LXXXI. THE BLOOD OF EQUINES.

BY CHRISTIAN PETRUS NESER.

Preliminary Communication¹ from the Division of Veterinary Education and Research, Onderstepoort, Union of South Africa.

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Technique. Without offering details of technique it may be briefly stated that blood, drawn from the jugular vein except where otherwise mentioned, was collected in 10 cc. bottles containing a measured adequate amount of sodium citrate solution. From this were determined, (a) percentage volume of erythrocytes by centrifuging in specially prepared uniform tubes of 2 cc. capacity, blown out slightly at the sealed end to facilitate rapid sedimentation; (b) corpuscle count after diluting 1 in 200 with Hayem's fluid for erythrocytes, and 1 in 10 with 0.5 % acetic acid for leucocytes, the Bürker chamber being used in both cases; (c) haemoglobin content by Sahli's method.

Smears were made by Craandyk's [1918] method and also by a modification of Ehrlich's method which is so simple and effective as to merit brief description. A small drop of blood is quickly transferred to a slide by means of a suitable platinum loop, and a cover slip, as broad as the slide, immediately lowered over it. The droplet at once spreads as a thin circular layer, and on drawing the slip lengthwise over the slide all the blood is left upon the latter as a thin film in which the distribution of leucocytes is remarkably uniform, and eminently suitable for differential count after Giemsa staining in the usual way.

Limits of error. From numerous observations expressly designed to determine the degree of accuracy of methods in conventional use, it was concluded that (a) the centrifuge gives very accurate and consistent data, (b) red counts are liable to an error of up to 10 % even with the improved Bürker chamber, (c) white counts are less liable to error, (d) the Sahli reading cannot be relied upon to within five scale units, (e) smears made by the described modification of Ehrlich's method show a very regular distribution of leucocytes.

THE RED CORPUSCLES.

Influence of work. Comparative study of the blood of different horses showed a remarkable difference in percentage volume of erythrocytes from individual to individual, but a fairly constant figure for any given individual over short intervals of time. The outstanding difference between the blood of different horses was at first very puzzling. Since all were fed alike, diet

¹ The full paper, giving detailed protocols, will appear in the forthcoming "Report of the Director of Veterinary Education and Research" (P.O. Box 593, Pretoria).

was not a factor. Age was excluded by statistical comparison of data. That sex played no part was evidenced by similar variation in mares and geldings. Since all the animals under observation had been purchased for the horse-sickness experiments of the institution, they were a mixed lot whose histories were generally unknown, and no mere inspection sufficed to explain the observed variations.

The clue, however, was given by two animals (laboratory Nos. 11144 and 11775) one of which was permanently stabled and showed a percentage volume of 23, while the other was used as a saddle horse and showed a figure of approximately 40. This remarkable difference at once suggested that *work* was the deciding factor in determining the percentage volume of erythrocytes in horse blood. Following up this clue, horses were grouped according to the work performed, as "fast working," "permanently stabled," and "other horses." It was at once noted, *without exception*, that the blood of fast-working horses showed a high percentage volume of erythrocytes, or high red count, while in horses stabled for six months or more the figures were correspondingly low. With the third group the data were variable, but it is probable that if the previous histories of these miscellaneous animals had been known, many of them would have been classified in one of the other groups. At a later date it was found possible to procure the blood of a few race-horses in training, and their high volume of red corpuscles bore out the earlier conclusions in most striking fashion. Graph I represents a summary of the data from over 200 animals from all sources, Graph II the data from three race-horses in various stages of training, while Graph III illustrates the change actually occurring during hard training of three young race-horses.

These data leave no doubt that hard fast work brings about a marked increase in the percentage volume of red blood corpuscles in the horse. The contrast shown in Graph II, for three race-horses from the same stable, is particularly striking, the figure for the fully trained animal showing that 52 %, or more than half the total volume of blood, may be made up of erythrocytes. This corresponds to a count of approximately 12 million as against an average count of about 8 million for the ordinary slow working horse, and less than 6 million for permanently stabled laboratory horses.

These already conclusive observations are further clinched by reference to Graph III, which shows a steady increase in numbers of red corpuscles during the actual course of hard training. In five weeks the count increased from less than 7 million to over 9 million in one case, and from 6.3 million to 8.8 million in a second case. In the third case training was interrupted owing to injury about the third week, the rapidly rising count then remaining stationary. Of special interest is the further fact that although the high count follows so rapidly in the wake of hard training, the reverse effect, or diminution of red blood corpuscles with rest, is a slow process. Thus in two young horses a decrease of two millions occurred only after three months, while in several old animals the decrease after five months was hardly noticeable at all.

Graph I.

A, Stabled horses.
 B, Medium horses.
 C, Fast working horses.
 D, Race-horses in full training.

Graph II.

Race-horses.
 A, Untrained.
 B, Partly trained.
 C, Fully trained.

Showing the influence of hard fast work upon the % volume of red corpuscles.

Graph III.

Showing the influence of time upon the red count in three young race-horses, during hard training.

Relationship between volume, count, and measurement of erythrocytes. In numerous cases, the percentage volume as determined by the centrifuge, the actual count in the Bürker chamber, and the diameter of the cells as measured in smears, were compared for the same horses.

The *diameter* of individual corpuscles varied between 4μ and 8μ : but, with one exception, the average of 200 cells remained fairly constant at 5.5μ . In respect, therefore, to average size of erythrocytes, different horses are very uniform.

The *ratio* between percentage volume and numerical count of red blood corpuscles varied between $4k$ and $4.7k$, with an average of $4.35k$ — k being a constant dependent upon the units of measurement used. From the relatively constant average ratio, the observed liability of the red count to an error of 10 %, and the observed constancy of centrifugal readings, it may be concluded that the variations observed are mainly due to errors in counting and not to real variations in the average size of the corpuscles. These considerations led to the conclusion that a count calculated from percentage volume on an assumed ratio of $4.35k$ is even more correct than an actual count; at least for the blood of healthy horses. It is quite certain that the percentage volume and the red count give the same information, and that the observed increase of the former with work is due to a real increase in the number of normal red cells per unit volume of blood; normal also in respect to haemoglobin content as determined by the Sahli method. This increase in erythrocytes is probably absolute, and a genuine response of the blood-forming tissues to the increased demands made upon the oxygen-carrying capacity of the blood.

Diurnal variations. Quite remarkable differences in percentage volume of erythrocytes were observed from day to day, and even from hour to hour. Numerous experiments in which horses were worked, fasted and then fed, allowed to go thirsty and then watered, all failed to account for the variations observed in jugular blood. The data obtained indicated that any change in the concentration of the blood, arising from these factors, is at most very transient. The real explanation of the variations, however, was at once found on tapping at different points in the circulation. In comparing blood from the jugular vein with that from the ear, it was found, with very few exceptions, that the latter gave a higher red count than the former, and was also subject to far greater variation. The difference between ear blood and jugular blood was greatest in animals feeding at rest, and least in animals excited or at work; an increase in one count being generally associated with a decrease in the other.

These observations indicated the mechanical state of the circulation as the important factor in determining distribution of erythrocytes throughout the body, and the few simple experiments recorded in Graph IV provided direct evidence of its operation.

Graph IV.

Showing the influence of the mechanical state of the circulation, upon the distribution of the red cells.

Fig. 1 of Graph IV represents the changes in red corpuscle count in an animal excited just prior to feeding; Fig. 2 those of an animal rested and fed immediately upon return from work; Fig. 3 those of an animal taken from the manger and threatened for 30 minutes by exhibition of a familiar whip. In all these cases the counts on peripheral and systemic blood converge most closely when the circulation is most active, and disagree most widely when the circulation is more sluggish. A less active circulation thus results in a concentration of the red corpuscles in the peripheral capillaries, possibly owing to relative increased lymph formation and possibly owing to sedimentation.

On many occasions it was also noticed that both ear and jugular red counts had increased or decreased together; observations which may be explained by assuming concentration or dilution in some other part of the circulation.

The general results, however, show that in horses the jugular blood is far more representative of the average circulation than is blood taken by ear puncture; that factors beyond the control of the observer may give very misleading results when ear blood is studied; but that even jugular blood is not free from variation.

Pregnant and nursing mares. The results obtained from a few animals of this class may be summarised by stating that no influence of gestation or lactation, as such, could be established. The red corpuscle counts varied greatly between individual mares, being always highest in those with a past history of active life, and such differences obscured possible variations centring around sex.

Foals. In foals the observed red count was very high just after birth, but in two months had decreased considerably. Two cases will serve to show that the most rapid fall occurs in stabled foals at rest:

Foal	Percentage volume of red corpuscles	
	At birth	After 2 months
(a) Permanently stabled	48	30
(b) Running with mother	42	36

Again the influence of physical activity is manifest.

Donkeys and Mules. The red cell picture of donkey and mule blood differs in an interesting way from that of the horse. The average diameter of the erythrocytes is approximately 6.2μ for both donkey and mule as against 5.5μ for the horse. For the donkey the ratio of percentage volume and numerical count was generally found greater than $5k$, while for mules it approximated $4.35k$, the figure for the horse. The inference is that the corpuscles of the mule are thinner than those of donkey and horse.

THE LEUCOCYTES.

The classification of the leucocytes adopted in this work is that which is generally accepted to-day, as fully discussed by du Toit [1917] in his article on bovine blood. Equine leucocytes are not unlike those of bovines, as studied in stained smears, with the exception of the eosinophiles and basophiles. The granules of the latter cells are very large and form a characteristic feature of equine blood.

The *number* of leucocytes in the blood of healthy horses is very variable, not only as between individuals, but also in the same animal from time to time. The general range is from 5 to 20 thousand per cubic millimetre. In any individual animal the hourly and daily variations are not great, although exceptions are not uncommon.

Several experiments were undertaken to ascertain the influence of ordinary non-pathological factors upon the white count. Results may be summarised as follows:

(a) Moderate variations in supply of food and water showed no definite effects, and the jugular blood certainly reflected no evidence of any "digestive leucocytosis."

(b) Exercise always increased the white count of jugular blood, in some cases by over three thousand per cmm. On cessation of exercise the numbers often decrease again very rapidly, even to a figure below the pre-exercise count.

(c) Simultaneous observations upon ear and jugular blood yielded no conclusive results, except with exercise. In this case the results obtained indicate that the distribution of leucocytes also depends largely upon the mechanical state of the circulation.

To ascertain the extent to which technique of sampling influenced the white count, variations were made upon the usual procedure of first slapping the ear and collecting from a large puncture. A small puncture was made and blood obtained by squeezing at the base and at the apex. The following data represent one case:

	Leucocyte count
Ear, ordinary technique	11.3×10^8
Ear, squeezed at base	7.4×10^8
Ear, squeezed at apex	14.3×10^8
Jugular blood for comparison	12.4×10^8

From these marked variations it may be concluded that the white cells cling to the walls of the capillaries and the wound, especially if the blood flow is slow. When the circulation is rapid, or when the blood is forced out under considerable pressure, the leucocytes become detached, or are prevented from clinging, with consequent increase in apparent numbers. This explains why the ear white count is usually lower than the jugular.

Differential Counts. Differential leucocyte counts, obtained by the useful method (modified Ehrlich) already described, showed the following general figures:

Differential count of equine leucocytes	Lymphocytes	Mono-cytes	Neutrophiles	Eosino-philes	Baso-philes
<i>Horses, average of 7 each:</i>					
(a) Stabled	36	4	54	5	1
(b) Medium	39	4	52	4	1
(c) Fast working	40	5	50	4	1
(d) General average	38	4	53	4	1
Normal variations	45-30	8-2	60-45	9-3	3-0
Extreme variations in 200 cases	50-25	9-0	62-54	15-1	3-0
<i>Donkeys, average</i>	53	4	34	8	1
<i>Mules:</i>					
(a) Clinically healthy	41	4	49	6	1
(b) Slight injuries	32	3	61	4	—
(c) Inoc. earlier against anthrax	52	3	39	6	1

The most interesting feature of this comparative table is the curious reversal of the figures for lymphocytes and neutrophiles, as between horses and donkeys. The horse shows lymphocytes 38 and neutrophiles 53 as general average, while the donkey shows neutrophiles 34 and lymphocytes 53. The count for mules approximates that of horses, when clinically healthy animals are considered. The slightly higher neutrophile count in group (b) mules may perhaps be accounted for by the slight injuries from which they were suffering. The high lymphocyte count in group (c) mules may perhaps be due to the fact that these animals had been inoculated against anthrax some months earlier.

Breeding mares and foals.

To these data may be added a few observations upon mares and foals. Taken just before or after parturition, the mares showed a very high neutrophile count, even over 70 %. An even higher neutrophile count, about 80 %, was characteristic of foals shortly after birth. After about two months this neutrophilia had disappeared, provided the animals remained healthy.

Influence of other factors.

Various minor deviations from the usual mode of life did not appear to influence the differential count in any regular way; except for the eosinophiles, which were generally increased by water-drinking after a period of thirst or fasting. Graph V offers a typical example of transient eosinophilia following large consumption of water after dry feeding.

Showing the influence of food and water upon the % of eosinophiles in one horse.

GENERAL DISCUSSION.

It is of interest to compare the findings recorded in this paper with the limited data already available in the same direction. References to healthy equine blood in the general haematological literature appear to be very scanty, and it is to be regretted that South African library facilities do not always allow of consultation of such references as can be tracked by abstract. Burnett quotes various authors as obtaining erythrocyte counts ranging from 6.3 to 8.5 million, and leucocyte counts varying between 5.6 and 15 thousand, for adult horses. The white count is approximately that recorded in this paper, but the red counts quoted by Burnett suggest that other workers have contented themselves too readily with animals of one class. The data now recorded show a much wider range, considerably below 6.3 million for permanently stabled horses and even up to 12 million for race-horses in hard training.

Frei [1909], working in this laboratory, compared the centrifuge and the counting chamber, obtaining 4.7*k* for the ratio between percentage volume and numerical count, and preferring the volumetric method for determining the proportion of erythrocytes. His ratio is thus about 8 % higher than the one now offered on a more extensive series of observations. The horses

available to Frei belonged to the same miscellaneous class as our own original lot, and showed variations from 22 to 43 for corpuscular volume. The astonishing thing is that no serious attempt was made to elucidate the nature of these differences, and the extraordinary effect of regular work seems to have wholly escaped his notice.

In regard to corpuscular dimensions, some authors give the average size of erythrocytes as 5.5μ , but others give figures as high as 5.8μ . The former figure is the average now established upon several hundred horses.

The differential counts quoted by Burnett for horses show higher neutrophile and lower lymphocyte percentages than those recorded in this paper. Stephens and co-workers [1921] have called attention to the variation in distribution of leucocytes in different parts of smears made in the ordinary way, and since our own technique has overcome this difficulty, the data now recorded would seem the more reliable.

By far the most striking observations recorded by us concern the remarkable influence of regular exercise upon the proportion of erythrocytes in horse blood. The available recent works upon horse blood all deal with pathological conditions, and nowhere does any reference seem to be made to the fundamental influence of normal work. In the various standard text-books upon Physiology and Haematology, the same omission is conspicuous. Dealing, as they do, largely with the human subject, in which normal variations are less noticeable, no stress is laid upon this factor, which plays so remarkable a rôle in the physiology of the horse. It is generally accepted that slight variations occur with mode of life, but whether or not these are specifically related to exercise is hardly discussed, although factors such as "altitude" are almost unduly stressed—perhaps because immediately detectable. Some workers on human blood have found that "robust" people have a higher, and "obese" people a lower, red count. The numbers are also lower for women, and it has been recorded that school children show a higher count at the end of a long vacation, than at the beginning. It is true that the variations generally recorded in healthy human individuals are registered in hundreds of thousands as against the tremendous variation of 4 million to 12 million between the stabled laboratory animal and the fully trained race-horse, but is it not possible that the minor differences in the human are due to differences in the amount of exercise rather than to other factors?

For the horse, the facts now recorded are perhaps not so surprising when considered in the light of its evolutionary history. The ancestor of the modern horse depended upon speed and endurance for its very existence; two qualities conditioned by a highly efficient circulatory system, and the demand for a high oxygen-carrying capacity of the blood. Is it then astonishing that the modern horse is born with a potential capacity for high speed and great endurance, and that during hard training his store of haemoglobin should be increased as fast as his muscles develop, so that finally more than half the total blood volume of a "finished race-horse" consists of erythrocytes?

In how far does this phenomenon of increase of red corpuscles with exercise, so strikingly shown here for the horse, apply to other animals? The problem at least seems a promising one for future investigation.

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