercise values, only at 13 m/s (Fig 3).

**Red blood cell filtration measurements**—Transient decrease in RBC filterability was observed during exercise. A significant ( $P < 0.001$ ) increase in the  $P_i$  of the RBC filtration curve was noticed at 13 m/s (Fig 4). Red blood cell filtration pressure returned to resting values within 10 minutes after exercise.

**Red blood cell density separation**—Density separation of RBC resulted in formation of 4 distinct layers in most samples (Fig 5). In all samples, RBC separated by size, with the largest cells on top and smallest cells at the bottom. Layers were designated a through d, in which a was the top layer, b and c the middle layers, and d the bottom layer. Electronic cell counter determination of RBC count in each layer of the density gradient was significantly correlated ( $r = 0.96$ ) with scanning densitometry determination of the percentage of total RBC in each band. In resting samples, mean values for layers b and c were 25 and 60% of total RBC, respectively. During and after exercise, there was a significant increase in the size of layer b (less dense RBC), with a corresponding decrease in the size of layer c (Fig 6). At 13 m/s, 68% of total RBC were found in layer b.

Mean values for MCV in RBC layers a, b, and d were significantly increased at 13 m/s, compared with preexercise values (Table 2). Echinocytes were < 1% of total RBC in resting samples and their numbers did not increase significantly during or after exercise. All of the echinocytes were type-I cells.

**Serum and RBC electrolyte determinations**—Serum sodium and potassium concentrations increased significantly during exercise (Fig 7). Mean serum sodium concentration increased to  $145.7 \pm 1.2$  mmol/L at 13 m/s, returning to resting values 10

Table 2—Mean cell volumes (fl) from each layer of RBC density gradient profiles for 5 Thoroughbreds before, during (9 and 13 m/s), and 10 minutes after treadmill exercise

Layer	Before exercise			Exercise		
	(30 min before exercise)	Immediately before exercise	Exercise		Recovery	
			9 m/s	13 m/s		
a	45 ± 1	45 ± 1	45 ± 3	47 ± 1*	46 ± 1	
b	42 ± 1	42 ± 1	44 ± 1	46 ± 1*	44 ± 1	
c	40 ± 1	40 ± 1	41 ± 1	42 ± 2	42 ± 2	
d	33 ± 2	33 ± 2	35 ± 2	38 ± 2*	35 ± 1*	

\*Significantly ( $P < 0.05$ ) different from resting values.  
 Values are mean ± SEM.

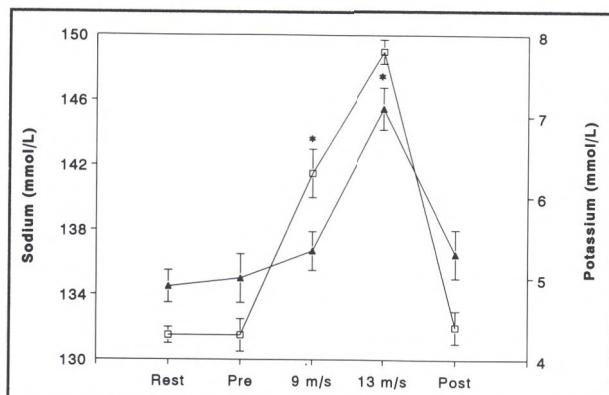


Figure 7—Effects of treadmill exercise on serum sodium (▲) and potassium (■) concentrations for 5 Thoroughbreds. Asterisk indicates significant ( $P < 0.01$ ) difference, compared with other values. See Figure 2 for key. Values are mean ± SEM.

minutes after exercise. Mean serum potassium concentration increased to  $6.2 \pm 0.1$  and  $7.9 \pm 0.2$  mmol/L at 9 and 13 m/s, respectively, returning to resting values 10 minutes after exercise. Serum chloride concentrations did not change. Mean values for serum chloride were  $98 \pm 2$ ,  $98 \pm 2.6$ , and  $97.5 \pm 1.9$  mmol/L before exercise, at 13 m/s, and at 10 minutes of recovery, respectively.

Exercise also induced significant changes in RBC electrolyte concentrations (Fig 8). Mean RBC potas-

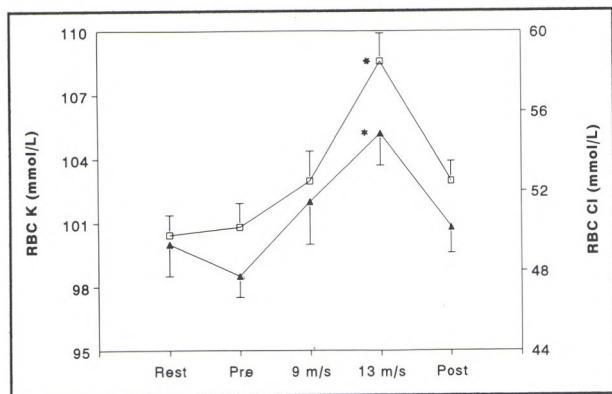


Figure 8—Effects of treadmill exercise on RBC potassium (RBC K, ▲) and chloride (RBC Cl, □) concentrations for 5 Thoroughbreds. Asterisk indicates significant ( $P < 0.05$ ) difference, compared with other values. See Figure 2 for key. Values are mean  $\pm$  SEM.

sium concentration increased from  $98.5 \pm 2.2$  mmol/L immediately before exercise to  $105.5 \pm 2.3$  mmol/L at 13 m/s. Mean RBC chloride concentration increased from  $49.9 \pm 1.9$  mmol/L at rest to  $58.4 \pm 1.9$  mmol/L at 13 m/s.

## Discussion

In this study, we evaluated alterations in blood flow properties associated with incremental treadmill exercise in Thoroughbreds. Our data were indicative of a consistent and repeatable pattern of changes, which included increased PCV, blood viscosity, RBC size, and RBC potassium and chloride concentrations, and decreased RBC filterability and density. The increase in PCV was largely attributable to splenic contraction, which results in a dramatic increase in the number of circulating RBC.<sup>4</sup> In addition, a decrease in plasma volume during exercise may have contributed to the observed increase in PCV.<sup>29</sup> The 21% increase in plasma TS concentration provided evidence for a decrease in plasma volume.

**Blood viscosity**—Our blood viscosity results were generally similar to those obtained in other studies in which the rotational cone-and-plate viscosimeter was used.<sup>1,20</sup> However, values for blood viscosity at low shear rates ( $11.25$  and  $23\text{ s}^{-1}$ ) in samples obtained before exercise were lower than would be predicted by the blood viscosity-shear rate relationship. Blood normally behaves as a strongly non-Newtonian fluid, in that apparent viscosity increases with decreasing shear rate.<sup>13,14</sup> In a previous study,<sup>20</sup> the cone-and-plate viscosimeter also lacked precision at low shear rates. This lack of precision at low shear rates may be attributable to aggregation and sedimentation of RBC in the viscosimeter cup (which would decrease blood viscosity readings) and by formation of a rigid film on the surface of the spindle (which would increase the viscosity reading). To minimize the effect of aggregation and sedimentation in our study, blood was subjected to high shear rates ( $450\text{ s}^{-1}$ ) for 2 minutes before determining blood viscosity at the lower shear rates.

Our data were consistent with those of previous studies that indicated an increase in blood viscosity associated with running in horses.<sup>1,2</sup> The increase in blood viscosity was greatest at low shear rates and was mainly attributable to the 47% increase in PCV. However, after adjustment of the PCV to 40%, blood viscosity values at a treadmill speed of 13 m/s were still significantly greater, compared with preexercise values, suggesting that factors other than RBC number contributed to the increase in viscosity. Other factors potentially contributing to the increase in viscosity included decreased RBC deformability, increased RBC aggregation, and increased plasma viscosity. The observed increase in plasma TS concentration may have contributed directly and indirectly, by increasing RBC aggregation, to the increase in viscosity.<sup>2,13,14</sup>

**Red blood cell filtration studies**—Deformability of RBC is a major determinant of resistance to blood flow in the microcirculation.<sup>10</sup> Deformability of individual RBC is determined by cell geometry (surface area-to-cell volume ratio), membrane viscoelasticity, and cytoplasmic viscosity.<sup>10,15,30</sup> Filterability of RBC depends mostly on the surface area-to-volume ratio of the cell, and is less influenced by other RBC characteristics.<sup>24</sup> In this study, RBC deformability was reduced during exercise, as evidenced by decreased RBC filterability through polycarbonate filters with a mean pore size of 3  $\mu\text{m}$ . Filterability of RBC returned to resting values within 10 minutes after exercise. The decreased RBC filterability was associated with increases in RBC size and RBC potassium and chloride concentrations, suggesting that the decreased RBC filterability was caused by increased uptake of potassium and chloride associated with cell swelling. In RBC, swelling reduces the surface area-to-volume ratio, which would reduce RBC filterability.<sup>10,15,30</sup> The relationship between cell surface area and volume determines the extent to which a cell can deform; an excess of surface area over that needed to enclose the cell volume is needed for deformation. An increase in cell volume, as observed in this study, will restrict the ability of the cell to pass through a narrow aperture.<sup>24,30</sup>

In a previous study in horses,<sup>31</sup> change in RBC deformability was not found with exercise. This difference between our study and the previous study may be attributable to the technique used to assess RBC deformability. Whereas RBC filterability depends primarily on cell geometry (surface area-to-cell volume ratio), ektacytometry (the method used by Smith et al<sup>31</sup>) depends more on the viscoelastic properties of the RBC membrane.<sup>32</sup>

Another explanation for decreased RBC filterability during exercise could be the release of less deformable RBC from the spleen.<sup>33</sup> The acidotic and hypoxic environment in the spleen has been hypothesized to induce echinocyte formation, and echinocytes have been found in the blood of horses after exercise.<sup>33,34</sup> Our data were not supportive of this hypothesis. Echinocyte numbers did not increase during or after exercise in our horses, and RBC associated with decreased filterability in this study were

large and had decreased density. Echinocytes and senescent RBC that are sequestered in the spleen are thought to be small, dense cells.<sup>33,34</sup>

**Red blood cell density**—In this study, RBC became transiently larger and less dense during exercise. Density of RBC primarily depends on hemoglobin concentration.<sup>35,36</sup> Decreased RBC density associated with increased RBC size was suggestive of water transiently moving into RBC during exercise, thus decreasing the hemoglobin concentration. The increase in RBC potassium and chloride concentrations was suggestive of water moving into RBC, secondary to electrolyte fluxes. In a recent study,<sup>1</sup> a decrease in MCV and an increase in mean cell hemoglobin concentration was observed during exercise, implying an increase in number of dense RBC. This difference between our study and the previous study may be attributable to the timing of sample collection. In the previous report, blood samples were collected 10 to 20 minutes after exercise. Thus, the increase in MCV would have been missed. In addition, determination of RBC indices by electronic cell counter in the previous study may have lead to spurious results. Addition of diluting fluid before determination of cell counts and indices adjusts cell volume, relative to the osmotic pressure of the diluent. To avoid this problem, we rapidly fixed RBC in glutaraldehyde before determination of MCV. This is a standard method for preservation of the shape and integrity of blood cells<sup>1,26</sup> and has proven to be reliable in our laboratory.

**Serum and RBC electrolytes**—Increases in serum sodium and potassium concentrations associated with exercise were consistent with those of previous studies.<sup>37-39</sup> Alterations in serum sodium concentration were not nearly as marked as those for potassium. If the decrease in plasma volume (on the basis of the increase in plasma TS) is corrected, circulating sodium actually has been lost. Some of this loss likely could have been attributed to the loss of sodium ions in sweat, in which the concentration is approximately 140 to 150 mmol/L.<sup>40</sup> The increase in serum potassium concentration during exercise is proportionally more than for other electrolytes, chiefly reflecting an efflux of potassium from working skeletal muscles.<sup>38,41,42</sup> Increase in serum potassium concentration has been implicated as a mechanism responsible for muscular fatigue during exercise.<sup>42,43</sup>

Because total RBC volume represents > 50% of the blood volume, total RBC volume constitutes a space where large amounts of potassium may be accumulated, without a large increase in serum potassium concentration. Our study revealed that potassium is sequestered in the RBC, which may help to maintain the extracellular- to-intracellular potassium ratio; this ratio has been implicated as a factor contributing to fatigue during exercise.<sup>42</sup> In other studies in which changes in RBC potassium concentration were examined in horses during exercise, no change<sup>37</sup> or a slight decrease in potassium concentration<sup>39</sup> was detected. This difference between our study and previous studies may be partly attributable to the timing

of the sample. Freestone et al<sup>37,39</sup> obtained samples after completion of exercise, by which time RBC potassium concentrations may have returned to preexercise values.

An increase in RBC chloride concentration during exercise was observed in this study. This finding is similar to that reported in human beings and may be explained by chloride-bicarbonate exchange (chloride shift), which serves to reduce plasma acidosis.<sup>41,44</sup> Results of this study and previous studies in human beings<sup>41,44</sup> suggested that RBC mechanisms are capable of inducing rapid ion flux during exercise that may maintain ion, acid/base, and volume homeostasis.

In this study, a consistent pattern of hemorheologic alterations were associated with exercise in Thoroughbreds, including increased PCV, blood viscosity, RBC size, and RBC potassium and chloride concentrations, and decreased RBC deformability and density. These data suggested that a single hemorheologic test cannot adequately define the complex hemorheologic alterations that develop during exercise in Thoroughbreds.

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