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HAEMATOLOGY

Physiological Factors Affecting Resting Haematology

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Introduction

Blood sampling for haematological examination is frequently carried out by many veterinarians, especially those involved with racing animals, both for assisting in the diagnosis of clinical or subclinical disease and for the possible assessment of fitness. It is its use in this last category that has given rise to considerable controversy, with some veterinarians believing in the usefulness of haemograms and leucograms, whilst many tests are performed because of pressures from trainers and owners. Unfortunately, there is little documentation on the usefulness of routine resting haematological investigation in the assessment of performance. In describing normal haemograms for Thoroughbreds, Stewart and Steel (1974) and Stewart *et al.* (1977) claim that subdivisions within the normal range may be useful in assessing fitness for optimal performance. These workers suggest that a haematocrit greater than one standard deviation below the population mean indicates a horse unlikely to perform well. On the other hand, Laufenstein-Duffy (1971) concluded that wide variations in resting haematocrit occurred in individual horses even when blood was collected under standardized conditions, and that performance was not related to differences in resting haematocrit. Persson (1969, 1975) also found that the reproducibility of haemoglobin concentration at routine sampling at rest was low. The problems of relying on measurement of resting red blood cell numbers or haematocrit when assessing fitness or work capacity have been well documented in a number of studies by Persson. Persson considers that the determination of total blood volume gives a more meaningful result (Persson 1983).

In addition to placing emphasis on resting red blood cell values, trainers now possibly lay even more reliance on an ideal white blood cell count, especially on the neutrophil/lymphocyte ratio. It would appear that at a ratio 1.5:1 (60% neutrophils, 40% lymphocytes) or thereabouts is considered ideal and that many horses are not raced, even in the absence of clinical problems, if the desired ratio is not seen on haematological examination. Unfortunately, examination of the literature reveals no scientific evaluation of the concept of such ratios and of their relationship to fitness. Furthermore, it has recently been shown that there is an alteration in the neutrophil/lymphocyte ratio with age, as the number of circulating lymphocytes decreases in the older, mature horse (Allen *et al.*, 1983).

One of the reasons for the unreliability of examining resting haemograms and leucograms is the number of physiological factors that can readily cause alterations in the

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resting values and thus contribute to the large normal daily fluctuations that may occur. It is the purpose of this review to consider some of these physiological factors and some drugs that may also cause these alterations.

Haemograms (red blood cell count, haematocrit, haemoglobin concentration)

In the horse, as in many other species except man, the spleen acts as a large reservoir for red blood cells (Persson *et al.*, 1973). The state of this organ therefore plays an important rôle in determining the number of circulating erythrocytes in the resting animal. The spleen has a smooth muscle capsule which is innervated by the sympathetic nervous system, and therefore any factor likely to alter sympathetic activity or plasma adrenaline concentrations will affect the number of circulating erythrocytes and the haematocrit. There are a number of factors that can alter sympathetic activity to the spleen.

Psychological factors. Excitement or apprehension in the horse due to awareness of being bled, or the presence of a stranger in the stable will cause increased sympathetic activity. Stewart *et al.* (1977) found that only visible excitement caused an elevation in haematocrit, whilst timidity and apprehension on blood sampling produced no alteration if samples were collected within 30 seconds of entering the stable.

Exercise. It is well known (Archer and Clabby, 1965) that any sort of exercise will cause an alteration in haematocrit, mainly due to the release of erythrocytes from the spleen. The number of cells released is not an 'all or none' phenomenon, but rather it is related to the extent of increase in sympathetic activity which is related to the workload. Following exercise, it can take one to two hours before the haematocrit returns to a baseline value. Irvine (1958) considered that it took several days after an exercise bout before true basal conditions were recorded.

Transport. The collection of blood samples shortly after a horse has been transported should not be undertaken, as both excitement and possible fluid shifts will cause alterations in the normal resting profile.

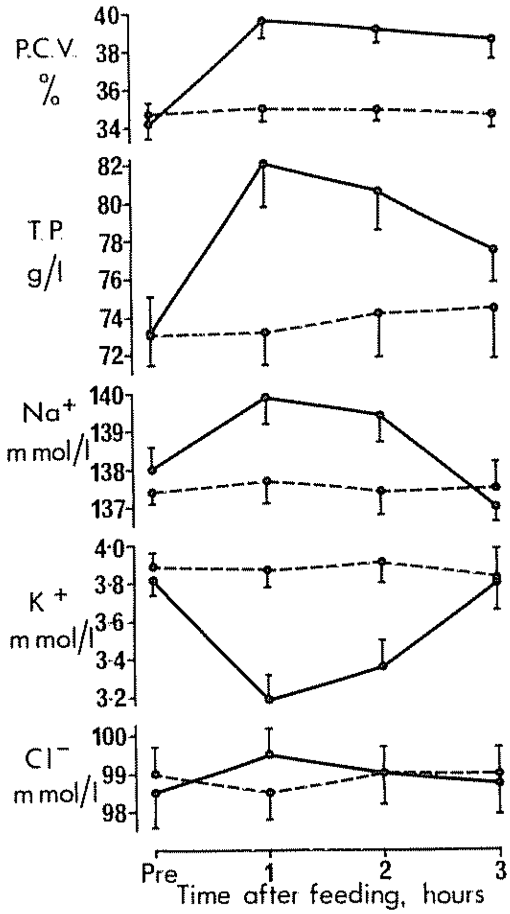
Fluid shifts. Alterations in plasma volume will also markedly affect the resting haemogram. Changes in fluid balance can be brought about by numerous factors including disease, a hot environment, exercise and feeding. Recently Kerr and Snow (1982) found that the feeding of hay, but not concentrates, will cause elevations in haematocrit. As shown in Fig. 1, return to normal values took a considerable time.

Tranquillizers. It is well documented that tranquillizers belonging to the phenothiazine (e.g. acepromazine) and butyrophenone (e.g. azaperone) groups cause a decrease in the normal resting haematocrit (Dalton 1972; Jeffcott 1974; MacKenzie and Snow, 1977). This effect is largely due to the α-adrenoreceptor antagonistic activity of the compounds, which leads to blocking of sympathetic activity on the splenic capsule and hence to relaxation of the smooth muscle, while their hypotensive effect leads to an increase in plasma volume.

Leucograms (white blood cell count and differential)

It is not commonly realized that an increase in sympathetic activity can also result in an increase in the number of circulating white blood cells. This occurs because in the intravascular system, leucocytes are found in both circulating and marginal pools, the latter representing cells sequestered in capillary beds and possibly the spleen. These pools

FIGURE 1. Effects of feeding hay on resting haematocrit, total plasma protein, sodium, potassium and chloride concentrations (from Kerr and Snow, 1982).



are in dynamic equilibrium so that factors which result in an opening-up of the capillaries or in the contraction of the spleen cause mobilization of cells from these marginal pools. Neutrophils enter the circulation mainly from previously shut capillaries and the spleen, whilst the lymphocytes are delivered from the spleen and possibly by increased lymph flow.

Exercise. In man, exercise results in increased input of leucocytes to the circulation, with a marked increase in white blood cell count (Garrey and Butler, 1929). This has been referred to as a physiological leucocytosis or pseudoleucocytosis, as formation of new leucocytes has not occurred. However, in the horse, leucocytosis does not result immediately following the initiation of exercise or adrenaline administration. This is because the mobilization of leucocytes is masked by the concomitant increase in red blood cells and hence the circulating volume. Although no or little change in total leucocyte number/litre occurs, alteration in the neutrophil/lymphocyte ratio can occur. Both Rossdale *et al.* (1982) and Snow *et al.* (1983) have found that immediately following a training gallop and following racing (Snow *et al.*, 1983) the neutrophil/lymphocyte ratio is decreased as relatively more lymphocytes are released into the circulation. Following maximal exercise, it then takes at least six hours before a return to a normal resting neutrophil/lymphocyte ratio occurs. In some cases it has been found that an increase in neutrophil number may occur during this post-exercise period, probably in response to the cortisol released during exercise.

The alterations described above are typical of short-term maximal exercise. At lower workloads lasting for longer periods, however, the changes in the leucogram are different in that a marked leucocytosis occurs which may persist for up to 48 hours (Rose 1982; Snow *et al.*, 1982). The leucocytosis is due to a neutrophilia, probably occurring in response to the high concentrations of plasma cortisol seen during this type of exercise. Cortisol causes both a reduction in margination and migration of neutrophils from capillaries and an increased input from the bone marrow. At the same time as the increase in circulating neutrophils occurs, there is also a decrease in the number of lymphocytes and eosinophils.

Transport. Transporting of horses also results in alterations in the leucogram, with the extent of the change being affected by the duration of travelling. With transport lasting up to five hours, Abbott (personal communication) found a marked leucocytosis due to a neutrophilia. This was related to the elevated plasma cortisol concentrations seen during the period of transportation.

Drugs. Drugs may also lead to alterations in the leucogram. The tranquillizer acepromazine has been found to cause a reduction in circulating leucocytes within 30 minutes of administration (Lumsden *et al.*, 1975). As well as endogenously released corticosteroids altering the white blood cell picture, profound changes can occur following exogenous administration. A resultant leucocytosis reflected in a neutrophilia has been well documented (Straub and Gerber, 1975). Lymphopaenia and eosinopaenia also occur with corticosteroid administration. In fact, before assays for measuring plasma concentrations of various synthetic corticosteroids were readily available, the duration of eosinopaenia was used to determine the duration of pharmacological activity. Zinkl and Carlson (1982) suggest that following the administration of a potent glucocorticoid, e.g. dexamethasone, the leucocyte count may increase up to 20 000/l for 12 to 24 hours, whilst long-term steroid therapy with less active compounds, e.g. prednisolone, has few effects on total or differential count. Rossdale *et al.* (1982) found that following intramuscular injection of ACTH, a change in neutrophil/lymphocyte ratio occurred four hours later due to an increase in neutrophil numbers and a decrease in lymphocyte number. Basal values were reattained by 24 hours after injection.

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The Significance of Haematological Data in the Evaluation of Soundness and Fitness in the Horse

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The fact that the total red-cell volume (CV) is a limiting factor for aerobic capacity and performance potential generates a perpetual interest in haematological values in the performance horse. This interest rests on the assumption that these data truly reflect the total red-cell content of the body. This is not valid, however, in the horse, as the function of the equine spleen causes a great variability of the haematological parameters at rest (Persson 1969). In the horse and in several other animal species, the spleen acts as a reservoir of erythrocytes by harbouring a large amount of highly concentrated blood at rest, and this blood store can be mobilized into active circulation when an increased demand for oxygen transport capacity arises. This is brought about by an adrenergically induced contraction of the abundant smooth muscle of the splenic capsule and trabeculae, following e.g. asphyxia, haemorrhage, excitation and exercise (Barcroft 1925; Scheunert and Krzywanek, 1926; Scheunert and Müller, 1926; Persson 1962, 1967). Consequently, exercise as well as excitation causes an increase of the circulating red-cell volume at an essentially unchanged plasma volume, resulting in rising values of the venous haematocrit (VH), haemoglobin concentration (Hb) and red blood cell count (RBC).

Because of the uneven distribution of the erythrocytes in the circulatory system due to the splenic storing, haematological values in the venous blood poorly reflect the size of the total red-cell volume at rest. Furthermore, varying degree of excitation at sampling induces unpredictable changes of these parameters, thus impairing their reproducibility (Persson 1969). This variability is related to the storage capacity of the spleen which, in turn, is dependent on the size of the red cell volume (Persson *et al.*, 1973). A way of eliminating this curtailment of the diagnostic value of haematological data in the horse would obviously be to do the blood sampling after mobilization of the splenic reservoir. This is readily achieved by exercise or intravenous adrenaline injection. The work or adrenaline dose must then be large enough to ensure maximal contraction of the spleen. In this way both reproducibility and diagnostic value of the erythrocyte parameters are greatly improved (Persson 1967, 1969).

Physical training induces adaptations to increased metabolic demands in several respects. One limiting factor for fitness and endurance is the oxygen transport capacity of the blood. This is improved by an increase in the total mass of red cells, and a relationship between state of training and CV is well established both in man (Holmgren *et al.*, 1960) and in the horse (Persson 1962, 1967, 1968). It is also conclusively documented that a