

## BLOOD PROTEINIC PICTURE OF THOROUGHBRED HORSES

S. FERRI \*; L. F. MARTINS \*\*; M. C. LEITE RIBEIRO \*\* & T. U. WORSMAN \*

*Laboratory of Hematology, Instituto Butantan, S. Paulo, Brasil*  
*Department of Histology and Embriology, School of Veterinary Medicine, USP*

Campello and col. (3) have revised the most important bibliographies about the proteinic picture of the blood serum and the several factors which may have influence on the results.

Patrushev (13) verified that even among Thoroughbred horses there are variations according to the lineage. Individual variations have also been demonstrated (11, 17).

The oscilation of the contents of proteinic fractions in the serum of the equine is such that Geinitz (7) advises that the normal mean values for the populational group we are dealing with, should always be established.

Dzialeszynski and Maciejewska (5) investigated the influence of sex. They could not find any difference for the total proteins between geldings and mares but, in the geldings, beta globulin apparently shows higher levels and albumin exists in greater quantity.

Concerning to serum proteins, Campello (2) did not find different results for male and female Thoroughbred horses.

According to Stankiewicz and col. (17), the rate of serum proteins is not influenced by the sex, the same occurring with the gamma globulins, according to Odebrecht and Branco (12).

There is no objective reference in literature indicating a research work to verify the influence of the proteinic picture of the serum on the performance of the animal.

The present study has been planned in order to analyse the influence of sex on the contents of total serum proteins and the various fractions in Thoroughbred horses, and the influence of the proteinic picture on the competitive capacity of the animal.

### MATERIAL AND METHODS

The material for the present work consisted of blood of 120 Thoroughbred horses, with ages ranging from 3 to 5 hippic years, submitted to regular physical exercises and similar regimen and lodged at the Jockey Club of São Paulo.

The animals were divided into two groups called "winners" and "losers", on the basis of times considered minimum and maximum for 1400 and 1500 meters

\* Department of Histology and Embriology of the School of Veterinary Medicine of the University of São Paulo (Caixa Postal 7064 — São Paulo — Brasil).

\*\* Department of Histology and Embriology of the School of Dentistry at Bauru, University of São Paulo.

Received for publication in october 15, 1968.



races, light sand; those which reached times inferior to the minimum were called winners, and losers those which went beyond the time established as maximum.

The indexes for classification of the animals as winners or losers were calculated according to the results obtained for 200 animals placed in the 1<sup>st</sup> and 2<sup>nd</sup> places and the same number in the two last posts in each of the two races mentioned above.

The data were obtained from the archives of the Comission of Races of the Jockey Club. As only the result of the 1<sup>st</sup> placed is expressed in "time" and that from the others, in "bodies" in relation to the first, we made a transposition from "bodies" to "time", employing the photochart which documents the races.

In order to verify the validity of the adopted criterion, the scores obtained by the firsts and the lasts were statistically analysed as a preliminar care in the design of a proper experiment. The comparison between the values showed a significant difference between the means of the two indexes at the level of 5% making it valid to use them in the selection of the animals.

After the 1400 or 1500 meters races, light sand, the time of the 1<sup>st</sup> placed was determined by a chronometer and that of the others by the photochart, thus selecting the competitors on the basis of pre-established characteristics for the sampling.

The animals rested for 16 — 20 hours after the race and the blood was then collected. The horses which we knew were not successful for adverse reasons, as for example, indocibility at the start, were rejected.

The blood was collected from the jugular vein in the morning, before the animals had received their meals, and anything that could excite them was avoided.

10 to 15 ml of blood were collected in 18x140 mm test tubes without the use of anticoagulant, in order to obtain the serum for a dosage of total proteins and electrophoretical analysis.

*Total proteins* — After coagulation the clot was detached and after retraction the serum was removed and centrifugated at approximately 1000 r.p.m., for 3 minutes, and then kept frozen in a test tube until the analysis could be done.

The Gornall's and col. (9) method was used for this dosage. The results were read through a spectrophotometer using a 540 milimicra wave length.

*Proteinic fractions* — The electrophoretic migration for the separation of proteinic fractions was made on a No. 1 Watmann's filter paper, in a "Elphor" set, using acetate veronal buffer with ionic force = 1 and pH 8,6.

0,7 ml of serum was placed at 9 cm from one end of the paper. The intensity of the current was equal to 2 mA per strip of paper, for 16 hours. The strips were then dried at 70 — 80°C.

They were developed with the use of Schwartz 10 B starch, after Grassman and Hanning's technic, modified by Ferri and col. (6); diaphanization was obtained with Elphor's special oil and an "Intergraph" set was used for reading the results.

*Statistical analysis* — The measures of position and variability were calculated for each group of the experiment.

The comparison between winners and losers, males and females, was accomplished through the Student's test, after Snedecor (16).

For the comparative analysis of the proteinic fractions, the  $\alpha_1$  globulin was not taken into consideration, since the values were calculated in percentual



terms and were interdependent, the last value becoming automatically known, and thus being lost one degree of freedom.

The rejection level for these comparisons was 5%.

## RESULTS

The results obtained are given on tables I, II, III and IV.

## DISCUSSION

Before discussing the results obtained we must point out that, although the number of fractions in the equine serum is variable, we have restrained the analysis to the main fractions, according to the authors taken into consideration (1, 3, 4, 7, 10, 15), thus achieving a more accurate dosage.

The proteins did not differ significantly between males and females of both categories, regarding to the total content and also to the fractions.

This confirms some works (2, 12, 17) and contradicts others (5, 14).

There is a significant difference between the two male groups, regarding to total proteins, beta and gamma globulins, which are present at a higher concentration in the losers. Between the female groups the same occurred, but only with the beta fraction.

Data have not been found in literature to make possible a comparative analysis.

The fact of the total proteins being present at a higher concentration in the losers might be accounted for a greater loss of liquid by these animals.

We must still point out that before the blood was collected the animals rested for a period of time considered sufficient for them to return to their resting condition.

The serum proteins are very important, its main function being the maintenance of blood volume, carrying and mobilizing antibodies, nourishment, transportation of lipids and other substances, and also mobilization of secreted hormones (8), being difficult to appraise precisely the real significance of the pointed differences.

The beta globulins are related mainly to the transportation of certain soluble lipids, with carotenoids and strogens; to the beta-globulins is also related a specific protein, the siderophilin, which serves for transporting the iron from the intestinal cells to the liver, spleen and bone marrow. Besides, they are related to a number of proteins which are important for the blood clotting and other activities, for example, the prothrombin, inhibitors of thrombin, plasminogen, serum lipase, vaso-pressor substances, etc. (8).

Further researches should be carried on in order to explain the differences we met with.

Antibodies, as it is well known, are related to the gamma-globulins. Speculating on the possible causes for these being found in a smaller number in the winners, we might presume that this is due to the fact that the winners are less challenged by antigenic elements, probably of infectious nature.

Concerning to the females, it is interesting to point out that only the beta globulin fraction presented a significant difference between winners and losers, but there is no available evidence to substantiate this verification.



TABLE I — SERUM PROTEINS IN MALE THOROUGHBRED HORSES

	Protein		Albumin		Globulins							
					Alfa <sub>1</sub>		Alfa <sub>2</sub>		Beta		Gamma	
	Winner	Looser	Winner	Looser	Winner	Looser	Winner	Looser	Winner	Looser	Winner	Looser
Mean .....	5,88	6,14	2,96	2,97	0,19	0,18	0,57	0,57	0,86	0,98	1,30	1,45
Standard deviation.	0,22	0,48	0,26	0,20	0,030	0,040	0,08	0,10	0,14	0,20	0,25	0,30
Coeff. of variation	0,04	0,08	0,09	0,07	0,15	0,22	0,14	0,18	0,16	0,20	0,19	0,21
Median .....	5,90	6,20	2,96	2,93	0,17	0,18	0,57	0,54	0,86	0,95	1,29	1,39

TABLE II — SERUM PROTEINS IN FEMALE THOROUGHBRED HORSES

	Protein		Albumin		Globulins							
					Alfa <sub>1</sub>		Alfa <sub>2</sub>		Beta		Gamma	
	Winner	Looser	Winner	Looser	Winner	Looser	Winner	Looser	Winner	Looser	Winner	Looser
Mean .....	5,97	6,10	3,01	2,97	0,19	0,19	0,61	0,62	0,87	1,00	1,29	1,33
Standard deviation.	0,49	0,41	0,25	0,20	0,045	0,030	0,10	0,14	0,20	0,20	0,26	0,28
Coeff. of variation	0,08	0,07	0,08	0,07	0,24	0,15	0,16	0,23	0,23	0,20	0,20	0,21
Median .....	5,80	6,10	3,02	2,94	0,17	0,19	0,60	0,57	0,83	1,00	1,30	1,31



TABLE III — SERUM PROTEINS IN THOROUGHBRED HORSES

	Protein		Albumin		Globulins							
					Alfa <sub>1</sub>		Alfa <sub>2</sub>		Beta		Gamma	
	Winner	Looser	Winner	Looser	Winner	Looser	Winner	Looser	Winner	Looser	Winner	Looser
Mean .....	5,92	6,12	2,99	2,97	0,19	0,19	0,59	0,59	0,86	0,99	1,29	1,39
Standard deviation.	0,46	0,45	0,20	0,20	0,045	0,030	0,09	0,14	0,20	0,20	0,28	0,30
Coeff. of variation	0,08	0,07	0,07	0,07	0,24	0,16	0,15	0,24	0,23	0,20	0,22	0,22

TABLE IV — SERUM PROTEINS IN THOROUGHBRED HORSES. *T* VALUES FOR THE DIFFERENT CONTRASTS

	Protein	Albumin	Globulins			Critic value of <i>t</i> — at 5%
			Alfa <sub>2</sub>	Beta	Gamma	
MW x ML .....	2,60	0,16	—	2,67	2,11	2,00
FW x FL .....	1,08	0,22	0,31	2,50	0,56	2,00
MW x FW .....	0,90	0,25	1,33	0,25	0,14	2,00
ML x FL .....	0,36	—	1,67	0,40	1,60	2,00
W(M+F) x L(M+F)	2,44	0,56	—	3,61	1,89	1,98

M = male; F = female; W = winner; L = looser



Comparisons were made between winners and losers for total proteins and fractions. The results obtained for males and females were grouped, as no difference was observed between them.

This analysis made it possible to verify that the total proteins and the beta globulins differed significantly in the two groups.

Through this fact we perceive that the differences between the male winners and losers have sufficiently influenced the results, regarding to the total proteins. The same did not occur in regard to the gamma globulins, where the differences observed before were masked.

### SUMMARY

The rates of total serum proteins and its eletrophoretic fractions were analysed in 120 Thoroughbred horses. The animals were divided into two groups, "winners" and "losers", and comparative studies were made between males and females, winners and losers.

The statistical analysis showed:

1 — The male winners have lower rates of total proteins, beta and gamma-globulins, than the losers;

2 — The female winners have lower levels of beta globulins than the losers;

3 — The male and female winners have lower levels of total proteins and beta globulins than the losers;

4 — The levels of total proteins and the other analysed fractions are the same for males and females, in both groups, winners and losers.

### REFERENCES

1. Ashton, G. C. — Serum proteins variations in horses. *Nature*, 182:1029-30, 1958.
2. Campello, A. P. — Teores de alguns compostos nitrogenados no sangue de cavalos P.S.I. de corrida, sob a influência do exercício muscular e da glicose. Tese. Escola Superior de Agricultura e Veterinária do Paraná, 1958.
3. Campello, A. P.; Cardoso, A. T. & Faria, A. M. — Perfil eletroforético do sôro de cavalos P.S.I. de corrida. *An. Fac. Med. Paraná*, 3:3-16, 1960.
4. Deutsch, H. F. & Goodloe, M. B. — An eletrophoretic survey of various animals plasma. *J. biol. Chem.*, 161:1-20, 1945.
5. Działoszyński, L. & Maciejewska, M. — Frakcje białkowe surowicy Krwi Kónskiej. *Méd. Vét.*, Varsovia, 13:173-7, 1957.
6. Ferri, R. G.; Mendes, E.; Cardoso, T. Y. B. & Tutiya, T. — Electrophoresis of serum protein in Asthma. Preliminary Report. *J. Allergy*, 27:494-503, 1956.
7. Geinitz, W. — Über Serumeiweisse von Tieren, die Häufig als Versuchstiere oder zur Gewinnung von Heilseren Dienen. *Klin. Wschr.*, 32:1108-1111, 1954.
8. Gonçalves, M. J. — Sistemas de proteínas. Alguns estudos. Tese. Faculdade Nacional de Medicina, Rio de Janeiro, 1951.
9. Gornall, A. G.; Baradawill, C. J. & David, M. M. — Determination of serum proteins by means of the Biuret reaction. *J. biol. Chem.*, 177:751-766, 1949.
10. Hirtz, J. — Étude électrophorétique du sérum de chevaux infectés expérimentalement d'anémie infectieuse. *Rev. Immunol.*, 16:397-405, 1952.



11. *Kunde, H.* — Die Papierelektrophorese des Blutserums bei verschiedenen Erkrankungen der Pferd. *Wiss. Z. Humboldt Univ.*, **10**:273, 1961.
12. *Odebrecht, S. & Branco, C. L.* — Determinação dos teores séricos normais de gama globulinas em cavalos P.S.I. de corrida. *An. Fac. Med. Paraná*, **5**:67-72, 1962.
13. *Patrushev, V. I.* — Physiological variation within the English race-horses. *Compt. Rendus Acad. Sci., URSS*, **23**:710-13, 1939.
14. *Patrushev, V. I.* — Physiological variation in horse as connected with age. Breed and Performance. *Compt. Rendus Acad. Sci., URSS*, **23**:718-21, 1939.
15. *Polson, A.* — Variation of serum composition with the age of horse as shown by electrophoresis. *Nature*, **152**:413-414, 1943.
16. *Snedecor, G. W.* — Statistical methods, 5th ed. Ames. Iowa, Iowa States University Press. 1956, p. 116-27.
17. *Stankiewicz, W.; Markiewiczowa, Z. & Malinowski, W.* — Hematologiczne badania koni pelnejkrwi i rasy Fiording. *Med. vet.*, **16**:594-98, 1960.
18. *Stockl, W. & Zackerl, M. K.* — Papierelektrophoretische Untersuchungen des Serums von Rind und Pferd. *Hoppe-Seylers Z. physiol. Chem.*, **293**:278-83, 1953.



