STUDIES ON THE INHERITANCE OF ELECTROPHORETIC FORMS OF TRANSFERRINS, ALBUMINS, PREALBUMINS AND PLASMA ESTERASES OF HORSES

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BY means of starch gel electrophoresis BRÆND and STORMONT (1964) demonstrated 16 different transferrin phenotypes in horses. Family data supported the theory that the observed differences were attributed to the action of six co-dominant autosomal alleles designated Tf^D , Tf^F , Tf^H , Tf^M , Tf^O and Tf^R . The theory of six alleles have been verified by BRÆND (1964) and GRÆTZER, HESSELHOLT, MOUSTGAARD and THYMANN (1965). Genetic variations of horse albumin were reported by STORMONT and SUZUKI (1963). They described three different albumin phenotypes A, AB and B controlled by a pair of codominant autosomal alleles, designated α^A and α^B . The albumin variations were also studied by BRÆND (1964) who used the phenotype symbols AA, AB and BB. Later BRÆND and EFREMOV (1965) decided to use the allele symbols Al^F and Al^S and called the albumin phenotypes FF, FS and SS. ASHTON (1959) observed at least three pre-albumin types in horses. Also BRÆND and EFREMOV (1965) found a variation in the prealbumin area in their gels used for albumin typing. However, no attempt has been made to classify the variants and study them genetically.

By using starch gel electrophoresis, several multiple forms of esterase have been reported to occur in horse plasma. KAMINSKI and GAJOS (1964) described two intensely stained esterase fractions and two faint zones of activity, but no individual differences were observed among 51 horses. OKI, OLIVER and FUNNEL (1964) found 11 esterase zones in zymograms from 94 horses and reported a variant form of cholinesterase which might be genetically controlled.

The present paper is concerned not only with a study of transferrin and albumin phenotypes in horses but also with evidence for several electrophoretic forms of prealbumins and plasma esterases and the basis for their inheritance.

MATERIALS AND METHODS

The horse material comprises four complete sire families with 36 dams and 107 offspring of the Salernitana horse breed in Italy. The breed originates from old importations of Arabian horses. During the 19th century the breed was upgraded by the use of stallions from English Thoroughbred stock and French Trotters. After the first World War, the breeding has been rather closed. Now the race occurs as a remnant concentrated to the old royal estate of Persano, where the horses are bred and trained for horse racing and military purposes.

All blood samples were collected from animals older than three months. The samples were taken in an anticoagulant solution (2% sodium citrate, 0.5% sodium chloride, 0.05% streptomycin).

cin). As soon as possible the blood samples were centrifuged and the plasma was pipetted off. The red cells were washed with saline and thereafter hemolysed with distilled water in the proportions 1:1. The plasma and the hemoglobin samples were subsequently sent by air to the laboratory in Uppsala.

The analyses of the plasma samples were carried out according to a modification of Smithies horizontal starch gel electrophoresis method (GAHNE 1963). Two kinds of buffer systems were used. The transferrins were analyzed by the following system. Electrolyte: pH 8.5, 0.06 M lithium hydroxide + 0.229 M boric acid. Gel buffer: pH 8.5, 1 volume electrolyte + 5.4 volumes Tris-citric acid buffer (0.079 M Tris(hydroxymethyl)aminomethane + 0.007 M citric acid).

In order to demonstrate the different albumin and prealbumin types a sodium-acetate-EDTA buffer, pH 5.4 was used. The composition of the buffer in the cathode vessel was 0.125 M sodium acetate + 0.0085 M EDTA (ethylenediamine tetraacetic acid). Some of the heat effects were avoided by halving the buffer concentration in the anode vessel. The gel buffer was a fourfold dilution of the cathode buffer.

In all experiments the starch concentration in the gels was 11.5% and the voltage gradient about 15 volts per cm. The electrophoresis time was 3.5 hours in experiments at pH 8.5 and 4 hours at pH 5.4. The starch gel apparatus, the cooling system and the sample insertion technique were the same as described by GAHNE (1963). The starch gel was always cut in three slices, each 2 mm thick. The middle slice was used for protein staining in a concentrated solution of amido black and nigrosin. One of the other two slices was stained for esterase in the following solution: 50 ml 0.1 M Tris-maleate buffer, pH 6.5, 50 ml methanol, 2 ml 1% alpha-naphthyl acetate, 50 mg Fast Garnet GBC salt. The gel slice was washed half a minute in the buffer and methanol mixture before the alpha-naphthyl acetate and the diazonium salt were added. The washing in the methanol-buffer mixture fixed the surface of the gel slice and gave more distinct zones of those esterases which showed the genetic variation. The other esterase fractions were somewhat inhibited by the methanol.

The hemolysates were used for the purpose of detecting possible electrophoretic variants of hemoglobin but no variants were observed (cf. BRÆND and STORMONT 1964). Therefore, there will be no further discussion of hemoglobin types in this report.

Terminology: The gene loci in horses considered in this study are represented by the symbols *Tf* for transferrin, *Al* for albumin, *Pr* for prealbumin and *Es* for esterase. An allele is indicated by the symbol of the gene locus followed by the superscript symbol assigned to the allele (e.g. *Pr^F*). In the albumin, prealbumin and esterase systems the allele symbol *F* (fast) is used for the allele which has the fastest migrating zones and the symbol *S* (slow) for the allele with the slowest zones (cf. BRÆND and EFREMOV, 1965). Other alleles are designated with symbols between *F* and *S*. This system of nomenclature allows new detected alleles to be designated in accordance with the migration rate of the zones determined by the allele.

When a protein zone is first distinguished, it is given a temporary designation until it can eventually be shown to belong to a genetic system. In such cases the zones are named according to which allele they are determined by. Capital letters are used for the main zones (e.g. *Pr-F*) and small letters for the faint accompanying zones (e.g. *Pr-f*). If several zones are determined by an allele, a numerical subscript designation may be used (e.g. *Pr-f₁*) to avoid misunderstanding. The number 1 is assigned to the fastest zone, the number 2 to the next one, etc.

The phenotypes are named according to their main zones (e.g. *Pr-FI*). In genetic systems, where all alleles act as codominants, the homozygous phenotypes are given a double designation (e.g. *Pr-FF*). For convenience the locus symbols (i.e., *Tf*, *Al*, *Pr* and *Es*) are omitted whenever there should be no question concerning the identity of the locus under discussion.

RESULTS

Transferrins: Of the six earlier described transferrin alleles all except the allele *Tf^M* were found in the Salernitan horses. In addition it was possible to divide the *Tf^F* allele into two different alleles designated *Tf^{F1}* and *Tf^{F2}*. The transferrin

FIGURE 1.—A photograph of the result of a starch gel electrophoresis experiment at pH 8.5 with horse plasma samples of different transferrin types.

types shown in Figure 1 represent all the alleles which occurred in this material. The difference in migration rate between the F¹ and F² bands was very slight but by including reference samples of known F types on the same gels, it was possible to make the classification.

The transferrin types found in this study were compared with samples from Norway, classified by BRÆND. With the exception of the subdivision of the F zone the transferrin patterns agreed with those described by BRÆND and STORMONT (1964).

The distribution of transferrin phenotypes among parents and offspring is shown in Table 1. Sixteen of the 21 possible phenotypes are represented. The following allele frequencies were computed by simple gene counting based on

TABLE 1

Distribution of transferrin types in 147 horses of the Salernitana horse breed

	DD	F ¹ F ¹	F ² F ²	OO	DF ¹	DF ²	Transferrin types										Total No.
							DH	DO	DR	F ¹ F ²	F ¹ H	F ¹ O	F ² H	F ² O	F ² R	HO	
Sires	1	..	..	..	1	1	1	..	..	..	..	..	..	..	..	..	4
Dams	4	2	1	1	7	5	..	7	1	4	..	1	..	2	..	1	36
Offspring	26	8	..	..	19	13	11	14	2	3	3	2	3	..	1	2	107

the parental material: $Tf^D = 0.41$, $Tf^{F^1} = 0.21$, $Tf^{F^2} = 0.18$, $Tf^H = 0.03$, $Tf^O = 0.16$ and $Tf^R = 0.01$.

The data of the four sire families were in all but one case in agreement with the hypothesis of six transferrin alleles. In the exceptional case a DH sire gave in mating to a DO mare an offspring of phenotype DF². This was most probably a result of illegitimacy or an accidental interchange of samples.

Albumins: It was possible to recognize the three different albumin phenotypes with the buffer system at pH 8.5 but the resolution of the albumin zones was increased much by lowering the pH to 5.4 (Figures 2 and 3). The homozygous albumin types FF and SS were characterized by a thick staining fraction called Al-F and Al-S respectively and a thinner zone behind the thick one. (Al-f and

FIGURE 2.—A photograph of the result of a starch gel electrophoresis experiment at pH 5.4 with five horse plasma samples. Three albumin types and four prealbumin types are represented.

FIGURE 3.—A diagram of albumin and prealbumin phenotypes.

Al-s), while the heterozygote FS type had accordingly four typical zones. In addition at least one zone was observed both in homozygous and heterozygous types just in front of the thick albumin zones.

Data on the inheritance of the albumin types are summarized in Table 2. Three sires were of the albumin type FS and one of type SS. The albumin types of the offspring were in all cases consistent with the theory of a pair of codominant, autosomal alleles. In the mating of FS sires $\times$ SS dams, there was a rather great deviation from the expected ratio. Also STORMONT and SUZUKI (1963) obtained a similar result in the mating FS $\times$ SS, namely 28 FS and 13 SS offspring. More data are needed in order to determine whether these deviations from the expected 1:1 ratio in FS $\times$ SS matings are due to something other than chance.

The frequencies of the two alleles Al^F and Al^S were respectively 0.34 and 0.66 as computed from the parental material.

Prealbumin: With the alkaline buffer system it was sometimes possible to detect one protein fraction, which migrated faster than the albumins. However,

TABLE 2
Inheritance of albumin phenotypes

Kinds of matings Sire Dam	No. of offspring of phenotypes			P of χ^2
	FF	FS	SS	
FS $\times$ FF	6	4	..	...
FS $\times$ FS	7	17	11	<0.70
FS $\times$ SS	..	27	15	<0.10
SS $\times$ FF	..	4	..	...
SS $\times$ FS	..	3	4	...
SS $\times$ SS	..	..	9	...

several fractions were demonstrated in the area in front of the albumins (Figure 2) by using the acid buffer system. The fastest migrating zones were called prealbumins and designated Pr. They showed a regular variation among the plasma samples. Eight different prealbumin phenotypes were observed (Figure 3). The designations F, I, L and S were used for the main zones.

Phenotype LL was the most common. It was characterized by a distinct zone L (or Pr-L) which was accompanied by two faint zones, one l_1 in front of the main zone and the other l_3 behind. The leading and trailing zones were often very faintly stained and at times were not visible. The phenotypes FF, II and SS had the same protein pattern as the LL type except that the zones had other migration rates. The zone I coincided with the l_1 zone, the F zone with i_1 zone and the S zone with the l_3 zone. In the phenotypes FI, IL and LS generally only the two main zones were visible while in the phenotype FL a third rather faint zone was observed between the F and L zones.

Data on the inheritance of the phenotypes are summarized in Table 3. Three of the four stallions were of the prealbumin type LL and one was of the FL type. The observed types of the offspring were in all but two cases consistent with the interpretation that the prealbumin phenotypes were controlled by four codominant autosomal alleles, designated Pr^F , Pr^I , Pr^L , and Pr^S . In the mating LL $\times$ FF two offspring had the type LL, which was not expected on the basis of the genetic theory. These deviations were probably caused by illegitimacy or by interchange of samples. At first there were four exceptional offspring, but two were corrected by taking new blood samples, while it was not possible to get new samples in the two remaining cases.

Another possible explanation for the deviations would be the occurrence of additional prealbumin alleles with protein zones, which migrated more slowly than the S zone and which were more or less covered by some of the several other protein zones in this area. This explanation is rather unlikely here, because all

TABLE 3

Inheritance of prealbumin phenotypes

Kinds of matings Sire Dam	No. of offspring of prealbumin phenotypes					Total No.
	LL	FI	FL	IL	LS	
LL $\times$ FF	2*	..	4	..	..	6
$\times$ II	..	..	..	4	..	4
$\times$ LL	38	..	..	..	..	38
$\times$ SS	..	..	..	..	3	3
$\times$ FI	..	..	2	1	..	3
$\times$ FL	1	..	5	..	..	6
$\times$ IL	15	..	..	9	..	24
$\times$ LS	5	..	..	..	2	7
FL $\times$ LL	4	..	8	..	..	12
$\times$ IL	2	2	..	..	..	4
Totals	67	2	19	14	5	107

* Not expected on the basis of the genetic theory.

the animals had typical prealbumin phenotypes. However, such prealbumin alleles with slow migrating protein zones may be difficult to detect.

The following allele frequencies were computed from the parental material: $Pr^r = 0.11$, $Pr^l = 0.19$, $Pr^L = 0.66$ and $Pr^S = 0.04$.

Esterases: The fastest migrating esterase zones designated Es in Figures 4 and 5, were intensely stained. A slow migrating zone Ch was also rather marked and was doubled in some samples. This zone was inhibited by eserine, $10^{-5}M$, so it was concluded that the zone contained cholinesterase. Between the Es and Ch areas some other faint esterase zones were observed, but they varied in staining intensity and needed a longer incubation time to become clear. With the present technique a reliable phenotypic classification seemed therefore possible only for the esterase zones Es.

Six different esterase phenotypes were observed. The phenotypic patterns were different at the two pH values which were used. At pH 8.5 (Figures 4 and 6) the phenotype F was characterized by a distinct zone F (or Es-F) followed by a fainter zone f_3 . A very faint zone f_1 was also observed just in front of the main zone. The phenotypes I and S showed a pattern similar to the F type but they had a slower migrating rate. The zone I coincided with the f_3 zone and the S zone with i_3 . The phenotypes FI and IS had accordingly three typical zones FIi_3 and

FIGURE 4.—A photograph of seven different esterase types from a starch gel electrophoresis experiment at pH 8.5. Ch., cholinesterase; Es., probably aliesterase.

FIGURE 5.—A photograph of six different esterase types from a starch gel electrophoresis experiment at pH 5.4.

IS₃. In the phenotype O there was no esterase activity in the Es area. In Figure 4, a seventh phenotype FS is represented, which was found in a Swedish horse.

At pH 5.4 (Figures 5 and 6) the phenotypes F, I and S were characterized by three zones and the fastest and the slowest zones had almost the same staining intensity. Moreover, the relative migration rates had changed so that the S zone coincided with the f₃ zone. The I zone migrated slower than the S zone but faster than the s₃ zone. The phenotype IS had therefore a blurred pattern, in which it was difficult to distinguish the different esterase zones. The phenotype FI was

FIGURE 6.—A diagram to illustrate the esterase phenotypes at pH 5.4 and pH 8.5.

TABLE 4

Inheritance of esterase phenotypes. All four sires were of the I phenotype, but two sires were presumed to be of the genotype IO and two of the genotype II

Esterase type of		No. of offspring of esterase types						Total No.
Sire	Dam	F	I	S	FI	IS	O	
I* × F		..	..	..	..	..	1	1
× I		..	38	..	..	..	..	38
× S		..	1	..	..	1	..	2
× FI		5	7	..	2	..	..	14
× IS		..	3	4	..	4	..	11
I† × F		..	3	..	1	..	..	4
× I		..	25	..	..	..	..	25
× FI		..	4	..	4	..	..	8
× IS		..	2	..	..	2	..	4
Totals		5	83	4	7	7	1	107

* Relio and Erone, genotype IO.

† Ermellino and Ercole, genotype II.

characterized by four typical zones, because the middle one was usually impossible to see.

Data on the inheritance of the esterase phenotypes are summarized in Table 4. All four sires were of the esterase phenotype I. However, two sires, called Relio and Erone, gave some offspring which lacked the I zone. Erone had also an offspring of the phenotype O. It is therefore supposed that there occurred an allele Es^o which gave no detectable esterase zone in the Es area and that the two sires Relio and Erone were of the genotype Es^I/Es^o . Some of the dams were also presumed to be heterozygous for the Es^o allele. The observed phenotypes of the offspring are consistent with the hypothesis that the esterase phenotypes are controlled by four autosomal alleles, designated Es^F , Es^I , Es^S and Es^o and that the three first alleles are codominant and the last one is recessive. Thus, the phenotypes F, I and S may be either homozygous Es^F/Es^F , Es^I/Es^I and Es^S/Es^S or heterozygous Es^F/Es^o , Es^I/Es^o and Es^S/Es^o .

Relio and Erone, in matings to mares of phenotype FI, produced offspring of the types F, I and FI in a ratio of 5:7:2. Under the assumption that the two sires were of the genotype IO the expected ratio would be 3.5:7:3.5. Likewise, the mating I × IS gave the observed ratio 3:4:4 of the types I, S and IS, respectively, against the expected 5.5:2.75:2.75.

The other two sires, called Ermellino and Ercole, had in the matings I × FI and I × IS a distribution of their offspring which agreed completely with the expected ratios, if the two sires were of the genotype II. In the mating I × F only two dams were represented and they seemed to be of the genotype FO. The matings I × I gave only offspring of the I type so the results supported the assumption that Ermellino and Ercole were of the genotype II.

The pedigree of animals which were assumed to carry the allele Es^o is shown in Figure 7. The individuals of the phenotypes F, I, S and O are here marked if possible with their presumptive genotypes, determined from their pedigree or

FIGURE 7.—A pedigree, where all known animals with the Es^o allele are included. The letters state the esterase genotype, if it is possible, and the figures give the numbers of animals of different types. The sires Relio and Erone are marked with A and B respectively in the diagram.

offspring data. In the mating $IO \times FO$, which gave the one offspring of type O both the sire and the dam had other offspring with the allele Es^o and the dam was also half-sister to the other sire of the IO type. There were no apparent contradictions to the genetic theory based on three codominant and one recessive allele.

As the Es^o allele had no observable effect in the zymogram of the heterozygous animals it was difficult to make an estimate of the frequencies of the esterase alleles. Because the population was rather small and also some inbreeding occurred, the population was not expected to be in genetic equilibrium. By assuming that animals of the type I are of the genotype II , if the genotype IO has not been possible to demonstrate from their pedigree or offspring data, an underestimation of the Es^o allele frequency is obtained. However, the following gene frequencies can then be estimated by simple gene counting in the parental group: $Es^F = 0.12$, $Es^I = 0.73$, $Es^S = 0.06$ and $Es^o = 0.09$.

Two dimensional starch gel electrophoresis: The protein fractions separated at pH 8.5 and pH 5.4 were compared by two dimensional starch gel electrophoresis. A diagram of the results is shown in Figure 8. At first the plasma proteins were separated by electrophoresis at pH 5.4. A gel strip with the separated proteins was then taken from this gel and placed in the starting line of a gel of pH 8.5 and run normally but for a somewhat shorter time.

The transferrins showed slow and dispersed migration in the anodic direction at pH 5.4 and any transferrin classification was therefore impossible. The slow alpha-globulin was easily found as a slow distinct migrating zone even at the acid pH. The different albumin fractions were not clearly separated at pH 8.5. The prealbumin zones Pr with the genetic variation demonstrated at pH 5.4

FIGURE 8.—A diagram of the results of a two-dimensional starch gel electrophoresis experiment with horse plasma of transferrin type FR, albumin type FS and pre-albumin type FI. The corresponding separations at pH 5.4 and pH 8.5 are shown below respectively to the right of the two-dimensional diagram.

migrated in the broad albumin zone at pH 8.5. The Es zones were located behind the albumin at pH 8.5 while at pH 5.4 they migrated just in front of the albumins.

In order to make a comparison possible, the minor protein zones were designated by letters from e to m according to a descending rate of migration at pH 8.5.

The fast migrating e zone at pH 8.5 was found behind the prealbumin zones Pr at pH 5.4. The e zone occurred only in some plasma samples and showed a rather wide variation in staining intensity. Probably the e zone is not related to the prealbumin zones Pr.

In the area between albumins and the transferrins several different zones were possible to demonstrate at pH 8.5. Most of the zones were located between the e zone and the albumins at pH 5.4. The k zone showed a uniform appearance in different samples. On the other hand the staining intensity and the migration rate of several other zones, for instance the f, g, and m zones, were rather varying

among the plasma samples, so it is difficult to make a good diagram of them. These variations may have a genetic basis. However, a reliable classification of the different types seemed not to be possible without some modification of the technique.

Neuraminidase treatment: When some plasma samples were used as references several times a small change was noticed in the patterns of the prealbumin and the esterase types. For instance, in a fresh sample of prealbumin type LL, the l_3 zone is very faint or lacking but after the sample has been frozen and thawed several times or stored in the refrigerator several days, the l_3 zone becomes stronger while the intensity of the l_1 zone decreases. A similar change in staining intensity so that the fast migrating zones became fainter and the slow ones more distinct occurred in other prealbumin types and also in the esterase types.

In order to interpret these results, a portion of some plasma samples was treated with the enzyme neuraminidase as described by GAHNE (1963). The treated portions were then taken for starch gel electrophoresis together with untreated samples as controls. The digestion reduced the migration rate of the prealbumin zones at least one step but the relative migration rates of zones of different types were not changed. A similar effect was demonstrated in regard to the esterase fractions (Figure 9).

Neuraminidase is known to remove selectively sialic acids from macromolecules such as glycoproteins. The conclusion may therefore be that the prealbumins

FIGURE 9.—A photograph from a starch gel electrophoresis experiment at pH 5.4 of a normal esterase type I (to the right) and the same sample after treatment with neuraminidase (to the left).

and the esterases contain sialic acids and that the migration rates are slowed down when the sialic acids are removed from the molecules by enzyme digestion or by a degrading treatment. Accordingly, caution is necessary when samples are classified which are not in good condition. On the other hand the neuraminidase treatment seemed not to have any influence on the albumin phenotypes.

DISCUSSION

In some plasma samples a somewhat deviating pattern of the albumin types was observed in that the normally rather faint zone in front of the thick albumin zone had increased much in staining intensity. For instance the homozygous type SS had a pattern closely similar to heterozygous type FS. All those deviating samples seemed to be collected on the same occasion. New samples were obtained from most of these animals and the albumin patterns were then quite normal. Therefore, the probable explanation is that some unknown conditions may have a certain effect on the albumin molecules so that the patterns of the albumin phenotypes are changed. ASHTON (1964) obtained similar deviating albumin phenotypes in cattle after storage of the sera. However, in horses normal storage conditions did not seem to have any influence on the appearance of the albumin phenotypes.

When samples from animals heterozygous for the Es^o allele were compared with samples from animals of the homozygous types FF and II , no differences could be observed in staining intensity of the esterase zones. In the esterase type O the loss of the Es zones did not result in an increased intensity of the Ch esterase zone or the other esterase zones in the area between the Es and Ch zones. Nor was any increase of esterase activity observed at the sample insertion place. Two blood samples at an interval of half a year were taken from the animal of the type O and both samples gave the same result at starch gel electrophoresis. The cause of the lack of the Es zones in the O type may therefore be that the Es^o allele changes the enzymatic characteristics of the Es esterase in regard to substrates and inhibitors or that the enzyme becomes especially sensitive. Another explanation could be that the Es^o allele gives rise to an enzymatically inactive protein.

Several different types of esterases are known to occur in animal plasma and they can be characterized by using different substrates and inhibitors. The only experiment made here was the adding of eserine to the substrate, which inhibited the slow moving esterase fraction Ch , while the genetically varying zones were not affected. OKI *et al.* (1964) demonstrated a broad rather fast migrating esterase zone, called B, which they characterized as aliesterase. When the zymograms of OKI *et al.* are compared with those obtained in the present study, it seems very likely that their B zone is identical with the Es zones, which should accordingly be aliesterases.

The main characteristics of the aliesterases are that they hydrolyze both the short and long chain fatty acid esters but are not particularly reactive with aromatic esters. They are inhibited by organophosphorous compounds but not by eserine, $10^{-5}M$, (AUGUSTINSSON 1961). However, little is known about their function. Therefore, it is impossible to predict the effect the Es^o allele may have

on its carrier, if the Es^o allele gives rise to an inactive enzyme. On the other hand the animal of the recessive esterase type O may show how the organism compensates the loss of the Es esterase zones, but as yet no physiological examination has been made of the only known animal of the type O.

In spite of the relatively small size of the Salernitan horse population, there is a great genetical variation in several biochemical characteristics. In the present paper six transferrin alleles, two albumin, four prealbumin and four esterase alleles have been demonstrated and in a study of the erythrocyte antigens PODLIACHOUK, SALERNO and LABERT (1965) reported the occurrence of 11 blood factors in the same breed.

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SUMMARY

By means of starch gel electrophoresis 16 transferrin, 3 albumin, 8 prealbumin and 6 esterase phenotypes are demonstrated in the plasma of 147 horses of the Salernitana horse breed. Family data are consistent with the interpretation that the prealbumin phenotypes are controlled by four codominant autosomal alleles, designated Pr^F , Pr^I , Pr^L and Pr^S and that the esterases phenotypes are controlled by four autosomal alleles, of which Es^F , Es^I and Es^S are codominant and Es^o is recessive. In the transferrin system the alleles Tf^D , Tf^F , Tf^H , Tf^O and Tf^R are found. It is shown that the allele Tf^F can be divided into two alleles designated Tf^{F^1} and Tf^{F^2} . An improved technique for the albumin separation is presented.

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