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QUANTITATIVE COMPARISONS OF ACIDIC PREALBUMIN (PR) PHENOTYPES IN HORSES

By

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EK, NILS and MIKAEL BRÆND: *Quantitative comparisons of acidic prealbumin (Pr) phenotypes in horses.* Acta vet. scand. 1980, *21*, 380—388. — Comparisons of Pr protein amounts in horse sera have been performed using *Mancini et al.*'s (1965) immunodiffusion technique. Relative values against a chosen standard of 100 % were determined for a total of 435 horses.

There was considerable variation between horses, the highest Pr value being 125 % and the lowest 50 % of the standard. In animals of the same Pr phenotype the mean Pr values were significantly higher ($P < 0.001$) in foals than in mares. In Norwegian Trotter horses the Pr value of Pr NN animals was significantly higher than that of Pr SS phenotypes, whereas the mean Pr values of Pr SS was significantly higher than that of Pr UU Warmblood Trotter horses, the Pr value of Pr SS being 90 % and the Pr UU 80 % of that of Pr NN.

No difference between sexes with regard to Pr values was found.

horse; serum; Pr phenotypes; relative Pr quantities.

When describing the great complexity of the horse Pr system, *Brænd* (1970) stated that the basic zone patterns controlled by the individual alleles varied not only with respect to number of zones but also with regard to staining intensity of the zones. During 11 years of routine parentage control with currently some 2000 samples per year these differences in strength between zones have been confirmed. Thus the major zone controlled by the Pr^F, the Pr^L and the Pr^N alleles generally appears stronger than the major zone of the Pr^S and the Pr^U alleles.

This called for quantitative studies of the various Pr phenotypes, results of which are given in the present report.

MATERIALS AND METHODS

The horse material included eight horses of mixed breeds. These were randomly chosen and sampled once a month over half a year. Also randomly chosen were the families of two Norwegian Trotter stallions with 27 and 30 dam offspring pairs each and two Warmblood Trotter stallions with 30 and eight dam offspring pairs respectively. Sixty-eight mares and 215 foals aged two—12 months were selected according to Pr phenotypes; of these, 17 mares and 31 foals belonged to the four sire families. Among the selected horses there were 86 Warmblood Trotter. Except for the eight horses first mentioned the samples were obtained in connection with routine parentage control. The horses were bled by veterinary practitioners. When the samples arrived at the laboratory, serum was pipetted off and stored at —20°C when not used immediately.

Determination of Pr phenotypes was carried out according to the technique of *Brænd* (1970).

Quantitation of the Pr protein was performed using the method of *Mancini et al.* (1965). Antiserum against the Pr protein was prepared as described earlier (*Ek* 1979). Of this antiserum 0.8 ml was preheated to 56°C and mixed with 7.2 ml melted agarose. The mixture was transferred to a glass plate, measuring 6 cm × 7 cm. After solidification, 12 wells, 3.0 mm in diam., were punched out, and each was filled with 10 µl of the sample to be analyzed. Three of the wells were filled with 1/2, 1/4 and 1/8 dilutions of the serum from a Pr FF horse which had been chosen as a standard. The quantity of the Pr protein of this horse (a 12 months old male) was given the value of 100 %. The remaining nine wells in the agarose plate were each filled with a 1/4 dilution of serum from horses to be examined. Every sample was tested twice. The test plates were placed horizontally in a humid chamber for 72 h, after which they were photographed. Control of similarity between wells was carried out by testing the same serum sample in all nine wells.

The Pr values were determined by measuring the diameter of the precipitate for each well, and then calculating the area. Because of the linear relationship between the precipitate area and the amount of protein (*Mancini et al.*) a standard line using the dilutions 1/2, 1/4 and 1/8 of the reference Pr FF serum was prepared for each agarose gel plate. By correlating the precipitate area for each serum sample to that of the standard, the

quantity of Pr protein could be determined as a percentage of the standard.

The standard deviation of the double tests was calculated from the formula $s = \sqrt{\frac{\Sigma \cdot d^2}{2n}}$ where d is the difference between the double test values and n the number of double tests. The standard deviation in the material was 2.6. Standard methods of t-tests and analyses of variance were employed.

RESULTS

Fig. 1 is a photograph of an agarose gel plate where 12 samples were tested. The three top samples (1, 2 and 3) show the precipitate areas for the standard dilutions of 1/2, 1/4 and 1/8. The 1/4 dilution is always given the value of 100 %. The nine other samples (four to 12) vary from 86 % to 110 % of the

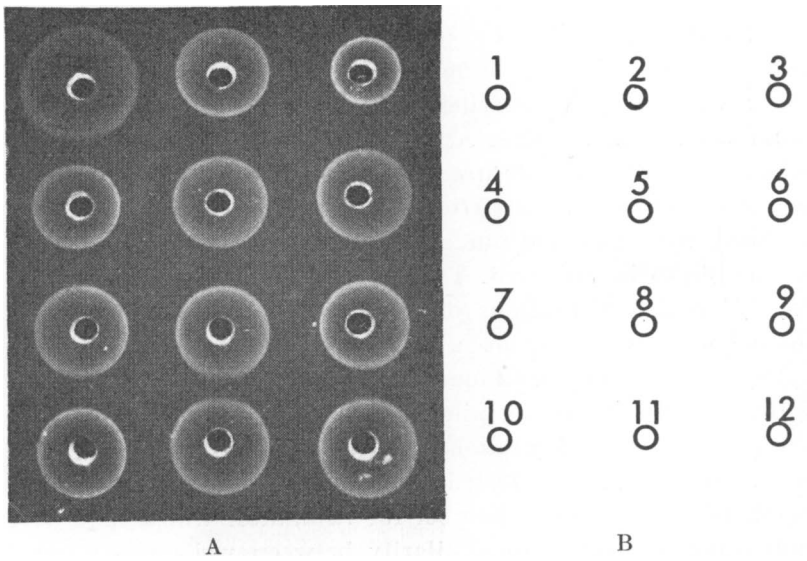

Figure 1. A. Photograph of an agarose gel plate where 12 samples have been quantitated for the Pr protein.
B. Diagram showing the numbered positions of wells.
The gel contained 0.8 ml antiserum against the Pr protein mixed with 7.2 ml melted agarose (1 %).
Wells Nos. 1, 2 and 3 were filled with 10 µl of 1/2, 1/4 and 1/8 dilutions, respectively, of a standard serum given the value of 100 %. Wells Nos. 4—12 were each filled with 10 µl of a 1/4 dilution of serum from horses to be examined.

standard. It can further be seen that only in positions Nos. 5 and 8 are the wells situated in the center of the precipitate; in the others the outer radius is larger than the inner one. This difference in diffusion does not, however, influence the total precipitate area. In a control plate with all wells filled with the same sample the Pr values were the same for all the wells.

Fig. 2 shows the Pr values of the eight horses of mixed breeds which were tested over a period of half a year. The animal being lowest with regard to Pr quantity varied from a low of 58 to a high of 65. The horse with the highest values varied between 97 and 114.

Further differences between horses appeared in the results from the studies of sire families (Table 1). All these animals were also tested for Pr phenotypes, but only those of the sires

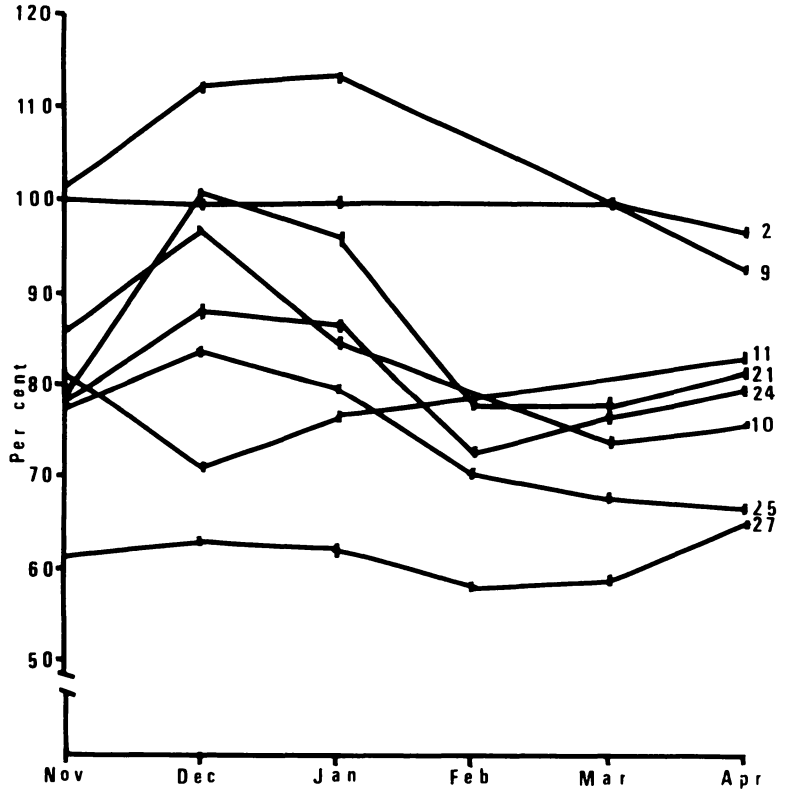

Figure 2. Pr values of eight horses of mixed breeds investigated over half a year. The figures represent identification numbers of the horses.

Table 1. Pr values in four sire families.

No.	Sire		Dam		Offspring	
	Pr type	Pr value	Pr value ± s	n	Pr value ± s	n
1	FS	83	78.4 ± 11.9	27	81.9 ± 7.4	27
2	NS	88	83.4 ± 12.4	30	92.1 ± 11.7	30
3	LU	76	69.5 ± 11.2	28	80.3 ± 13.1	30
4	UU	60	74.9 ± 8.6	8	73.9 ± 5.8	8

are given in the table. These investigations showed significant differences ($P < 0.01$) between the mean values of dams and their offspring for the families of Sires 2 and 3, the offspring being higher.

Results from investigations of adult mares and of foals aged up to 12 months but selected according to Pr phenotypes are presented in Table 2. Among the mares the highest Pr values were found in the Pr NN animals. Their mean differed significantly from that of the Pr UU mares ($P < 0.001$) and also from that of the Pr SS mares ($0.02 > P > 0.01$). The difference between the Pr SS and the Pr UU mares did not reach statistical significance. Between foals of Pr phenotypes SS and UU, however, the difference was highly significant ($P < 0.001$) as was

Table 2. Relationships between Pr phenotypes and Pr concentrations.

Category of horse	Breed	Age in months	n	Pr type	Pr value ± s	Range	
						max.	min.
Mare	NT	> 48	23	NN	83.7 ± 10.0	108	63
Mare	NT	> 48	25	SS	76.4 ± 9.1	101	60
Mare	WT	> 48	20	UU	70.7 ± 10.9	93	50
Foal	NT	2—11	26	NN	97.6 ± 11.1	114	80
Foal	NT	2— 9	31	SS	88.0 ± 10.8	115	72
Foal	WT	2—12	29	UU	78.5 ± 8.4	102	65
Foal	NT	3—10	27	FN	94.1 ± 13.4	118	60
Foal	NT	3—12	42	LN	97.5 ± 15.0	125	78
Foal	NT	3—10	23	NW	99.4 ± 9.6	124	79
Foal	WT	5—11	19	*)	98.4 ± 11.6	114	80
Foal	WT	4—10	18	SU	88.0 ± 8.6	100	70

NT = Norwegian Trotter.
WT = Warmblood Trotter.
*) = There were 10 LN, 4 IL, 2 IN, 2 GL, 1 GN.

also the case for the Pr NN foals against the Pr SS foals. There were also significant differences between mares and foals for these three categories of Pr phenotypes ($P < 0.001$). Foals of Pr FN, Pr LN and Pr NW did not differ significantly from foals of Pr NN phenotype.

Table 2 also shows that the mean Pr value of a group of Warmblood foals with various heterozygous phenotypes did not differ from that of Pr NN in Norwegian Trotter foals. Furthermore, Pr SU Warmblood foals had a mean Pr value which differed significantly ($P < 0.001$) from that of Pr UU foals, as well as from that of the group with high value Pr genes.

In Table 3 Pr values in foals classified according to sex are given. The highest difference between means occurred for the Pr FN phenotypes, but was not significant.

The same significances were obtained in t-tests as in analyses of variances.

Table 3. Relationships between Pr concentration and sex in foals.

Sex	n	Pr type	Pr value ± s
♂	16	NN	98.1 ± 9.9
♀	10	NN	96.9 ± 13.9
♂	15	SS	85.5 ± 8.4
♀	16	SS	90.4 ± 12.4
♂	18	UU	77.4 ± 8.2
♀	10	UU	80.6 ± 9.2
♂	11	FN	90.0 ± 13.3
♀	14	FN	96.4 ± 12.8
♂	14	LN	97.0 ± 12.0
♀	27	LN	99.0 ± 11.0
♂	13	NW	99.8 ± 10.9
♀	9	NW	100.2 ± 7.2

DISCUSSION

In our material highly significant differences between mean Pr values were established between horses homozygous for the Pr^N, Pr^S and Pr^U alleles. There were not enough animals of the other homozygous categories to arrive at significant conclusions. The Pr values of the Pr^F, Pr^L and Pr^W alleles were, however, indirectly deduced from phenotypes in which they occurred together with Pr^N. As long as the mean Pr values of these three categories of heterozygous horses did not differ from the mean

of Pr NN we assume that the Pr FF, the Pr LL and the Pr WW phenotypes do not differ from that of Pr NN.

We did not find any significant difference between the mean Pr value of a group of Warmblood Trotter foals representing various heterozygous Pr phenotypes and the mean Pr value of Pr NN phenotypes in Norwegian Trotter foals. Our results are therefore, consistent with the view that there are high value Pr genes of the same order and same types in Warmblood Trotter horses as in Norwegian Trotters, even though limited numbers of these phenotypes were investigated in Warmblood horses. Supporting this view is also the mean Pr value of Pr SU Warmblood foals which fits into the pattern established through the mean Pr value of homozygous Pr SS in Norwegian Trotter foals and that of Pr UU in Warmblood foals.

Between mares the Pr value of Pr SS was 91 % of that of Pr NN. In foals the corresponding figure was 90 %. These two figures, therefore, show good agreement. The mean of Pr UU mares was 84 % of Pr NN mares, whereas the corresponding figure for foals was 80 %. The difference between these last two figures is not statistically significant. Since the material for foals includes larger numbers than for mares we are inclined to consider 80 % as the best figure. In making these comparisons we have considered the standard Pr value of Pr FF as a unit and not as a percentage.

The biological reason for the observed variation in Pr values can as yet only be a matter of speculation, but we may ask why the differences between Pr phenotypes are not more pronounced. Thus in man the product of the Pi^Z allele comprises only 15 % of that of the Pi^M allele (*Ganrot et al.* 1967). A significant proportion of individuals homozygous for the Pi^Z allele suffer from lung emphysema (*Ganrot et al.*). In horses, however, natural selection against non-favourable alleles would be expected to be more efficient than in man, because it would appear that horses depend more on healthy lungs for survival. Whether Pr values of 50 to 80 % might affect the health, particularly that of the lungs, is doubtful as there is a high frequency of the Pr^U allele in Warmblood trotters. Furthermore, in man individuals of Pi SS type have only 63 % of the normal Pi value but do not seem to be affected by this lower Pi value (*Fagerhol* 1969).

Besides the variation correlated with Pr phenotypes and age, there was additional variation within and between horses. Some

of this variation is environmental and we have reason to believe that diseases such as infections may cause higher Pr values (*Ek* 1980). Whether there are interferences by other environmental factors, for instance season or feeding, we do not know.

Such unknown factors may explain the very wide variation observed between individual horses. They may also be the reason why the mean Pr value of the offspring of Sire No. 1 was so low and was not significantly different from the mean of their dams. Even though there were four more Pr^S genes among the offspring than among their dams this is not enough to account for the low mean of the offspring but we may say that there is a trend towards significant differences. The non-significant difference between the mean Pr value of dams and offspring in the family of Sire 4 could be explained by small numbers investigated.

The higher Pr values in foals are difficult to interpret. To our knowledge a similar situation does not exist in man. For this matter the amount of other protease inhibitors in horse serum might be of significance. *Matthews* (1979 a) showed that besides the Pr protein there are two other protease inhibitors in horse serum, the acidic prealbumin Xc which he suggested to be the same as esterase (Es) and an $alpha_2$ macroglobulin (*Matthews* 1979 b). It might be that these three protease inhibitors are interdependent and change with age.

In the introduction it was mentioned that the Pr S and Pr U zones generally appear much fainter than the major zones of the faster Pr phenotypes. The lower Pr values of the Pr SS and Pr UU types is in agreement with previous observations. But the reduced values of 90 and 80, respectively, do not seem to account for the pronounced differences as seen when judged by eye. An explanation has been given through *Ek*'s (1979) studies using immunological techniques. He showed that there are slower Pr zones in addition to those described by *Brænd* (1970). For the Pr^S and Pr^U alleles these slower zones are generally considerably stronger than the slower zones of the faster alleles. Thus the Pr U pattern has three peaks of about the same size. Only the fastest of these was diagnosed by the starch gel technique of *Brænd. Scott* (1978) also reported additional Pr bands.

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SAMMENDRAG

Kvantitative sammenligninger av sure prealbumin (Pr) fenotyper hos hester.

Ved bruk av *Mancini et al.*'s (1965) immundiffusjons-teknikk er det foretatt sammenligninger av konsentrasjoner av Pr-protein i hestesera. Relative verdier i forhold til en valgt standard på 100 % ble bestemt for i alt 435 hester.

Det var stor variasjon mellom hestene. Den høyeste Pr-verdi var 125 % og den laveste 50 % av standarden. Hos dyr med den samme Pr-fenotype var middelverdiene signifikant høyere hos føll enn hos hopper. Hos norske traverhester var Pr-verdiene signifikant høyere hos NN-dyr enn hos dyr med Pr-fenotype SS, mens middelverdiene hos SS-dyr var signifikant høyere enn hos varmblods-traverhester med Pr-fenotype UU. Pr-verdiene for Pr-SS var 90 % og for Pr-UU 80 % av tilsvarende verdi for Pr-NN.

Det ble ikke funnet noen forskjell på Pr-verdiene mellom kjønnene.

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