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TRAINING AND BLOOD CHEMISTRY
I. Resting

The Biochemistry and Haematology of Inherent Performance

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Summary

Between 1974 and 1981 blood samples were analyzed from horses at varying intervals of time for over 28 components. Many horses exhibited marked individuality, and individual normal values for many blood components were established. When these data were computerized and animals assessed as having sub-clinical disease were removed from the survey, a study was undertaken to examine whether the remaining results which would reflect the inherent biochemistry and haematology of the horse were related to their Timeform rating at the end of a season. The results indicated that there was a relationship between plasma sodium concentration and red blood cell count in blood samples taken six hours after exercise with their Timeform rating. There was a small but significant relationship with plasma urea concentration. These observations suggest that apparently the inherent performance of the Thoroughbred is associated with its water balance and possibly its capacity to utilize protein.

Introduction

This study was undertaken to ascertain whether there is a relationship between the concentration of haematological and biochemical components of blood and the inherent performance of Thoroughbreds in training and racing.

It was essential in a study of this nature, when comparing analyses of blood samples in differing horses, to ensure that results were not biased by sampling effects, analytical variation, natural physiological variation, sub-clinical or clinical disease, effects of training and diet and the more obvious effects of age and sex. The examination of these effects has been previously reported (Blackmore 1975), and the concept of individual normality for each subject under study was introduced. The analysis to be reported presents an extension of that study to confirm the individuality hypothesis and a comparison of blood chemistry and haematology with performance as assessed by Timeform rating at the end of the racing season.

Materials and Methods

Series One: to examine individual variance

Eight Thoroughbred yearlings were sampled at 14-day intervals for a period of ten months. All samples were taken at 08.30 h on Monday morning following a Sunday rest day and analyzed with 24 hours.

Series Two: determination of normality

Ninety two-year-old Thoroughbreds were sampled at intervals of five to six weeks on 13 occasions over a period of 15 months. All samples were taken between 16.00 h and 17.00 h to standardize the effects of stress and diurnal variation. Those samples that could not be analyzed within 24 hours were stored at −24 °C.

Series Three: extension of normality and relation to performance

Over 600 two-year-old, three-year-old and four-year-old Thoroughbreds were sampled during a five-year period from ten stables in the United Kingdom. All samples were taken when the animals were at rest and had not been exercised for the previous four hours. Samples that could not be analyzed within 24 hours were stored at −24°C. These samples had been submitted to the laboratory as part of an 'unfitness' stable screening programme and the results were analyzed in retrospect. All routine haematological and biochemical components were analyzed within 24 hours of sampling. Copper, zinc and selenium were analyzed on samples that had been stored at −24°C for periods up to two years, and vitamin A and E samples were stored for up to three years.

Analysis for aspartate aminotransferase (E.C. 2.6.1.1.), creatine kinase (E.C. 2.7.3.2.), sorbitol dehydrogenase (E.C. 1.1.1.14), alkaline phosphatase (E.C. 3.1.3.1.), 'intestinal' phosphatase (Blackmore and Palmer, 1977), and gamma-glutamyl transferase (E.C. 2.3.2.2.) activity, calcium, inorganic phosphorus, urea and bilirubin concentrations were measured by centrifugal, continuous flow and reaction rate analysis. Sodium and potassium were measured by flame photometry and chloride by potentiometric titration. Total proteins were determined by Biuret and differential proteins by electrophoresis. Selenium, copper and zinc concentrations were determined by electrothermal atomic absorption spectroscopy (Blackmore *et al.*, 1982; Stubley *et al.*, 1983), vitamins A and E by high performance liquid chromatography (Butler and Blackmore, 1982; Butler and Blackmore, 1983) and haematological parameters (haemoglobin, leucocytes, erythrocytes, packed cell volume, plasma viscosity, red cell indices and differential leucocytes) by the standard methods described for the horse by Allen and Archer (1976).

Results were analyzed by standard statistical techniques for multiple and linear regression, multiple correlation, principal components analysis (Cooley and Lohnes, 1971), cumulative sum and Student's 't' comparison following application of Chauvenet's criteria to eliminate outliers.

Results

Individuality

Following repetitive analysis of the blood from eight Thoroughbreds in Series One, it became apparent that the concentration of many parameters varied little for a given individual, and it was the total of the individuals that formed the normal range, rather than day-to-day variations of each. The results were extended to cover Series Two and Three. Fig. 1 shows the range of the standard deviation within an individual for seventeen components, compared to that of the total population. The standard deviations are given in brackets for those components with a normal distribution and have been omitted in those with a logarithmic distribution, since the resulting numbers would be misleading. It was of interest that sodium, calcium and urea concentrations and the total number of erythrocytes in circulating blood had individual variations that were less than 50% of that of the total population. The results from the measurement of haematological parameters were further analyzed by analysis of variance, and Table 1 indicates the factors that contributed to each individual value. It can be seen that, with the exception of the plasma viscosity, analytical error contributed 10% of the variation, the time of year between 4.7 and 19%, with the location, age and sex having little effect. The individual variation, due to 'genetic' factors, varied between 33 and 49% of the total, with the residual unexplained variation varying between 29 and 39%.

FIGURE 1. The standard deviation of the observed range for an individual over a fifteen-month period, compared with that of the total population for 17 blood components.

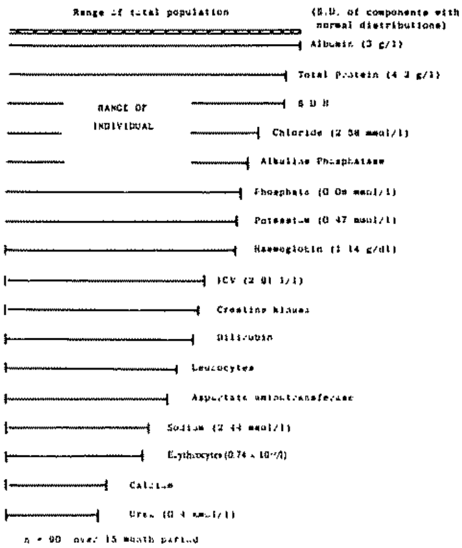


TABLE 1. The degree by which some factors are responsible for an individual measurement of five blood components.

	Hb	RBC	PCV	Viscosity	WBC
Analysis	10	11	9.5	19	9
Time of year	12	4.7	19	14	6
Location	5	5.5	3.5	2	7
Age	5.6	0.3	5	0.2	0
Sex	2.7	0.5	2	2	0.5
Individuality (genetic)	35	49	33	29	37
Unexplained	29	29	29	34	39

It was apparent, therefore, that prior to further analysis of the data, it was essential to ascertain the individual normal range for each animal under study and, in particular, to eliminate from analysis all subjects with values falling outside this range.

Differences between half-siblings

It has previously been shown (Blackmore 1975) that during training there is considerable variation in serum aspartate aminotransferase (AST) activity between half-siblings. Fig. 2 shows the increase in AST following natural infection with equine herpes virus 1. It is apparent that the response to the infection, as reflected by the AST in the blood, was related to the sire of the offspring. Due to the small numbers and large differences in variance between the groups, statistical evaluation was not undertaken as it would have been misleading.

FIGURE 2. Changes in serum aspartate aminotransferase activity following natural infection with EHV 1 in four groups of half-siblings.

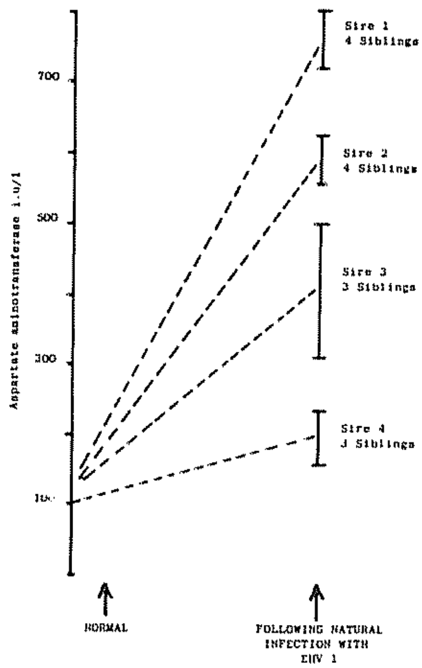


Fig. 3 presents the results of the analysis in relation to the differences observed between seven groups of half-siblings (four to six in each group). Should all groups of half-siblings have been significantly different from each other, then 100% of the subjects would have indicated a difference at a level giving a probability of <0.01. The results indicate that many of the components present in blood were significantly different in not less than 30% of the population. It was of interest that it was only the protein components of blood that showed significant differences in this analysis and that the concentration of these in circulation appears to be related to genetic control.

FIGURE 3. Components measured in blood that demonstrated significant differences between seven groups of half-siblings sampled on ten occasions. The horizontal axis indicates the percentage of groups showing significant differences.

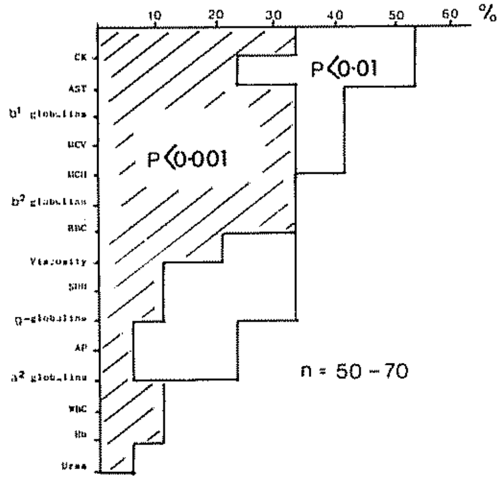
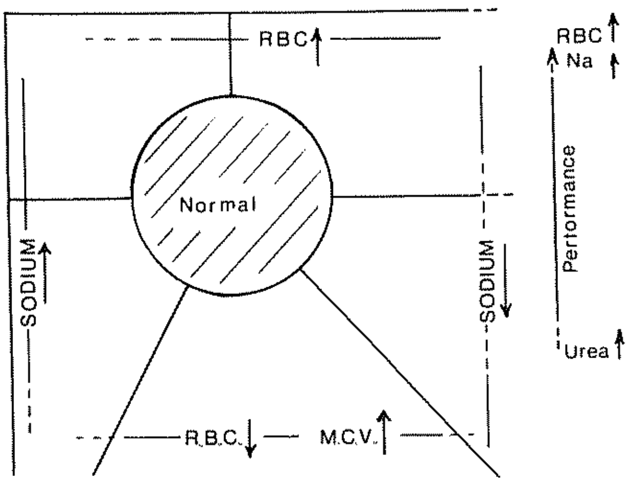


FIGURE 4. Simplified diagram prepared following principal components analysis of the results from blood determination of 28 components sampled from 86 horses housed in seven different stables on ten occasions.
(The analysis was undertaken and the original diagram prepared by Bryant, T. N. and Smith, J. E., Department of Microbiology, University of Surrey).



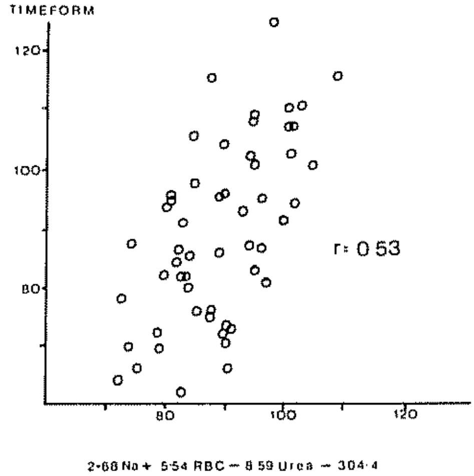
Principal components analysis

Fig. 4 is a simplified summary of the results of principal components analysis on the data observed in Series Two, and it indicates that when the total population was examined the subjects fell into five conditions outside normal. The sodium concentration and number of circulating erythrocytes were the major cause for the polarization observed, with animals on the left of the diagram having higher serum sodium concentrations than those on the right, and those in the upper section having higher erythrocyte counts than those in the lower section.

Relationship with performance

Following application of Chauvenet's criteria to the results of Series Two to eliminate outliers, multiple regression analysis of the 28 components indicated that the Timeform rating of the animals was positively correlated to sodium concentration, and the number of circulating erythrocytes with an inverse relationship to the urea concentration (Fig. 5). This observation has been included on Fig. 4 for comparison with the results of principal components analysis.

FIGURE 5. Regression equation derived from the results of 28 blood components in 56 Thoroughbreds in training sampled on ten occasions, compared to the 'Timeform' rating at the end of the season. Timeform rating was only available on the 56 subjects.

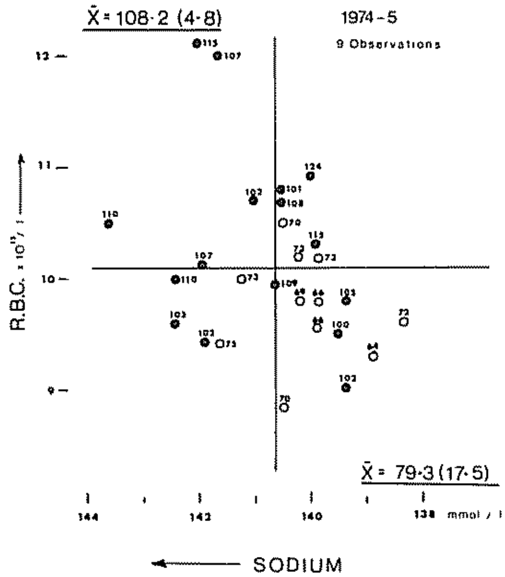


The use of Timeform as a numerical indicator of inherent ability is open to question, but an analysis of 100 random ratings indicated a true logarithmic distribution that is unlikely to have been observed if the effects had been entirely random.

Sodium, RBC and Timeform

Following the previous observations, it was interesting to note the results when the sodium and red cell counts were plotted similarly to the form derived from the results of principal components analysis (Fig. 4) and compared with the Timeform rating in the areas of the resultant graph. Fig. 6 shows the results for the 1974/75 data (Series Two). The heavy lines represent the mean of the sodium and red cell count. When comparing the quadrants, it can be seen that the upper left-hand quadrant, i.e. those samples with high red cell counts and high serum sodium concentrations, had a mean Timeform rating of 108.2, while those samples in the lower right quadrant had a mean Timeform rating of 79.3. These results were prepared by taking the mean of nine observations. A further survey was undertaken on the data obtained during 1981, in which six consecutive observations were compared and outliers omitted following application of Chauvenet's criteria. A similar pattern was observed (Fig. 7), with the upper left-hand quadrant having a mean Timeform of 102.8 and the lower quadrant a mean Timeform of 76.3. Fig. 8 compares the combined data from both these studies. It can be seen that there was a significant difference ($p < 0.0001$) between the top left-hand quadrant with a mean Timeform of 105.1 and the lower right-hand quadrant with a mean Timeform of 77. The apparent small number of horses available for such analysis was due to the absence of Timeform ratings for the majority of two-year-olds at the end of a season.

FIGURE 6. Graph showing the relationship between serum sodium concentrations and erythrocyte counts in the blood of two-year-old Thoroughbreds in training during 1974–75 related to their Timeform rating at the end of the season. The solid lines represent the mean of the sodium concentrations and number of red blood cells. Each point represents the mean of nine observations when 'outliers' have been excluded.



In 1982, in an attempt to see whether a single observation of the serum sodium concentration and red cell count was related to Timeform, ten animals with Timeform ratings of greater than 100 were compared with seven of less than 65. These were three-year-old animals, and their Timeform rating was based on that at the end of their two-year-old season. No such relationship was observed, and the use of a single observation to determine inherent performance in this manner was not viable. A further series of single observations on blood taken from 19 three-year-old Thoroughbreds with Timeform ratings greater than 100 was compared with 13 three-year-olds with Timeform ratings of less than 65. There were no significant differences between the groups, confirming the unreliability of single observations in such comparisons.

FIGURE 7. Graph showing the relationship between serum sodium concentrations and erythrocyte counts in the blood of two-year-old Thoroughbreds in training during 1981, related to their Timeform rating at the end of the season. The solid lines represent the mean of the sodium concentrations and number of red blood cells. Each point represents the mean of six observations.

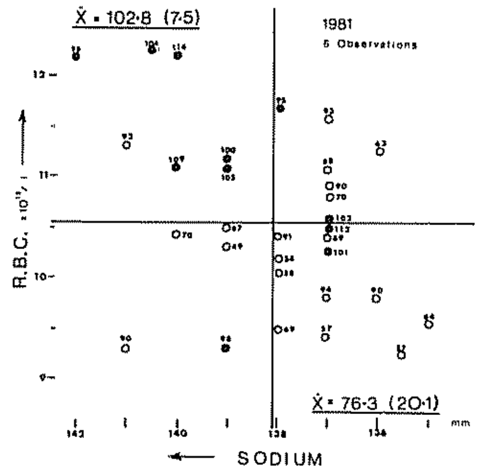
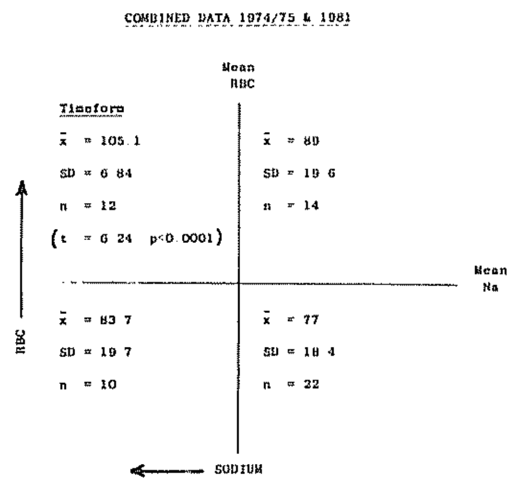


FIGURE 8. Simplified figure showing the relationship between sodium concentrations and erythrocyte counts of Thoroughbreds in training, combining the results obtained during 1974–75 and 1981.



Discussion

This investigation had indicated that the normal range for the blood constituents of individual Thoroughbreds was less than that of the total population. Individuality in other species has been observed and recorded in the normal human by Williams (1971) who showed that the variation of results due to analytical variation of 'noise' was greater than the physiological variation of the constituents measured in blood. Krause *et al.* (1975), following earlier work by Cotlove and his co-workers (Cotlove *et al.*, 1970; Williams *et al.*, 1970; Harris *et al.*, 1970; Young *et al.*, 1971) utilized a Bayesian model to overcome the problems of analytical error. In the Thoroughbred, it was not found necessary to use Bayesian statistics, because normality was better defined since there were fewer dietary and environmental variations.

From this study, it would appear that individuality is a normal state within the Thoroughbred and that some blood components are related to inherent performance. The influence of genetics on both is important, and recent work by Woolliams *et al.* (1982) has indicated that in sheep the response to dietary copper is a function of genetic variation. They demonstrated significant differences in liver copper concentrations when studying a series of half-siblings from males of different breeds mated to black-faced females. Vessel (1975) also commented on the polygenic control for the large individual variations in metabolism of some commonly used drugs when examining human families and twins. Rowlands *et al.* (1973) have also shown that the levels of haemoglobin, glucose, potassium, calcium, phosphorus, magnesium and urea in blood are inherited in healthy calves.

Undoubtedly, the breeding of the Thoroughbred depends upon the prior performance of the horse and has resulted in a degree of selectivity (Leicester 1964). It is not surprising therefore that observations relating biochemical and haematological parameters to performance are apparent. The results in no way suggest that a single estimation will demonstrate the inherent performance expected of an individual, but repetitive sampling and the establishment of the animal's 'individual normal' could provide an indicator of its potential performance. In addition, the observations that the concentration of sodium in serum, coupled with the number of circulating red cells at rest, is apparently related to inherent performance, suggests a profitable area for future research into an indication of lack of performance when no clinical reason can be given.

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Plasma Biochemical Values in Thoroughbred Horses in Training

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Summary

Fifteen biochemical tests were performed on plasma samples from Thoroughbred horses in training. The tests were conducted over a period of one year on horses selected from a large stable in the country and from six smaller stables in the Adelaide metropolitan area. Preliminary studies ascertained the stability of the biochemical constituents in stored blood and the effects of diurnal variations.

Of the 128 horses initially bled, only 63 were bled on a sufficient number of occasions during the two-month 'conditioning' period and the first two months of racing to be acceptable for statistical analysis. This analysis showed that stage of training had a significant effect on the majority of plasma constituents tested, although trends were apparent for only a few: increases in creatinine and bilirubin and decreases in urea, phosphorus and lactate dehydrogenase during the training period. Location-related influences also had a significant effect on the majority of plasma constituents, whereas sex-related influences and age did not have such a general effect. It was found that total protein, lactate dehydrogenase and aspartate aminotransferase differed between the sexes and that inorganic phosphorus, alkaline phosphatase and lactate dehydrogenase tended to decrease with age, whereas bilirubin and total protein tended to increase with age.

Introduction

It has become increasingly common among diagnostic laboratories to offer a panel of biochemical tests on blood. A number of these tests can assist in assessing the fitness and health of horses in training. Serum enzymes have a particular application in judging the status of the muscular system during training, and abnormalities in serum electrolytes have been noted by Williamson (1974) in horses with poor performance. Interpretation of these biochemical tests, however, has often been restricted by inadequate information on appropriate reference ranges.