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STUDIES ON BLOOD AND SERUM TYPES OF THE ICELANDIC HORSES

By
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In recent years immunogenetic investigations of the red cell antigens and starch gel electrophoretic separations of polymorphous blood proteins have shed light on several genetic systems in cattle, swine, horses, sheep and chickens. Because of their well known mode of inheritance these characters are now widely used in the registration control and in general genetical studies.

Extensive blood and serum type studies of cattle in Scandinavia have been carried out in Norway (*Brænd* 1959), Sweden (*Rendel* 1958; *Gahne* 1961), Iceland (*Brænd et al.* 1962), Finland (*Maijala & Lindstrøm* 1965; *Vasenius* 1965), and Denmark (*Neimann-Sørensen* 1958; *Brummerstedt-Hansen et al.* 1963; *Hesselholt & Moustgaard* 1965). Likewise, investigations on the porcine blood group and serum protein polymorphism have been performed in Denmark (*Andresen* 1963; *Moustgaard & Hesselholt* 1966).

Research on the identification of the equine blood and serum types has been carried out at the same time in our laboratory. This report is concerned with results from these studies. Family data will also be given which will elucidate the genetic transmission mode of the equine blood and transferrin types. Finally, the distribution of the causative genes in a population of Icelandic horses will be presented.

BLOOD TYPES

The blood group research of horses, in the first half of this century, was strongly influenced by the extensive blood group investigations in man, which followed Landsteiner's discovery in 1900 of naturally occurring isoagglutinins in human serum against erythrocytes (*Landsteiner* 1900). Several attempts were made to classify equine erythrocytes by means of naturally occurring isoagglutinins. This period in the history of horse blood grouping, which is characterized by a lack of an international nomenclature, is described in detail elsewhere (*Podliachouk* 1957; *Hesselholt* 1961).

Podliachouk has isolated 13 specific agglutinins corresponding to the blood group factors A—M. Genetic data tended to show that the blood factors belonged to 13 one-factor, two-allele blood type systems, although a strong association was observed between the A and F systems.

It was discovered that hemolytic disease of the newborn foal was due to transplacental isoimmunization of mares during pregnancy caused by red cell incompatibility between mares and affected foals (*Coombs et al.* 1948; *Bruner et al.* 1948); this initiated blood group studies of horses in England. From sera of such immunized mares 11 blood typing reagents, anti 1 — anti 11, were isolated. Family investigations showed close linkage between the 1, 2, 5, 8, and 11 genes and between the 6 and 10 genes, which were identical with Podliachouk's A and F genes (*Franks* 1959, 1962).

Preliminary Danish investigations of normal horse sera showed that the naturally occurring isoagglutinins seemed to be only slightly active and gave non-specific agglutination reactions. Furthermore, as their number seemed to be limited, it was decided to produce, by means of iso- and heteroimmunizations, specific antibodies to be used for blood typing of horses. Seven blood typing reagents, anti A — anti G, were isolated. All of the specific immune antibodies were found to be complete agglutinins applicable for the direct agglutination test in saline medium (*Hesselholt* 1961). During 1961 at the Pasteur Institute, comparative studies were made between French and Danish reagents, and a nomenclature was established (Table 1). This nomenclature is now used in horse blood grouping (*Podliachouk & Hesselholt* 1962a). Since 1961 further 3 reagents have been produced in our

Table 1. Comparison between French and Danish reagents and the nomenclature proposed for the equine blood groups. (*Podliachouk & Hesselholt* 1962 a).

France	A	B	C	D	E	F	—	H	—	J	—	L	M
Denmark	B	—	A	D	—	—	C	E	G	—	F	—	—
Nomenclature prop.	A_2	B	C	D	E	F	G	H	I	J	K	L	M

Table 2. Reagents used for blood typing of horses in the Danish laboratory.

Reagent	Nature of antibody	Source of antibody
A_2	agglutinin	isoimmune
	hemolysin	isoimmune
C	agglutinin	heteroimmune (cattle)
	hemolysin	isoimmune
D	agglutinin	isoimmune or swine normal serum (*Podliachouk & Hesselholt* 1962 b)
E	agglutinin	isoimmune
G	agglutinin	isoimmune
H	agglutinin	isoimmune
I	agglutinin	isoimmune
J*)	agglutinin	horse normal serum
K	agglutinin	isoimmune
Da 1	agglutinin	isoimmune
Da 2	agglutinin	isoimmune

*) The J reagent was kindly provided by Dr. L. Podliachouk.

laboratory, namely the E, Da_1 and Da_2 reagents (Table 2). Da_1 and Da_2 are preliminary designations (*Hesselholt et al.* 1965).

In a comprehensive study on blood groups of the Shetland pony and the Thoroughbred horse *Stormont et al.* (1964) described 16 blood group factors detected by means of iso- and heteroimmune sera. Eight blood group systems were later established. The A system contained the factors A_1, A_2, A′, and H; the D system had the factors D and J; the P system had P_1, P_2 and P′ and the Q system had the factors Q, R and S. The blood factors C, K, T, and U represented one-factor, two-allele systems (*Stormont & Suzuki* 1964).

MATERIAL AND METHODS

Serological tests

Blood samples for type determinations were drawn in either Alsever solution or a cyanide-citrate anticoagulant (sodium citrate 40 g, sodium chloride 25 g, sodium cyanide 2 g, dist. water 5 l).

Initially the direct agglutination test was made as a traditional tube test (*Andresen* 1963). Later the test was performed in a rectangular plastic sheet with a grid of 96 cylindrical cells moulded in it for erythrocytes and antiserum. One drop (0.05 ml) of an approximately 2 per cent cell suspension was thoroughly mixed with one drop of the antiserum. After 2 hours of incubation at room temperature the aggutination reactions were read macroscopically. Second readings were performed 1 hour later. During incubation the plastic sheets were covered with damp cloths to prevent evaporation of the mixtures. The agglutination reactions were designated by the system proposed by *Andresen* according to which '4' symbolized one complete mass of agglutinated cells. The minor grades of agglutination were designated 3+, 3, 2+, 2, 1+, 1, tr. Reactions which were barely visible were described with 0+. 0 was negative reaction. Emphasis was placed on the need to prepare blood typing reagents which under the above conditions showed 3+ or 4 reactions.

Production of blood typing reagents

31 isoimmunizations and 11 heteroimmunizations (2 in cattle and 9 in rabbits) were performed. The donors were Icelandic, Norwegian Fjording, Frederiksborg and Oldenborg horses.

The isoimmunization procedure was carried out by intramuscular injection with 10 ml of the 3 times washed and packed red cells in the cranial part of the superficial pectoral muscle. The intramuscular technique was used in preference to the intravenous injection to prevent serious transfusion reactions. Only a few cases of urticaria were observed following intramuscular injections. The immunizations were carried out twice weekly for 2—3 weeks, depending on the time of appearance of antibodies. Reimmunizations were performed by 2 weekly injections, 1 year after the initial immunizations. Generally, a satisfactory antibody titer (1/16—1/128) was obtained 10—14 days after the last injection. Since a significant decline in the titer of the complete immuneagglutinins could occur in 48—72 hours, it was essential to follow the antibody formation in the recipients by taking samples for titer tests 3—4 times weekly. The heteroimmunizations in cattle were carried out in the same way as the isoimmunizations. The rabbits were injected with 2 ml of the 3 times washed and packed erythrocytes intramuscularly every second day. The courses consisted of from 7—10 injections and the animals were usually bled by heart puncture 1 week after the last injection (*Neimann-Sørensen* 1958).

In order to destroy complement activity the polyvalent immune sera were incubated at 56°C for 30 min., and they were subsequently

fractionated by traditional absorptions. The absorptions took place in 2 steps. As a rule equal amounts of 3 times washed and packed erythrocytes and antiserum were mixed. During the first step the mixture was incubated at room temperature for 30 min. The second absorption involved incubation at room temperature for 30 min. followed by incubation at 4°C for 30 min. Due to differences in the strength of antibodies this technique was often varied, especially the relative amounts of cells and serum. The test for unity was performed with 20—25 positive individuals.

By means of these methods 10 monovalent antisera, blood typing reagents, were isolated (Table 2). The specific antibodies are complete agglutinins applicable for the direct agglutination test in saline medium. The immune sera were tested against donor cells by means of the agglutination test in bovine albumin medium and the hemolytic test where normal guinea-pig serum diluted 1/10 was used as complement; it was observed then that some sera contained incomplete agglutinins and hemolysins. No attempt was made, however, to isolate these antibodies.

By means of the reagents listed in Table 2 blood type determinations were made of a family material comprising 28 stallions and 263 mares with 313 offspring. A population material of 925 horses, randomly sampled from 3 localities in Iceland, were also typed. All the animals belonged to the Icelandic horse breed.

RESULTS AND DISCUSSION

Genetics of horse blood groups

In order to show whether there is dominant or recessive inheritance of the 11 blood factors the family data were classified in mating classes in Table 3. The table demonstrates that the distribution of the progeny blood factors in all the matings, except 7, is in agreement with the law of dominant inheritance. It is of special importance that the mating type, negative $\times$ negative, can give rise to negative offspring only. Exceptions to this rule were actually observed in 6 matings (marked in the table with an asterisk). These exceptions, which must be ascribed to erroneous registration, will be discussed in a later section of the present report. The H factor was not observed in Icelandic horses, but American investigations have shown that this factor is transmitted as a dominant character (*Stormont & Suzuki* 1964).

In Table 4 analyses of stallion families in which heterozygous stallions were mated to double recessive mares are listed. Because of its rare occurrence the G antigen is omitted in the table. The expected number of progeny phenotypes was estimated by means of *Bernstein*'s (1929) "a priori" method. It is observed that a

Table 3. Inheritance of the single blood type factors. A study of 313 matings. For the I antigen only 201 matings have been observed.

Blood group factor	Mating type	No. of matings	No. of progeny	
			+	—
Da_2	+ × +	2	1	1
	+ × —	55	31	24
	— × —	256		256
A_2	+ × +	26	19	7
	+ × —	92	50	42
	— × —	195	1*	194
C	+ × +	128	119	9
	+ × —	148	98	50
	— × —	37		37
D	+ × +	14	8	6
	+ × —	84	39	45
	— × —	215	2*	213
E	+ × +	10	9	1
	+ × —	77	40	37
	— × —	226	3*	223
G	+ × +			
	+ × —	11	3	8
	— × —	302		302
I	+ × +	108	95	13
	+ × —	47	23	24
	— × —	46		46
J	+ × +	1		1
	+ × —	56	24	32
	— × —	256		256
K	+ × +	21	15	6
	+ × —	142	70	72
	— × —	150		150
Da_1	+ × +	102	89	13
	+ × —	158	79	79
	— × —	53	1*	52

good agreement was found between the expected and the observed number of progeny phenotypes.

From Tables 3 and 4 it was concluded that each of the 11 blood factors is controlled by a single, dominant gene.

The problem of allelism or nonallelism of the causative genes was solved by means of analyses of family and population data. The following 8 blood group systems were tentatively established.

Table 4. Analysis of stallion families in which positive stallions were mated to one or more negative mares. The material is taken from stallion families in which at least one offspring was negative.

Mating type	No. of offspring				χ^2 1 d. f.
	lacking the factor		having the factor		
	obs.	exp.	obs.	exp.	
Da_2 positive ♂ × Da_2 negative ♀ .	16	14.0	12	14.0	0.57
A_2 positive ♂ × A_2 negative ♀ .	10	8.0	6	8.0	1.0
C positive ♂ × C neagtive ♀ .	18	19.8	18	16.2	0.36
D positive ♂ × D negative ♀ .	20	18.9	17	18.1	0.13
E positive ♂ × E negative ♀ .	12	12.1	12	11.9	0.00
I positive ♂ × I negative ♀ .	13	17.4	19	14.6	2.44
J positive ♂ × J negative ♀ .	15	15.2	15	14.8	0.01
K positive ♂ × K negative ♀ .	35	38.1	40	36.9	0.49
Da_1 positive ♂ × Da_1 negative ♀ .	39	36.3	30	32.7	0.42

The A system. Blood type determinations of the above mentioned material gave the evidence that the antigens A_2 and Da_2 were related to each other through a linear subgroup relationship. Absorptions of the A_2 reagent and the subsequent tests exhibited a pattern, which was typical for a linear subgroup relationship between the 2 factors. As pointed out by *Stormont et al.* (1964) a linear subgroup relationship between 2 blood factors provides sufficient evidence for the conclusion that the factors belong to the same blood group system. Maximum information about allelism or nonallelism is provided by studies of data from double backcross matings. Such matings are listed in Table 5. The table also shows an interdependent segregation of the factors A_2 and Da_2.

Table 5 demonstrates that the transmission of the antigens A_2 and Da_2 is independent of the factors E, I, K, and Da_1. The lack of a relevant family material necessitated the use of two-by-two contingency tables with the application of population data, in order to determine the association between the factors A_2 and Da_2 and the remaining antigens. The following results were obtained: A_2—C (278 horses), $\chi^2 = 0.26$; A_2—D (278 horses), $\chi^2 = 0.02$; A_2—G (539 horses), $\chi^2 = 0.32$.

These results support the theory that A_2 and Da_2 belong to the same blood group system, the A system.

It has been mentioned above that the H factor was not obser-

Table 5. Distribution of progeny after 23 types of double back-cross matings. Only factor designations relevant to the test for allelism are indicated.

Mating type	+	+	+ +	− −
A_2/−, Da_2/− × −/−, −/−			14	12
A_2/−, E/− × −/−, −/−	5	3	10	7
A_2/−, I/− × −/−, −/−	1	1	1	1
A_2/−, K/− × −/−, −/−	5	2	1	1
A_2/−, Da_1/− × −/−, −/−	2	5	2	3
C /−, D/− × −/−, −/−	5	2	1	4
C /−, E/− × −/−, −/−	3	2	2	5
C /−, G/− × −/−, −/−	1	1	1	2
C /−, I/− × −/−, −/−	3	1	1	2
C /−, J/− × −/−, −/−	5	4	3	3
C /−, K/− × −/−, −/−	6	5	5	9
C /−, Da_1/− × −/−, −/−	6	4	6	5
D /−, I/− × −/−, −/−	5	2	2	2
D /−, K/− × −/−, −/−	7	3	4	5
D /−, Da_1/− × −/−, −/−	5	7	5	4
I /−, K/− × −/−, −/−	4	2	1	3
I /−, Da_1/− × −/−, −/−	3	6	3	5
J /−, K/− × −/−, −/−	4	5	3	2
J /−, Da_1/− × −/−, −/−	7	8	7	7
K /−, Da_1/− × −/−, −/−	3	7	6	4
E /−, Da_1/− × −/−, −/−	3	3	2	4
I /−, J/− × −/−, −/−	2	1	1	2
E /−, I/− × −/−, −/−	1	2	1	1

ved in Icelandic horses. The factors H and A_2 were therefore treated in two-by-two tables. A material consisting of 186 unrelated Danish horses was used. The χ^2 value obtained, 17.78, supports the theory put forward by *Stormont & Suzuki* that the H antigen belongs to the A system.

The C system. The above mentioned investigations showed that the C antigen is transmitted independently of the A system. Table 5 indicates that there is no association between the C factor and the other 10 blood factors under investigation. Thus the C antigen belongs to 1 blood group system, which consists of C^c and C^-. C^- symbolizes the recessive allele.

The D system. According to *Stormont & Suzuki* the D system contains the factors D and J. These antigens were relatively rare among Icelandic horses, so our material is insufficient for a definite establishment of the D system.

The results from the decription of the A and C systems and the data listed in Table 5 show that the factors D and J are inherited independently of the antigens A_2, Da_2, C, I, K, and Da_1. The relationship to the remaining factors was elucidated by means of two-by-two tables. D—G (278 horses), $P^* = 0.31$ (Fisher-Yates exact treatment); D—E (278 horses), $\chi^2 = 0.79$.

The E system. Table 5 and the results already mentioned demonstrate a lack of association between the E antigen and the factors A_2, Da_2, C, D, J, I, and Da_1. Two-by-two tables with the remaining factors gave the following results. E—G (401 horses), $\chi^2 = 1.99$; E—K (401 horses), $\chi^2 = 0.002$. The E antigen apparently belongs to one blood group system, which consists of 2 alleles, E^e and E^-.

The G system. The G antigen seems to segregate independently of the above mentioned systems. The relationship to the antigens I, K and Da_1 was studied by means of two-by-two tables. G—I (401 horses), $\chi^2 = 0.56$; G—K (401 horses), $\chi^2 = 0.11$; G—Da_1 (401 horses), $\chi^2 = 0.58$. Until further data are obtained the G antigen is considered to belong to one blood group system, with the alleles G^g and G^-.

The T, K, and Da_1 systems. Table 5 and the results already mentioned tended to show that the blood factors I, K and Da_1 belong to 3 one-factor, two-allele blood group systems.

Exceptions to the genetic theory

It has already been mentioned in the description of Table 3 that 7 exceptions to the genetic theory were observed. The exceptions were found in 6 matings. In all of the 6 matings the progeny possessed blood factors which were not detected in the parents. During our investigations in Iceland more cases of exceptions to the genetic theory were observed. When repeated type determinations, however, were carried out with new samples, or when additional information was provided, causal explanations were always found. In the 7 matings, listed in Table 6, neither new samples nor additional information were provided. The exceptions were therefore considered to be caused by erroneous registrations. The cases are demonstrated in Table 6 in order to show the application of horse blood groups in the parentage control.

In case 1 (Table 6) the foal had the factor E and in case 2 the

Table 6. Exceptions to the genetic hypothesis (see text).

Case	Individuals in matings		Blood group systems								
			A	C	D	E	G	I	K	Da_1	Tf type
	Stallion Si.	(80)	–/–	–/–	D/–	–/–	–/–	I/–	–/–	–/–	FR
1	Mare Gu.	(87)	A_2/	C/	D/	–/–	–/–	I/	K/	Da_1/	OR
	Foal Fr.	(88)	A_2/	C/	–/–	E/	–/–	–/–	–/–	–/–	DH
2	Mare Gu.	(94)	A_2/–	C/	–/–	–/–	–/–	I/	K/–	–/–	OO
	Foal Th.	(95)	–/–	C/	–/–	E/	–/–	I/	K/	Da_1/	RR
	Stallion Sv.	(153)	–/–	C/–	D/–	–/–	–/–	–/–	K/–	–/–	FO
3	Mare So.	(58)	–/–	–/–	–/–	E/	–/–	I/	–/–	Da_1/	DF
	Foal Bl.	(59)	A_2/	–/–	–/–	E/	–/–	I/	–/–	Da_1/	DD
	Stallion Gr.	(91)	–/–	–/–	–/–	–/–	–/–	–/–	–/–	Da_1/	FF
4	Mare Hr.	(10)	–/–	C/	J/	–/–	–/–	I/	–/–	Da_1/	HR
	Foal Bi.	(11)	–/–	–/–	J/	E/	–/–	–/–	–/–	–/–	DR
	Stallion Ba.	(319)	A_2/–	–/–	–/–	–/–	–/–	I/–	–/–	–/–	DF
5	Mare Lj.	(299)	A_2/	C/	–/–	E/	–/–	I/	–/–	–/–	FH
	Foal Li.	(300)	A_2/	C/	D/	E/	–/–	I/	–/–	–/–	FH
	Stallion Od.	(215)	–/–	C/–	–/–	–/–	–/–	I/–	K/–	Da_1/–	DD
6	Mare R. Sk.	(180)	–/–	C/	–/–	–/–	–/–	–/–	–/–	Da_1/	DF
	Foal Sk.	(179)	–/–	C/	D/	–/–	–/–	–/–	K/	Da_1/	DD

factors E and Da_2 were observed in the foal; neither factors were present in the parents. These foals were from the same stallion family. In case 3 the foal possessed the factor A_2, in case 4 the factor E, in cases 5 and 6 the factor D; these factors were not found in the respective parents. Table 6 also shows that the transmission of the transferrin types in cases 1—4 was not in agreement with the Mendelian inheritance law of these characters.

Gene frequencies in Icelandic horses

Estimations of gene frequencies were carried out on the basis of blood type determinations of 925 unrelated horses. These samples were taken from 3 localities in Iceland. 292 samples were from the neighbourhood of Skagafjord (subpopulation I), 201 samples were from the neighhourhood of Borgfjord (subpopulation II) and 432 samples were from the neighbourhood of Blønduos (subpopulation III). Because of a shortage of the J reagent only 138 horses from Skagafjord (subpopulation V) and 140 horses from Borgfjord (subpopulation VI) were typed with this reagent. 150 unrelated parental horses (subpopulation IV)

Table 7. χ^2-test for homogeneity of 3 horse subpopulations with respect to the blood group factors A_2, C, E, G, I, K, Da_1 and Da_2.

Sub-populations	No. of animals in sub-populations	No. of positive animals in subpopulations							
		Da_2	A_2	C	E	G	I	K	Da_1
I	292	10	53	182	75	1	137	91	186
II	201	6	36	129	44	8	115	61	106
III	432	23	99	254	122	6	214	109	243
χ^2 for 2 d.f.		2.25	3.41	1.88	3.05	2.7	5.16	3.52	4.9

were therefore incorporated in the gene frequency determinations of the alleles belonging to the D system. The subpopulations were subjected to χ^2-tests for homogeneity with respect to the blood group factors (*Snedecor & Irwin* 1932). The Tables 7 and 8 demonstrate the homogeneity of the subpopulations with respect to the blood factors under study. Consequently the subpopulations were added together and the entire population was used as the basis for gene frequency estimations.

Table 8. χ^2-tests for homogeneity of 3 horse subpopulations with respect to the blood group factors D and J.

Sub-populations	No. of animals in sub-populations	No. of positive animals in subpopulations	
		D	J
IV	150	23	12
V	138	21	8
VI	140	19	9
χ^2 for 2 d.f.		0.23	0.52

The gene frequencies are listed in Table 9. The frequencies of the one-factor, two-allele blood type systems were estimated by means of the simple square root method. The effective method of *Bernstein* was applied for the alleles of the D system.

Test for Hardy-Weinberg equilibrium was not made in the blood group systems, but the entire population was found to be in equilibrium with respect to the alleles for the transferrin system.

Table 9. Estimates of gene frequencies of 8 equine blood group systems in the Icelandic horse.

Blood group system	No. of animals tested	Alleles	Gene frequencies
A	925	A^{da_2}	0.02
		A^{a_2}	0.11
		A^{h}	0.00
		A	0.89
C	925	C^{c}	0.38
		C	0.62
D	428	D^{d}	0.08
		Dj	0.03
		D	0.89
E	925	E^{e}	0.14
		E	0.86
G	925	Gg	0.01
		G	0.99
I	925	I^{i}	0.30
		I	0.70
K	925	K^{k}	0.15
		K	0.85
DA	925	DA^{da_1}	0.35
		DA	0.65

SERUM TYPES

Individual serum protein variations in horses were first observed by *Ashton* (1958) in a starch gel electrophoretic study of 19 horse sera. Although some of the differences seemed to be grouped, no attempt was made to determine the genetic basis of the observed variations. Six different transferrin phenotypes in horse sera were later demonstrated, and a hypothesis of 3 alleles as the basis for the observed polymorphism was advanced (*Schmid* 1962). Comprehensive starch gel electrophoretic studies of American and Norwegian horse sera revealed 16 transferrin phenotypes. Genetic investigations supported the theory that the observed differences were attributed to the action of 6 codominant alleles, designated Tf^{D}, Tf^{F}, Tf^{H}, Tf^{M}, Tf^{O}, and Tf^{R} (*Brænd & Stormont* 1964; *Brænd* 1964). In preliminary studies on transferrin types of Icelandic horses, 21 phenotypes were observed. Analyses of a limited family material supported the above six-allele theory (*Hesselholt* 1964; *Graetzer et al.* 1965).

MATERIAL AND METHODS

Plasma and serum samples from 28 stallions, 263 mares and from 313 of their progeny, all of the Icelandic horse breed, were subjected to horizontal starch gel electrophoresis.

A modification of the discontinuous buffer system originally described by *Poulik* (1957) was used. Gel buffer: 1.73 g/l Sigma 7—9 (tris) and 0.58 g/l citric acid, pH: 7.6. Vessel buffer: 0.3 M boric acid and 0.1 M sodium hydroxide, pH: 8.6; starch conc. (Danish starch): 14 %. Voltage gradient: 15—25 v/cm. Staining procedure: The gels were immersed either for 15 min. in a saturated solution of amido-black 10 B or for 15 min. in a 0.05 % solution of nigrosin. As solvent and washing solution, methanol-water-glacial acetic acid was used (*Smithies* 1959).

During the initial investigations it was found essential to standardize the electrophoretic procedure. The voltage gradient was about 15 v/cm during the first 30 min. The voltage was then increased to about 25 v/cm. No attempt was made to cool the gels during electrophoresis. The electrophoresis was discontinued when the boundary was 2 cm from the anodic bridge. Three reference samples were inserted in each gel.

RESULTS AND DISCUSSION

After starch gel electrophoresis of the above mentioned material 21 different types of transferrin pattern were observed (Fig. 1). Six electrophoretically different transferrin components occurred in the horse sera investigated. The fast component was designated D according to the nomenclature of *Brænd & Stormont* (1964). With decreasing mobility the remaining components were called F, H, M, O, and R. Each component consisted of a relatively broad band followed by a narrow band. The first 6 samples in Fig. 1 represent sera with only one transferrin component. Samples no. 7—21 represent the different combinations between the first 6 types. No samples were devoid of transferrin.

On the basis of the above findings the theory was advanced that the synthesis of serum transferrin in horses is determined by 6 codominant alleles. As our observations were in good agreement with those of *Brænd & Stormont*, the alleles were designated in accordance with the nomenclature proposed by these authors.

In order to test the genetic hypothesis, the family data were analysed. In 304 of the 313 matings, which represented 85 mating types, we found in no case progeny phenotypes, which were not expected from the parental transferrin types. The remaining 9 cases were exceptions to the genetic theory. Four of the 9 cases were also exceptions to the genetic theory with respect to the

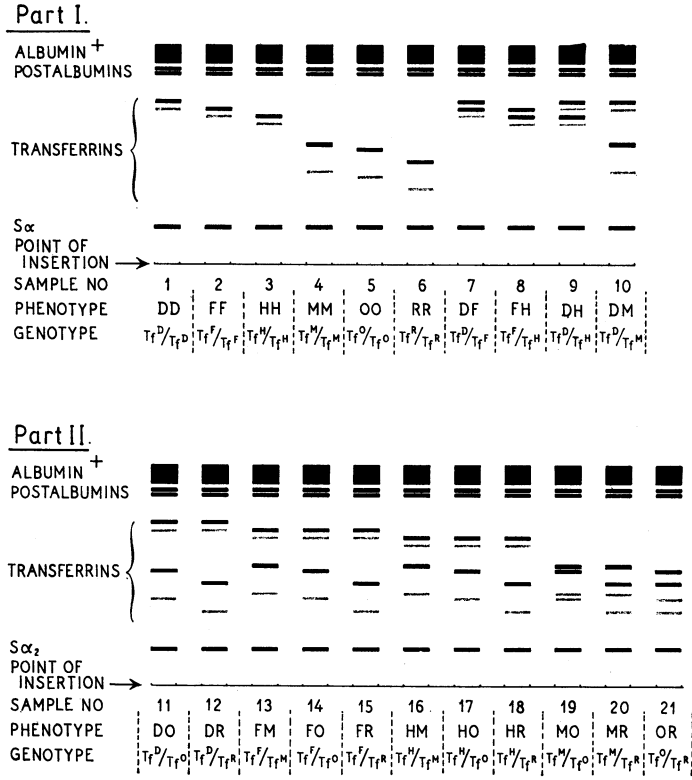

Figure 1. Diagrammatic representation of the 21 transferrin phenotypes observed in horse sera by means of starch gel electrophoresis.

inheritance of the red cell antigens under study (see Table 6). In none of the 9 cases neither new samples nor additional information of the pedigree were obtained. The cause of the exceptions was therefore ascribed to erroneous registration.

According to the above mentioned six-allele theory, samples no. 1—6 in Fig. 1 represent homozygous individuals, while samples no. 7—21 represent heterozygotes.

On the basis of the genetic theory, gene frequencies were estimated by means of simple gene counting.

The following frequencies were obtained among 925 Icelandic horses:

$$q_{Tf}D = 0.20$$
$$q_{Tf}F = 0.27$$
$$q_{Tf}H = 0.07$$
$$q_{Tf}M = 0.01$$
$$q_{Tf}O = 0.25$$
$$q_{Tf}R = 0.20$$

Table 10. The observed and on basis of gene frequencies expected distribution of transferrin phenotypes among 925 Icelandic horses.

DD		FF		HH		MM		OO		RR		DF	
obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.
39	37.0	64	67.4	3	4.5	2	0.9	60	57.8	41	37.0	104	99.9

DH		DM		DO		DR		FH		FM		FO	
obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.
24	25.9	3	3.7	90	92.5	71	74.0	37	35.0	6	5.0	120	124.9

FR		HM		HO		HR		MO		MR		OR	
obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.
105	99.9	1	1.3	29	32.4	28	25.9	3	4.6	4	3.7	91	92.5

$\chi^2 = 3.51$ 2 d.f.

In Table 10 the observed and, on the basis of gene frequencies, expected distribution of transferrin phenotypes in the population material are listed. The deviations, which are observed in Table 10, were not significant ($\chi^2 = 3.51$; 2 d.f.).

The haemoglobin-binding α_2-globulins, the haptoglobins, were studied in about 100 horse sera by using the method described elsewhere (*Hesselholt* 1963). One haptoglobin band was observed in the sera. The band, which showed the same electrophoretic mobility in all cases, was situated on the starch gel in the area of the fast transferrin. These observations were in good agreement with those of *Brænd & Stormont.*

The equine ceruloplasmin was investigated in about 100 sera by means of the method described by *Graetzer et al.* (1965). One ceruloplasmin band, which was situated on the gel between the fast transferrins and albumin, was observed in each serum under study.

Starch gel electrophoresis of about 300 sera did not reveal individual variations of serum amylase in the Icelandic horse breed. The amylase pattern observed consisted of 2 bands, a fast faint band and a slow relatively strong band. The bands were situated in the area between the starting line and the slow transferrin (*Hesselholt et al.* 1966).

The above findings of identical haptoglobin, ceruloplasmin and serum amylase pattern in the Icelandic horse do not exclude the possibility of polymorphism in other horse breeds.

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SUMMARY

By means of isoimmunizations and heteroimmunizations 10 equine blood typing reagents were isolated. The specific antibodies were complete agglutinins, which were used in the direct agglutination test in saline medium. The reagents were designated A_2, C, D, E, G, H, I, K, Da_1, and Da_2 reagent. Da_1 and Da_2 are preliminary designations.

The data obtained from blood typing of a family material and a population material of Icelandic horses showed that the occurrence of each blood type factor is controlled by a single, dominant gene. The family data tended to show that the blood factors under investigation belonged to 8 blood type systems. The A system contained the antigens A_2 and Da_2. These antigens are related to each other through a linear subgroup relationship. The D system had the factors D and J. The C, E, G, I, K, and Da_1 systems are one-factor, two-allele blood type systems. The H factor was not observed in Icelandic horses. In connection with the establishment of the 8 blood type systems it must be emphasized that the problem of allelism or nonallelism of 2 genes can only be solved by means of relevant family data. Because of the rare occurrence of some of the blood factors in the Icelandic horse such data were in some cases not available. Thus some conclusions were based on results from two-by-two contingency tables with the use of population data. This was used particularly for the D and G systems, and additional family data are necessary for a definite establishment of these systems.

Exceptions to the genetic theory, apparently caused by erroneous registration, were presented.

Finally, estimates were given of gene frequencies of the causative genes among Icelandic horses.

Starch gel electrophoresis of sera from Icelandic horses revealed the existence of 21 transferrin phenotypes. The data obtained sup-

ported the theory advanced, that transferrin polymorphism in horses is controlled by 6 autosomal codominant alleles: Tf^D, Tf^F, Tf^H, Tf^M, Tf^O, and Tf^R.

925 randomly selected Icelandic horses were typed for serum transferrin and the gene frequencies were estimated.

Starch gel electrophoresis of about 100 horse serum samples did not reveal individual variation of the equine haptoglobin and ceruloplasmin. Studies on approximately 300 sera showed an identical serum amylase pattern.

ZUSAMMENFASSUNG

Studien von Blutgruppen und Serumtypen bei isländischen Pferden.

Mit Anwendung von Iso- und Heteroimmunisierungen sind 10 Blutgruppenreagenzien, alle komplette Agglutinine verwendbar bei direkten Agglutinationstesten, isoliert worden. Die Reagenzien haben die Bezeichnungen A_2, C, D, E, G, H, I, K, Da_1 und Da_2 bekommen. Da_1 und Da_2 sind vorläufige Bezeichnungen.

Blutgruppenbestimmungen eines Familienmaterials und eines Populationsmaterials von isländischen Pferden zeigten, dass die Vererbung jedes Blutgruppenfaktors von einem einzelnen dominanten Gen kontrolliert wird. Genetische Daten haben bezeigt, dass die Blutgruppenfaktoren voraussichtlich 8 Blutgruppensystemen angehören. Das A-System umfasst die beiden Antigene A_2 und Da_2, die durch eine lineare Untergruppenverwandtschaft verwandt sind. Die Faktoren D und J gehören dem D-System an. Die C-, E-, G-, I-, K- und Da_1-Systeme sind ein-Faktor, zwei-Allel Blutgruppensysteme. Das H-Antigen wurde bei isländischen Pferden nicht observiert. Im Anschluss an die Etablierung der 8 Blutgruppensysteme soll stark hervorgehoben werden, dass das Problem Alleli oder nicht-Alleli zweier Gene nur mit Hilfe einer Analyse von relevanten Familiendaten gelöst werden kann. In einigen Fällen waren diese nicht vorhanden, weil bestimmte Antigene bei den isländischen Pferden sehr selten vorkommen. Einige Konklusionen wurden daher auf populationsgenetische Daten basiert. Dieses gilt besonders dem D- und G-System.

Die Genfrequenz wurde auf Grundlage der Populationsdaten berechnet.

Mit Hilfe der Stärkegelelektrophorese in Seren von isländischen Pferden wurden 21 Transferrinphänotypen nachgewiesen. Die angeführten Daten unterstützen die früher hervorgesetzte Hypothese, dass die Variationen, welche beim Pferd festgestellt worden sind, von 6 autosomalen kodominanten Allelen: Tf^D, Tf^F, Tf^H, Tf^M, Tf^O und Tf^R bedingt sind.

Die Genfrequenzbestimmungen wurden auf Grundlage der Daten von 925 zufällig ausgewählten Pferden berechnet.

Individuelle Variationen in bezug auf Haptoglobin, Ceruloplasmin und Serumamylase wurden nicht gefunden.

SAMMENDRAG

Studier over blod- og serumtyper hos islandske heste.

Ved hjælp af iso- og heteroimmuniseringer er der isoleret 10 blodtypereagenser, som alle er komplette agglutininer anvendelige i den direkte agglutinationstest. Reagenserne har fået betegnelserne A_2, C, D, E, G, H, I, K, Da_1 og Da_2 reagenser. Da_1 og Da_2 er foreløbige betegnelser.

Blodtypebestemmelse af et familiemateriale og et populationsmateriale af islandske heste viste, at nedarvnigen af hver blodtypefaktor er under kontrol af et enkelt dominant gen. Genetiske data har sandsynliggjort, at blodtypefaktorerne tilhører 8 blodtypesystemer. A-systemet indeholder 2 gennem et lineært undergruppeslægtskab beslægtede antigener A_2 og D_2. D-systemet består af faktorerne D og J. C-, E-, G, I-, K- og Da_1-systemerne er een-faktor, to-allel blodtypesystemer. H-antigenet blev ikke observeret hos islandske heste. Det må i forbindelse med etableringen af de 8 blodtypesystemer stærkt understreges, at problemet alleli eller ikke-alleli for 2 gener kun kan løses ved analyse af relevante familiedata. Sådanne har i nogle tilfælde ikke været tilstede på grund af visse antigeners sjældne forekomst blandt islandske heste. Nogle konklusioner er derfor baseret på populationsgenetiske data. Dette er især tilfældet med D- og G-systemet.

Der er givet nogle eksempler på undtagelser fra den genetiske teori, forårsaget af forkert registrering.

Genfrekvenserne er udregnet på grundlag af populationsdata.

Ved stivelsegelelektroforese af sera fra islandske heste fandtes 21 transferrinfænotyper. De fremkomne data understøtter den tidligere fremsatte hypotese, at de påviste variationer hos hesten er betinget af 6 autosomale kodominante alleler: Tf^D, Tf^F, Tf^H, Tf^M, Tf^O, Tf^R.

Genfrekvensbestemmelser blev udregnet på grundlag af data fra 925 tilfældigt udvalgte heste.

Der blev ikke fundet individuelle variationer i haptoglobin, ceruloplasmin og serumamylase.

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