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Effects of Training and Time of Day of Blood Sampling on the Variation of Some Common Haematological Parameters in Normal Thoroughbred Racehorses

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Summary

The effect of 20 weeks of training on various haematological parameters was determined in 32 healthy, previously untrained, two-year-old Thoroughbreds. Haemoglobin, erythrocyte count, packed cell volume and total bilirubin increased significantly after training, but serum folate, erythrocyte folate and serum vitamin B_{12} values were significantly lower. Total and differential leucocyte counts, platelets, plasma viscosity and fibrinogen remained constant. The fall in serum folate levels during training was confirmed in eight other healthy Thoroughbreds bled at regular intervals during a two-year training period.

Blood samples for routine haematological parameters were collected from 15 Thoroughbreds in training, aged 2–4 years, at 08.30 h and 16.00 h on a day of no exercise and again seven days later when strenuous exercise was undertaken at 09.00 h. On the day of no exercise, results obtained at 08.30 h agreed well with those recorded at 16.00 h except for the absolute lymphocyte count which was significantly higher in the afternoon. The results on the day of exercise showed that total leucocyte counts and absolute neutrophil counts were significantly higher at 16.00 h than at 08.30 h, suggesting that a time lapse of about seven hours is insufficient for these parameters to return to resting values.

Introduction

There are relatively few reports establishing the haematological response to training in the Thoroughbred racehorse (Jeffcott 1977). Most investigations have concentrated on comparing erythrocyte and occasionally leucocyte parameters before and after various training programmes. The evidence tends to suggest that erythrocyte counts increase following intensive training, particularly in those racehorses with low values when training commenced (Kitchen *et al.*, 1965; Clarkson 1968; Stewart *et al.*, 1970; Allen 1978; Catling 1978). Rose and Hodgson (1982), however, found erythrocyte counts to be fairly stable in horses subjected to endurance training and concluded that the type and intensity of training may be an important factor in stimulating increased erythrocyte production.

A prolonged demand for increased erythropoiesis, as seen during training, may alter normal production of other cell types and also result in consumption of essential nutrients. It was of interest, therefore, to assess the haematological status of two-year-old Thoroughbreds before and after training for racing on the flat. Fifteen parameters including erythrocytes, leucocytes, platelets, proteins and vitamins were measured in 32 previously untrained two-year-old horses approximately two weeks before training commenced and again 20 weeks later.

In addition to assessing the changes which occurred in the blood due to training, the influence of time of venepuncture on the variations in some haematological values was investigated. It is usual practice to bleed Thoroughbreds in training either in the mornings before exercise or in the late afternoon several hours after exercise. For this reason, fifteen racehorses were bled at 08.30 h and 16.00 h on the day of no exercise and again seven days later when they were subjected to strenuous exercise at 09.00 h. The variations in the haematological parameters are presented in this paper.

Materials and Methods

The following groups of Thoroughbreds in training were used for this study:

1. Thirty-two healthy two-year-old Thoroughbreds (18 colts and 14 fillies) from three racing stables in Newmarket were bled in March (approximately two weeks before training commenced) and again in August after 20 weeks of training in preparation for racing on the flat. The training was along 'traditional lines' based upon daily submaximal work (walking and cantering) with maximal exercise (galloping) once or twice a week when the horses were nearly fit enough to race. All horses raced at least once during the period under study.
2. Eight other healthy two-year-old Thoroughbreds were bled several times before the commencement of training and again at regular intervals during training, over a total period of about 24 months.
3. Fifteen Thoroughbreds, aged 2–4 years, which had been in training for 12 weeks were bled at 08.30 h and 16.00 h on day 1 and again on day 7. On day 1 no exercise was allowed during the period under study. On day 7 all horses were subjected to strenuous exercise (a canter for 500 metres followed by a gallop for 1000 metres) at 09.00 h. Food and water were allowed *ad libitum*.

Blood was obtained by jugular venepuncture and dispensed into potassium EDTA (1.5 mg/ml) for haematology into sterile universal containers for vitamins and bilirubin, and into 3.8% trisodium citrate for fibrinogen. Unless otherwise stated, all samples were collected by the same individual before the horses were exercised at 08.30 h. Animals which became excited when handled were not used for this investigation.

Haemoglobin (Hb), erythrocytes (RBC) and packed cell volume (PCV) were estimated using standardized semi-automated methods (Allen and Archer, 1973). Total leucocytes, neutrophils, lymphocytes and eosinophils were counted using electronic volume analysis by the Coulter Channelyzer system (Allen 1981). Monocyte counts were obtained from spinner slides (Wenk 1976). Platelets were counted using the isopycnic centrifugation technique (Archer *et al.*, 1978). Serum and red cell folate were measured using *Lactobacillus casei* by the methods described by Allen (1978), and vitamin B_{12} was measured by using *Lactobacillus leichmannii* (Spray 1955). Total bilirubin was measured by continuous flow analysis (Wahlefeld *et al.*, 1972), plasma viscosity by the Harkness capillary viscometer, and fibrinogen by the clot weight method (Ingram 1961).

The results after training were compared with those before training, using the paired Student's t-test. The same test was used to assess the degree of difference between samples collected at 08.30 h (X_1) and 16.00 h (X_2) when the animals were sedentary, and also at 08.30 h (X_3) and 16.00 h (X_4) when the animals had been exercised. The time effect was obtained from X_1 versus X_2 and the exercise effect from (X_4–X_3) versus (X_2–X_1).

Quality assurance checks were made by using commercially available standards and own-made preparations, and by regular participation in inter-laboratory quality control comparability trials. In addition, blood counts were controlled using erythrocyte indices (Bull and Elashoff, 1974) from two-year-old and three-year-old Thoroughbreds in training. The means of the erythrocyte indices, mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV), calculated from batch sizes of approximately 20 horses, were plotted day by day on control charts. These results must fall within 5% of the target means for the laboratory to be in control. The target means are the values for MCH, MCHC and MCV which have been previously determined on several hundred samples from two-year-old and three-year-old Thoroughbred horses.

Results

As there was no sex difference, all results for the 32 two-year-olds have been considered together, and these are presented in Table 1 (erythrocytes, leucocytes and platelets) and Table 2 (fibrinogen, protein, folate, vitamin B_{12} and bilirubin).

TABLE 1. Results of statistical analysis of erythrocyte, leucocyte and platelet values in 32 two-year-old Thoroughbreds before and after 20 weeks of training.

Investigation	Training	Mean	SD	Significance*
Hb (g/l)	Before	136.0	9.8	$p < 0.001$
	After	152.0	16.8	
RBC ($\times 10^{12}$/l)	Before	9.22	0.80	$p < 0.001$
	After	10.23	1.23	
PCV (l/l)	Before	0.37	0.02	$p < 0.001$
	After	0.41	0.04	
WBC ($\times 10^9$/l)	Before	9.84	1.30	NS
	After	9.65	1.11	
Neutrophils ($\times 10^9$/l)	Before	5.02	0.68	NS
	After	4.78	0.81	
Lymphocytes ($\times 10^9$/l)	Before	4.05	0.79	NS
	After	3.93	0.60	
Monocytes ($\times 10^9$/l)	Before	0.547	0.157	NS
	After	0.594	0.227	
Eosinophils ($\times 10^9$/l)	Before	0.220	0.105	NS
	After	0.207	0.078	
Platelets ($\times 10^9$/l)	Before	112.0	17.3	NS
	After	111.0	14.1	

* Paired t-test. NS not significant. SD standard deviation. WBC white blood cells.

TABLE 2. Results of statistical analysis of some metabolites in 32 two-year-old Thoroughbreds before and after 20 weeks of training.

Investigation	Training	Mean	SD	Significance*
Fibrinogen (g/l)	Before	2.96	0.50	NS
	After	2.97	0.38	
Plasma viscosity (c/P)	Before	1.50	0.06	NS
	After	1.51	0.06	
Serum folate (μg/l)	Before	4.50	1.67	$p < 0.005$
	After	3.60	0.93	
Erythrocyte folate (μg/l)	Before	78.0	22.0	$p < 0.0005$
	After	64.0	17.0	
Vitamin B_{12} (ng/l)	Before	2398.0	198.0	$p < 0.005$
	After	1840.0	538.0	
Total bilirubin (mmol/l)	Before	31.5	11.1	$p < 0.0001$
	After	40.4	11.9	

* Paired t-test. NS not significant. SD standard deviation.

TABLE 3. Changes in haematological values in 15 Thoroughbreds sampled at 08.30 and 16.00 h on day of no exercise (day 1) and on day of exercise† (day 7).

Parameter	08.30 h	16.00 h	Condition
Hb (g/l)	144.0 ± 13.2	147.0 ± 9.1	No Exercise
	144.0 ± 11.4	153.0 ± 14.8	Exercise
RBC ($\times 10^{12}$/l)	9.15 ± 1.14	9.33 ± 0.87	No Exercise
	9.27 ± 0.94	9.81 ± 1.13	Exercise
PCV (l/l)	0.39 ± 0.03	0.40 ± 0.02	No Exercise
	0.39 ± 0.03	0.42 ± 0.04	Exercise
WBC ($\times 10^{9}$/l)	8.4 ± 1.42	8.8 ± 1.50	No Exercise
	8.3 ± 1.61	11.4 ± 2.33**	Exercise
Neutrophils ($\times 10^{9}$/l)	4.1 ± 0.95	4.2 ± 0.82	No Exercise
	4.1 ± 0.94	6.5 ± 1.32**	Exercise
Lymphocytes ($\times 10^{9}$/l)	3.6 ± 1.11	3.9 ± 1.20*	No Exercise
	3.4 ± 1.23	4.0 ± 1.22	Exercise
Monocytes ($\times 10^{9}$/l)	0.61 ± 0.24	0.56 ± 0.20	No Exercise
	0.59 ± 0.24	0.68 ± 0.18	Exercise
Eosinophils ($\times 10^{9}$/l)	0.13 ± 0.03	0.15 ± 0.03	No Exercise
	0.11 ± 0.04	0.11 ± 0.03	Exercise

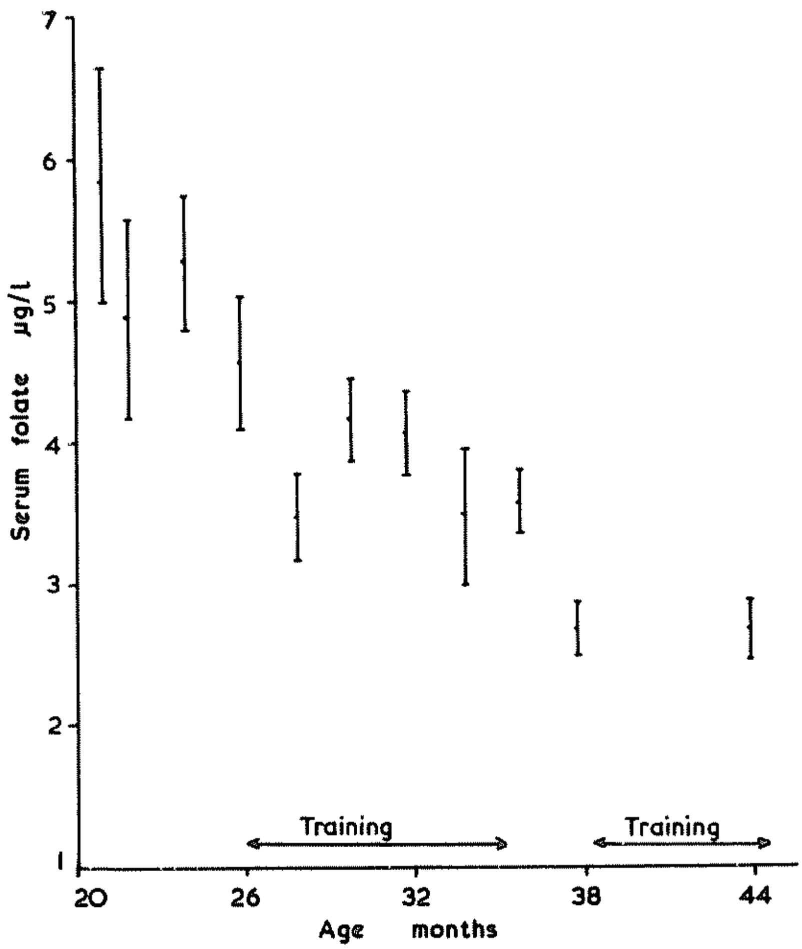
† Exercise consisted of 500 metres canter and 1000 metres gallop at 09.00 h.
* Significant ($p < 0.002$).
** Significant ($p < 0.0005$).

There were significant increases in Hb ($p<0.001$), RBC ($p<0.001$), PCV ($p<0.001$) and total bilirubin ($p<0.0001$) following training. There were also significant decreases in serum folate ($p<0.005$), erythrocyte folate ($p<0.0005$) and serum vitamin B_{12} ($p<0.005$). Total and differential leucocyte counts, platelets, plasma viscosity and fibrinogen remained constant.

Fig. 1 shows the fall in mean serum folate levels in eight Thoroughbred racehorses during a two-year training programme. The highest serum folate levels (mean 5.1 μg/l) were found before the horses were trained, i.e. when they were two-year-olds, and the lowest (mean 2.6 μg/l) after approximately 14 months of training over two racing seasons.

Table 3 presents the haematological results obtained at 08.30 h and 16.00 h on the day of no exercise (day 1) as well as results obtained when strenuous exercise was undertaken

FIGURE 1. Changes in mean serum folate values ± SD (n = 8) over a two-year training programme for racing on the flat.



at 09.00 h (day 7). Lymphocyte counts were significantly higher ($p<0.002$) at 16.00 h than at 08.30 h on the day of no exercise. On day 7 total leucocyte counts and absolute neutrophil counts were significantly higher ($p<0.005$) at 16.00 h than at 08.30 h. Erythrocyte parameters tended to be slightly higher at 16.00 h than at 08.30 h on day 7, but these changes were not significant.

Discussion

The results of the first part of this study show significant changes in several haematological parameters, following 20 weeks of regular non-standardized physical training in 32 previously untrained two-year-old Thoroughbreds.

After 20 weeks of training there was a significant increase in mean Hb, RBC and PCV, which both confirms earlier work by several authors (Kitchen *et al.*, 1965; Clarkson 1968; Catling 1978) and suggests that these horses had shown a normal response to prolonged regular exercise. The mean increase in haemoglobin of 17 g/l agrees well with the 28 g/l increase found by Stewart *et al.* (1970). Since blood volume measurements were not undertaken in this study, it was not possible to assess whether the increase in haemoglobin values reflects a true increase in total body haemoglobin as shown by Persson (1967). In contrast to the increase in erythrocytes, total and differential leucocyte counts remained remarkably constant. Rose and Hodgson (1982), working with endurance horses, also found that the total and differential leucocyte count remained fairly stable during training. Although platelet counts in this study were found to be low when compared with non-Thoroughbreds (Schalm *et al.*, 1975), they remained unchanged by training.

The significant increase in total bilirubin observed after training was probably due to decreased caloric intake rather than to an abnormal rate of erythrocyte destruction. In man, severe prolonged exercise produced marked increases in serum bilirubin values caused by a combination of haemolysis and decreased caloric intake. (Lindemann *et al.*, 1978). In the horse, haptoglobins remained constant during training (Allen 1978), suggesting no increase in erythrocyte turnover. Fasting, on the other hand, is known to cause considerable increases in plasma bilirubin concentrations (Gronwall and Mia, 1975).

Plasma viscosity and fibrinogen levels were unaffected by training. It is interesting to note, however, that plasma viscosity values in the Thoroughbred are lower than in other breeds of horses and all other animals so far studied (Archer and Allen, 1970). This may be an important factor to consider when assessing the relationship between blood viscosity, exercise tolerance and fitness.

The fall in serum and erythrocyte folate levels together with a fall in serum vitamin B_{12} levels is consistent with an increased demand for the vitamins during a prolonged training programme. Since folate levels are low and vitamin B_{12} levels are high in the Thoroughbred compared with other species, supplementation of the diet with folic acid rather than with B_{12} should improve the horse's haematological status. Furthermore, it has been shown in this investigation that the mean serum folate level gradually fell in a group of eight Thoroughbred racehorses during a two-year training period. This would tend to suggest that older horses, rather than previously untrained younger animals, may be more prone to folic acid deficiency states. Chanarin *et al.* (1969) have suggested that there is a developing economy in the use of folates when body stores reach low levels. In the Thoroughbred there is the possibility that normal body functions can still be maintained on very low levels of this vitamin.

The second part of this study investigated the within-day changes in several haematological parameters in a group of healthy Thoroughbreds in training. Venepunctures were undertaken at 08.30 h and 16.00 h on two separate days, one when the horses were subjected to strenuous exercise at 09.00 h and the other when they were not. On the exercise day total leucocyte and absolute neutrophil counts were significantly higher at 16.00 h than at 08.30 h, suggesting that a time lapse of approximately seven hours is insufficient for these parameters to return to pre-exercise levels. These findings have previously been reported in horses subjected to maximal exercise and would tend to be indicative of a stress response (Carlson 1975; Catling 1978; Snow *et al.*, 1982). Conversely, on the day of no exercise leucocyte values obtained at 08.30 h agreed well with those at 16.00 h, with the exception of the absolute lymphocyte count which was significantly higher at 16.00 h. Erythrocyte parameters remained fairly constant when the horses were sedentary, but on the exercise day values were higher at 16.00 h than 08.30 h, presumably as a result of prior splenic contraction or a decreased plasma volume. There is thus strong evidence to suggest that blood samples should not be collected in the afternoon following strenuous exercise in the early morning. In addition, because of the diurnal variation of the lymphocyte count there is a need to standardize the time of venepuncture, especially for studies involving daily blood counts.

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Exercise-Induced Alterations in Haemostasis in Thoroughbred Horses

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Summary

The effects of exercise on blood coagulation, platelet function and fibrinolysis were studied in five fit Thoroughbred horses which were galloped at maximal speed for 1200 m. Blood samples were collected four hours before exercise (resting sample), after saddling the horse and walking him to the track (track sample) and five minutes after exercise (post-exercise sample). Activated partial thromboplastin times, one-stage prothrombin times and thrombin times did not significantly differ in any of the samples, nor were fibrinogen/fibrin degradation products detected at any stage. The slopes of the ADP-induced platelet aggregation curves were reduced, but not significantly, when results from track samples were compared with resting sample findings. Exercise induced a significant reduction in the slopes of all ADP-induced aggregation curves. It was hypothesized that this response was an indirect effect of increased plasma catecholamine levels, possibly mediated via the release of increased amounts of prostacyclin from vascular endothelium.

Introduction

Normal haemostasis depends on a balanced interplay between the vessel wall and the factors responsible for blood coagulation, platelet function and fibrinolysis, which together comprise the haemostatic system. Considerable study has been made of the effects of physical exercise on blood coagulation and fibrinolysis in people, especially in relation to myocardial and vascular disease. In humans, exercise results in a shortening of clotting time (Ikkala *et al.*, 1963), an increase in platelet number and aggregation (Poller *et al.*, 1971; Warlow and Ogston, 1974), and an increase in fibrinolytic activity (Egeberg 1963). The extent of these changes varies with the length and intensity of the exercise and the physical condition of the subject. The effects of exercise on platelet adhesiveness are more variable, being either unchanged or reduced, depending on the exercise (Bennett 1972).

Little is known of the effects of exercise on the haemostatic system in horses. The effects of exercise on haemostasis are of particular interest in this species because of the