GENETIC SYSTEMS OF BLOOD GROUPS IN HORSES[1]

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Received June 25, 1964

ALTHOUGH there are numerous reports on blood groups in horses, there is still relatively little information on the genetics of equine blood groups. The studies of PODLIACHOUK (1957, 1958) are undoubtedly the most extensive and, like those of FRANKS (1962), have the advantage of being contemporaneous. Utilizing naturally occurring isoagglutinins as her main source of blood-typing reagents, PODLIACHOUK described ten blood factors (A, C, D, E, F, G, H, I, J, and K) peculiar to horses and mules but apparently not found in asses. She concluded that none of the genes controlling those ten blood factors are alleles. Although she observed a strong association between blood factors A and F, that association was attributed to linkage. FRANKS, utilizing antisera from transplacentally isoimmunized mares as a source of blood-typing reagents, detected 11 equine blood factors which he designated by the numbers 1 through 11. He observed that 1, 2, 5, 8 and 11 were associated both in distribution and inheritance, and attributed the association to close linkage of the causative genes. He also mentioned that the genes for 6 and 10 belong to the A-F system described by PODLIACHOUK, but he made no statement relative to the inheritance of 3, 4 and 7.

In view of the extensive allelism associated with the control of blood groups in such species as man, cattle, chickens and sheep, the question has arisen whether multiple alleles might also be involved in the control of some of the blood groups in horses. Several years ago we undertook a program to develop our own battery of equine blood-typing reagents, one of the objectives being the use of those reagents in exploring for evidence of multiple alleles. We succeeded in developing 16 specifically different equine blood-typing reagents which are described in a foregoing report (STORMONT, SUZUKI and RHODE 1964). As pointed out in that report, our 16 reagents have been compared in parallel tests with 11 reference reagents provided by PODLIACHOUK and HESSELHOLT. Six of our reagents had equivalents among the 11 reference agents. The corresponding blood factors were therefore named in accordance with the international standards. They are A_1 (or A), C, D, H, J and K. The blood factors identified by our ten remaining reagents were given names which would not confuse them with previously described equine blood factors. They are A_2, A′, P_1, P_2, P′, Q, R, S, T and U. The purpose of the present report is primarily that of elucidating the genetic systems to which these 16 blood factors belong.

[1] Supported by grants from the American Shetland Pony Club, the California Thoroughbred Breeders Association and the Jockey Club.

MATERIALS AND METHODS

Blood samples for the family studies were obtained from one Appaloosa stallion, his 11 offspring and their mares, five Arabian stallions, their 26 offspring and their mares, three Quarter Horse stallions, their 14 offspring and their mares, 46 Shetland Pony stallions, their 351 offspring and their mares, 45 Thoroughbred stallions, their 214 offspring and their mares, and three Welsh Pony stallions, their 23 offspring and their mares; in all a total of 639 offspring. Because of the small number of representatives from the breeds Appaloosa, Arabian, Quarter Horse and Welsh Pony, the population studies were restricted solely to Shetland Ponies and Thoroughbreds. The samples for the population studies (391 Shetlands and 276 Thoroughbreds) consisted of all parental animals not related as siblings plus additional unrelated animals not involved in the family studies.

All blood samples were typed in accordance with procedures given by Stormont, Suzuki and Rhode (1964).

RESULTS

Inheritance of the individual blood factors: Table 1 is provided to show that each of the 16 equine blood factors, in contrast with its absence, appears to be inherited as a dominant trait. The few exceptions in Table 1 are accounted for elsewhere in this report.

Frequencies of the blood factors: Data on the frequencies of the blood factors in Thoroughbreds and Shetland Ponies are contrasted in Table 2. The two breeds differed significantly with respect to the frequencies of each of the 16 blood factors. Although the small size of the samples from the other breeds precludes

TABLE 1

Inheritance of the individual blood factors

	Mating types of parents					
	+ × +		+ × −		− × −	
	Number of offspring with (+) and without (−) blood factors					
Blood factors	+	−	+	−	+	−
A_1	294	30	154	88	0	73
A_2	504	13	105	15	0	2
A′	148	28	120	83	0	260
C	461	39	106	26	0	7
D	16	4	98	69	1*	451
H	14	2	80	79	2*	462
J	55	7	123	122	0	332
K	63	16	111	94	1*	354
P_1	207	48	157	119	0	108
P_2	250	50	158	119	0	62
P′	2	6	72	80	0	479
Q	183	41	197	92	0	126
R†	168	11	75	33	1*	23
S†	83	18	65	61	0	84
T	332	45	125	92	0	45
U	98	20	165	110	0	228

* Exceptions to the genetic theory (see text).
† Necessity to perform absorptions accounts for reduced totals on R and S.

TABLE 2

Frequencies of 16 blood factors in Shetland Ponies and Thoroughbreds

Blood factors	Shetland Ponies		Thoroughbreds	
	Number typed	Frequency	Number typed	Frequency
A_1	391	0.534	276	0.927
A_2	391	0.869	276	0.927
A′	391	0.583	276	0.057
C	391	0.879	276	0.927
D	391	0.250	276	0.000
H	391	0.181	276	0.007
J	391	0.227	276	0.278
K	391	0.327	276	0.123
P_1	391	0.567	276	0.369
P_2	391	0.621	276	0.503
P′	391	0.094	276	0.173
Q	391	0.519	276	0.797
R	316	0.911	185	0.611
S	316	0.354	185	0.632
T	391	0.698	276	0.884
U	391	0.534	276	0.275

comparison at this time, it should be mentioned that Arabians, as might be expected, appear to be much like Thoroughbreds. For example, the Arabians and Thoroughbreds were the only two breeds in which blood factor D was not encountered.

As may be noted in Table 3, our data on the frequencies of blood factors A_1 (or A), C, D and H in Thoroughbreds are in rather close agreement with the data obtained by PODLIACHOUK (1958) on French Thoroughbreds and PODLIACHOUK, KACZMERAK and ZWOLINSKI (1962) on Polish Thoroughbreds.

The A system of equine blood groups: As pointed out elsewhere (STORMONT 1961), subtyping relationships provide the strongest kind of evidence relating blood factors to the same genetic system. In a previous report (STORMONT *et al.*,

TABLE 3

Comparison of the frequencies of blood factors A_1 (or A), C, D, H, J, and K in three populations of Thoroughbreds

Blood factors	U.S. (276)	French* (162)	Polish† (100)
A_1	0.93	0.79	1.00
C	0.93	0.94	0.93
D	0.00	0.00	0.00
H	0.01	0.00	0.00
J	0.28	. . .	0.17
K	0.12	. . .	0.22

* Data of PODLIACHOUK (1958).
† Data of PODLIACHOUK *et al.* (1962).

1964), we have shown that blood factors A_1 and A′ are related through A_2 as nonlinear subtypes. It was expected, therefore, that A_1, A_2 and A′ would segregate as the products of allelic genes, as shown for A_1 and A′ in Table 4.

Family data also showed that blood factor H belongs to the same genetic system as A_1, A_2 and A′. With respect to the tests with reagents for blood factors A_1, A_2, A′ and H, eight phenotypes, namely, A_1, A′, H, A_1A', A_1H, $A_1A'H$ and — (no A or H), are defined. (In these notations blood factor A_2 is omitted because the reactions of A_2 antibodies were confined almost solely to bloods possessing either or both A_1 or A′. Occasionally A_2 antibodies reacted weakly with bloods lacking both A_1 or A′, but for the purposes of this report those weak reactions are ignored.) Twenty-seven of 36 possible kinds of matings involving the eight A system phenotypes were encountered in the family studies. The results are summarized in Table 4. The segregation of the phenotypes or groups in the various matings is consistent with an explanation based on five alleles

TABLE 4

Summary of the inheritance of phenotypes in the A system of equine blood groups. (Data on 639 offspring)

Kinds of matings	Numbers of offspring of phenotypes							
	A_1	A′	H	A_1A'	A_1H	A′H	$A_1A'H$	–
1. A_1 × A_1	189	0	0	0	0	0	0	5
2. A_1 × A′	9	6	0	11	0	0	0	2
3. A_1 × H	7	0	1	0	3	0	0	4
4. A_1 × A_1A'	34	8	0	21	0	0	0	1*
5. A_1 × A_1H	2	0	2	0	2	0	0	0
6. A_1 × A′H	3	5	0	5	1	3	8	1
7. A_1 × $A_1A'H$	4	0	0	0	0	1	7	0
8. A_1 × —	30	0	0	0	0	0	0	4
9. A′ × A′	0	13	0	0	0	0	0	1
10. A′ × H	0	5	2	0	0	4	0	2
11. A′ × A_1A'	5	32	0	15	0	0	2*	0
12. A′ × A′H	0	9	1	0	0	5	0	0
13. A′ × $A_1A'H$	0	0	0	3	0	0	0	0
14. A′ × —	0	9	0	0	0	0	0	1
15. H × A_1A'	1	3	0	0	4	1	0	0
16. H × A′H	0	1	0	0	0	1	0	0
17. H × —	0	0	2	0	0	0	0	0
18. A_1A' × A_1A'	10	11	0	9	0	0	0	0
19. A_1A' × A_1H	0	0	0	1	0	1	0	0
20. A_1A' × A′H	3	8	0	11	7	6	8	0
21. A_1A' × $A_1A'H$	0	0	0	1	0	0	2	0
22. A_1A' × —	17	10	0	0	0	0	0	0
23. A′H × A′H	0	0	0	0	0	8	0	0
24. A′H × $A_1A'H$	0	0	0	0	0	2	0	0
25. A′H × —	0	1	1	0	0	7	0	0
26. $A_1A'H$ × $A_1A'H$	1	0	0	0	0	1	2	0
27. $A_1A'H$ × —	0	0	0	0	0	1	0	0

* Exceptions to the genetic theory (see text).

a^{A_1}, $a^{A'}$, a^{H}, $a^{A'H}$ and a. (In this symbolism the superscripts represent the blood factors controlled by the alleles.) The three exceptions to the genetic theory (lines 4 and 11 in the table) are discussed elsewhere in this report.

The D system of equine blood groups: The family data summarized in Table 5 are consistent with the conclusion that blood factors D and J belong to the same genetic system, a system which presently appears to involve only three alleles d^D, d^J and d and four phenotypes or groups DJ, D, J and — (no D or J). The two exceptions to the genetic theory (lines 5 and 9 in the table) are discussed elsewhere in this report.

The P system of equine blood groups: As shown in the previous report (STORMONT *et al.*, 1964), blood factors P_1 and P′ are related through P_2 as non-linear subtypes. Accordingly, we should expect that P_1 and P′ would be inherited as alternatives in the progeny of any animal of phenotype P_1P'. Data on the inheritance of P_1 and P′ are summarized in Table 6. As may be seen by examining that table, the four phenotypes P_1, P′, P_1P' and — (no P) are readily explained on the basis of three alleles p^{P_1}, $p^{P'}$ and p. (The symbol P_2 is omitted from the phenotype designations for much the same reason we omitted A_2 from the designations in the A system. That is, the reactions of P_2 antibodies were confined almost solely to bloods possessing either or both P_1 and P′. We have encountered a few bloods lacking both P_1 and P′ which react to some degree with P_2 antibodies, but none were encountered amongst the animals involved in the family studies.)

The Q system of equine blood groups: Although all our data agree in indicating that blood factors Q, R and S belong to the same genetic system, this has been a particularly difficult system to work with primarily for serological reasons discussed by STORMONT *et al.* (1964). As pointed out in that report, the antibodies in the R and S reagents (for which we have only one source each) produce sub-threshold reactions (in this case lysis) with many of the bloods which possess those blood factors. The only reliable way to distinguish between these cryptic

TABLE 5

Summary of the inheritance of phenotypes in the D system of equine blood groups. (Data on 639 offspring)

Kinds of matings	Numbers of offspring of phenotypes			
	D	J	DJ	–
1. D × D	15	0	0	4
2. D × J	14	11	8	10
3. D × DJ	1	0	0	0
4. D × —	69	0	0	41
5. J × J	0	49	1*	6
6. J × DJ	1	3	2	0
7. J × —	0	99	0	94
8. DJ × DJ	0	0	0	0
9. DJ × —	3	4	1*	0
10. — × —	0	0	0	203

* Exceptions to the genetic theory (see text).

TABLE 6

Summary of the inheritance of phenotypes in the P system of equine blood groups. (Data on 639 offspring)

Kinds of matings	Numbers of offspring of phenotypes			
	P_1	P′	P_1P'	–
1. $P_1 \times P_1$	167	0	0	33
2. $P_1 \times P'$	15	9	20	15
3. $P_1 \times P_1P'$	17	7	9	0
4. $P_1 \times$ —	122	0	0	95
5. $P' \times P'$	0	0	0	2
6. $P' \times P_1P'$	4	1	1	0
7. $P' \times$ —	0	20	0	24
8. $P_1P' \times P_1P'$	0	0	0	0
9. $P_1P' \times$ —	9	7	0	0
10. — × —	0	0	0	62

positives and bona fide negatives is by means of absorptions. It was not possible to perform absorptions using all bloods which were not lysed by R and S reagents. Accordingly, this will account for the reduction of information on those two blood factors in Tables 1, 2, 7, 14 and 15.

In Table 7 are summarized family data on 27 of 36 possible kinds of matings involving the phenotypes Q, R, S, QR, QS, RS, QRS and — (no Q or R or S). Those data are reasonably consistent with an interpretation based on six alleles q^Q, q^R, q^S, q^{QR}, q^{RS} and q. The one exception to the genetic theory (line 5 in Table 7) is discussed elsewhere in this report.

The genetic systems C, K, T and U: In examining both family and population data, it became apparent that each of the blood factors C, K, T and U must belong to a separate genetic system. Accordingly, the genetic systems C, K, T and U are designated. Each is at present a one-blood factor, two-allele, two-phenotype system.

Exceptions to the genetic theory: In family data of the kind encompassed in the present report, it is unlikely that the parents assigned to the numerous offspring would be the correct parents in every instance. If misassignments of parentage are made, some of those misassignments will result in exceptions to the genetic theory. Our approach to inconsistencies suggestive of misassignment of one or both parents has been to call such inconsistencies to the attention of the owners and, where indicated, we also suggest an alternative stallion.

In the present study, there were ten exceptions to the genetic theory which we regarded as having resulted from misassignment of the male parent alone. There were none which we regarded as exceptions resulting from misassignment of both parents or the female parent alone. In each of the ten exceptions, there was always an alternative stallion within the same herd which would qualify as the true sire. Six of these ten cases were readily resolved when the owners responded by stating that their records or other evidence indicated that the alternative stallion suggested by us was in fact the true sire. In four of the cases, there

TABLE 7

Summary of the inheritance of phenotypes in the Q system of equine blood groups. (Data on 311 offspring)

Kinds of matings	Numbers of offspring of phenotypes							
	Q	R	S	QR	QS	RS	QRS	–
1. Q × Q	4	0	0	0	0	0	0	0
2. Q × R	5	1	0	21	0	0	0	0
3. Q × S	0	0	0	0	8	0	0	0
4. Q × QR	7	1	0	8	0	0	0	0
5. Q × QS	5	0	0	0	1	0	1*	0
6. Q × RS	1	0	0	7	0	0	11	0
7. Q × QRS	10	0	0	2	0	0	7	0
8. R × R	0	2	0	0	0	0	0	0
9. R × QR	1	8	0	6	0	0	0	1
10. R × RS	0	7	0	0	0	6	0	0
11. R × QRS	0	0	0	2	0	1	0	0
12. S × S	0	0	1	0	0	0	0	0
13. S × QR	0	0	0	0	1	1	0	0
14. S × QS	0	0	0	0	2	0	0	0
15. S × RS	0	0	0	0	0	4	0	0
16. S × QRS	0	0	0	0	2	1	1	0
17. QR × QR	0	9	0	10	0	0	0	0
18. QR × QS	1	0	0	0	1	0	0	0
19. QR × RS	0	8	0	11	1	11	8	0
20. QR × QRS	2	0	0	4	0	4	3	0
21. QS × QS	0	0	1	0	1	0	0	0
22. QS × RS	0	0	0	0	0	1	1	0
23. QS × QRS	4	0	0	1	1	4	2	0
24. RS × RS	0	6	0	0	0	26	0	0
25. RS × QRS	0	0	0	3	2	12	9	0
26. RS × —	0	1	0	0	0	0	0	0
27. QRS × QRS	4	0	0	0	0	3	9	0

* Exception to the genetic theory (see text).

was no response. It seemed, therefore, that we had no alternative other than that of including these exceptions in the body of family data. They are shown as cases numbered 1 through 4 in Table 8.

In the first case in Table 8, and according to the genetic theory advanced in this study, the stallion SP29W is excluded as the sire of offspring 29JJ on two counts. According to the genetic theory, no animal of phenotype A_1A' in the A system can give rise to an offspring lacking both A_1 and A′, and no animal lacking both of the blood factors D and J in the D system can give rise to an offspring possessing both of those blood factors. We also mention, although the data are not shown in Table 8, that stallion SP29W was excluded as the sire of 29JJ on still a third count. His transferrin phenotype (HR) was genetically incompatible with the transferrin phenotype (OO) of 29JJ, according to the genetic theory advanced by BRAEND and STORMONT (1964).

In case number 2 in Table 8, the offspring (SP12I) possessed blood factor D not

TABLE 8

Unresolved exceptions to the genetic theory

Matings	Blood groups in the systems*							
	A	D	P	Q	C	K	T	U
1. Male SP29W	A_1A'	—/—	P_1	—/—	C	—/—	T	—/—
Female SP29II	A_1	DJ	P_1	—/—	C	—/—	T	U
Offspring male 29JJ	—/—	DJ	—/—	—/—	C	—/—	T	U
2. Male SP2A	A_1	J	P_1	Q	C	K	—/—	—/—
Female SP2J	A_1H	J	—/—	—/—	C	—/—	T	U
Offspring female SP12I	A_1H	DJ	P_1	—/—	C	K	T	U
3. Male SP14–1	A_1A'	—/—	P_1	Q	C	—/—	T	U
Female SP14–17	A'	—/—	P_1	—/—	—/—	—/—	T	U
Offspring female SP14–18	A'H	—/—	—/—	Q	C	K	T	U
4. Male SP14–1	A_1A'	—/—	P_1	Q	C	—/—	T	U
Female SP14–32	A'	J	P_1	Q	C	K	—/—	—/—
Offspring male SP14–54	A'H	—/—	—/—	—/—	C	K	—/—	U
5. Male MMC–6	A_1	—/—	P_1	Q	C	—/—	T	—/—
Female MMC–41	A_1	—/—	P_1	QS	C	—/—	T	—/—
Offspring female MMD–F	A_1	—/—	P_1	QRS	—/—	—/—	T	—/—

* Underscored systems in the offspring indicate the exceptions to the genetic theory.

present in either putative parent. In the third case, the offspring (SP14–18) possessed blood factors H and K not present in either putative parent. In the fourth case, the offspring (SP14–54) possessed blood factor H not present in either putative parent. Cases 3 and 4 were both from the same herd. Although mutation is most unlikely as an explanation for the two exceptions to the genetic theory in case number 3, mutation cannot be dismissed entirely as a possibility in cases 2 and 4.

Cases 1, 2, 3 and 4 in Table 8 will account for all the exceptions to the genetic theory indicated in Tables 1, 4 and 5. Those cases are also illustrative of how such results are applicable in the solution of problems of questionable parentage.

In case number 5 in Table 8, the authors are of the opinion that the inconsistency (the appearance of R in the offspring of presumptive R-negative parents) resulted from misclassification of the Q system phenotype of the mare. We believe that the R blood factor went undetected in the mare which was typed one year prior to typing the foal. When the inconsistency was noted, it was no longer possible to obtain another blood sample from the mare. There was no question concerning the Q system phenotype of the stallion because he bred as if homozygous for allele q^9. This apparent discrepancy in classifying Q system phenotypes will account for the one exception to the genetic theory indicated for blood factor R in Tables 1 and 7.

Tests for independence and sex linkage: Although there was no evidence suggestive of linkage between any of the eight blood group loci, the possibility of linkage, especially loose linkage, cannot be excluded. In the absence of any evi-

TABLE 9

Estimates of the frequencies of alleles at the eight blood group loci in Shetland Ponies and Thoroughbreds

Loci	Alleles	Frequencies in Shetlands	Frequencies in Thoroughbreds
A	a^{A_1}	0.3107	0.7050
	$a^{A'}$	0.2852	0.0290
	a^{H}	0.0358	0.0036
	$a^{A'H}$	0.0601	0.0000
	a	0.3082	0.2624
D	d^{D}	0.1392	0.0000
	d^{J}	0.1215	0.1503
	d	0.7394	0.8497
P	p^{P_1}	0.3415	0.2058
	$p^{P'}$	0.0483	0.0910
	p	0.6102	0.7031
Q	q^{Q}	0.1519	0.5082
	q^{R}	0.3869	0.0000
	q^{S}	0.0103	0.1038
	q^{QR}	0.1306	0.0756
	q^{RS}	0.1893	0.3125
	q	0.1310	0.0000
C*	c^{C}	0.6521	0.7317
K*	k^{K}	0.1796	0.0635
T*	t^{T}	0.4505	0.6594
U*	u^{U}	0.3174	0.1485

* The frequency of the alternative allele(s) at these loci is simply 1 minus the figures shown.

dence for sex linkage, it follows that all of the eight loci are most probably autosomal.

Gene frequencies: Data on the frequencies of alleles at the eight loci in both Shetland Ponies and Thoroughbreds are summarized in Table 9. Those for the two-allele systems C, K, T and U were computed from the data in Table 2 using the square root method. Also, we note the same for the D system in Thoroughbreds. The three-allele systems, D in Shetlands and P in both Shetlands and Thoroughbreds, are analogous to the classical ABO system of man. Accordingly, BERNSTEIN's efficient equations were used in obtaining the estimates. As pointed out by COTTERMAN (1954), explicit equations are apt to be increasingly difficult to discover with increasing numbers of alleles. This is true for the systems A and Q. Accordingly, we obtained preliminary estimates using square root formulas and then adjusted those preliminary estimates by allocation somewhat after the method of NEIMANN-SØRENSEN (1958). We are particularly indebted to DR. JAN RENDEL for his assistance, not only in arriving at the estimates for the Q

system, but also in performing the chi-square tests for goodness of fit presented in the following section of this report. The population data used in estimating the frequencies of *A*, *D*, *P* and *Q* alleles in Shetland Ponies are shown respectively in Tables 10, 11, 12 and 14, whereas those used for the P and Q systems in

TABLE 10

Comparison of observed frequencies of A system phenotypes with expected frequencies in a sample of 391 Shetland Ponies

	Phenotypes							
	A_1	A′	H	A_1A'	A_1H	A′H	$A_1A'H$	—
Observed	105	99	12	77	7	32	20	39
Expected	112.642	100.517	9.134	69.294	8.704	38.970	14.604	37.135

$\chi^2=5.96$; degrees of freedom=3; 0.20>P>0.05.

TABLE 11

*Comparison of observed frequencies of D system phenotypes with expected frequencies in a sample of 391 Shetlands**

	Phenotypes			
	D	J	DJ	—
Observed	10	91	79	211
Expected	13	88	76	214

$\chi^2=1.25$; degrees of freedom=1; 0.50>P>0.20.
* Chi-square was computed using STEVENS' formula for direct calculation as cited by COTTERMAN (1954).

TABLE 12

*Comparison of observed frequencies of P system phenotypes with expected frequencies in a sample of 391 Shetland Ponies**

	Phenotypes			
	P_1	P′	P_1P'	—
Observed	16	206	21	148
Expected	13	208	24	146

$\chi^2=1.18$; degrees of freedom=1; 0.50>P>0.20.
* See footnote to Table 11.

TABLE 13

Comparison of observed frequencies of P system phenotypes with expected frequencies in a sample of 276 Thoroughbreds

	Phenotypes			
	P_1	P′	P_1P'	—
Observed	11	91	37	137
Expected	10	92	38	136

$\chi^2=0.05$; degrees of freedom=1; 0.95>P>0.80.
* See footnote to Table 11.

TABLE 14

Comparison of the observed frequencies of Q system phenotypes with expected frequencies in a sample of 316 Shetland Ponies

	Phenotypes						
	Q	R	S + QS	QR	RS	QRS	—
Observed	22	73	1 + 0	104	86	25	5
Expected	19.859	79.346	0.885 + 0.988	97.831	77.026	34.644	5.422

$\chi^2 = 5.066$; degrees of freedom = 1; $0.05 > P > 0.02$.

TABLE 15

Comparison of the observed frequencies of Q system phenotypes with expected frequencies in a sample of 185 Thoroughbreds

	Phenotypes				
	Q	S + QS	QR	RS	QRS
Observed	51	1 + 20	17	36	60
Expected	47.781	1.991 + 19.509	15.267	30.058	70.394

$\chi^2 = 2.912$; degrees of freedom = 1; $0.20 > P > 0.05$.

Thoroughbreds are shown respectively in Tables 13 and 15. The population data used in estimating the frequencies of A system alleles in Thoroughbreds are given as follows: There were 276 animals in the sample, 239 of which were phenotype A_1, one phenotype H, 16 phenotype A_1A', one phenotype A_1H and 19 phenotype — (no A or H). There were no animals in the sample which were of phenotypes A′, A′H and $A_1A'H$. Accordingly, this sample could not be used to test goodness of fit in Thoroughbreds.

The estimates in Table 9 show that the two breeds of horses are markedly different on the basis of these parameters. From those data, it is obvious that the Thoroughbred horse is much more homozygous for blood groups than the Shetland Pony. Indeed, had the present study been confined solely to Thoroughbreds much of the information relative to the systems A, D, P and Q could not have been obtained.

Tests of goodness of fit: Comparisons of observed phenotype frequencies with expected frequencies in the A, D, P and Q systems are made in Tables 10, 11, 12, 13, 14 and 15. With the exception of the data on the Q system in Shetland Ponies (Table 14), the observed frequencies were in good agreement with the expected frequencies, thereby confirming the genetic theories advanced to explain the inheritance of blood groups in the A, D, P and Q systems. Some qualification to this statement is necessary in the case of the Q system where chi-square for goodness of fit in the Shetland Pony data (Table 14) had a probability less than 0.05 but greater than 0.02. In both Shetland Ponies and Thoroughbreds, the deviations accounting for most of the chi-square were in the two classes RS and QRS (see Tables 14 and 15) and were in the same direction, namely, a deficiency of animals of phenotype QRS and an excess of animals of phenotype RS. This

observation focused attention on the possibility that some animals of phenotype QRS were actually misclassified as RS. We, therefore, had another look at all our blood-typing data involving tests for the blood factors Q, R and S. What the statistical data in Tables 14 and 15 indicated turned out to be true. At least six of the Shetland Ponies classified as of phenotype RS could just as well have been classified QRS, and at least three of the Thoroughbreds classified as phenotype RS could just as well have been classified as QRS. On the other hand, there were no animals actually classified as QRS that appeared to be erroneously listed as Q-positive. We also noted that there were at least 13 Shetland Ponies classified as of phenotype R which could have been classified as QR.

One of our problems in typing the Q system is that the reagents for blood factor Q produce all degrees of lytic reactions ranging from a faint trace of lysis to complete lysis. We also know that they can be converted into a variety of subtyping reagents by absorbing them with red cells which show the weaker grades of reactivity, but the subtypes so delineated are not sharply defined. The existence of various intergrades of blood factor Q indicates that the Q system is much more complex than what we now have data to prove. Undoubtedly there are many alleles yet to be recognized at the Q locus, but their analysis must await the development of reagents which will define new specificities in the Q system. Also compounding the difficulties is the evidence that our present Q reagents are partial dosage reagents as indicated by the fact that the preponderance of bloods exhibiting complete lysis is from animals of phenotype Q. (Most animals of phenotype Q are homozygous for allele q^9 and therefore have a double dose of blood factor Q.) In view of the problems encountered in typing the Q system, the authors were surprised to find that the observed frequencies of the various phenotypes shown in Tables 14 and 15 agreed with the expected frequencies as well as they did. Indeed, without these data we would have much less confidence in the six-allele theory.

DISCUSSION

Although we would like to contrast the present results with those obtained in the analysis of blood groups in other species, this discussion, because of limitations on space, will be confined solely to a comparison of the present data with those published by PODLIACHOUK (1957, 1958) and FRANKS (1962).

As already stated, six of the blood factors examined in this study were the same as six of the ten studied by PODLIACHOUK. They are A_1 (or A), C, D, H, J and K. Although our data agree with those of PODLIACHOUK in showing that blood factors C and K are independent of each other and also of A, D, H and J, they do not agree with PODLIACHOUK's conclusion to the effect that each of the blood factors A, D, H and J also belong to separate genetic systems. The present data show, beyond reasonable doubt, that A and H belong to the system which we have called A, and that D and J belong to the system which we have named D.

Concerning the blood factors A and H, we were unable to find anything in PODLIACHOUK's data which would suggest that they belong to separate genetic systems. On the contrary, her data provide additional information confirming

certain of the observations made in the present study. For example, in a two-by-two analysis of the distribution of A and H in 260 horses at the Garches Annex of the Pasteur Institute, PODLIACHOUK (1957) obtained a chi-square of 26.68 which is highly significant. It was largely on the basis of a two-by-two analysis of the distribution of A and F in the same population that PODLIACHOUK concluded that A and F belong to a system controlled by two pairs of linked genes. Although no tests were made for blood factor F in the present study, we did note on examining PODLIACHOUK's two-by-two tables that the chi-square on the distribution of F and H in 260 horses at the Garches Annex was 6.26, which can be interpreted as further evidence that H belongs to the same genetic system as A.

PODLIACHOUK examined the distribution of blood factors D and J in 215 horses at the Garches Annex and found that the chi-square (0.41) was not significant. However, considering the rarity of D and J in the horses sampled, this was not surprising. One of the chief fallacies in the use of two-by-two contingency tables is drawing conclusions on insufficient data. At the most, such data are useful only as a lead to further studies.

PODLIACHOUK also published data on the distribution of D and J in mules. In one of her tables (No. 15 in the 1957 report), she showed that there were three mules of type D, eight of type J, and 27 which lacked both D and J. In another table (No. 33 in the 1957 report), there were three of type D, ten of type J, and 31 which lacked both D and J. It is not known to us whether the data in her Table 33 include the animals in Table 15, but the significant thing is that no mules of type DJ were encountered, which is precisely what one would expect, that is, if the male parents of all mules are DJ-negative and the three-allele theory which we have advanced to explain the D system groups in horses is correct.

Although we have not had the opportunity of comparing our equine blood-typing reagents in parallel tests with those of FRANKS (1962), there are several things which suggest to us that his 1–2–5–8–11 system may be the same as the Q system described in this report. For example, three of the five reagents, namely, anti-1, anti-2 and anti-5, behaved much like our R and S reagents; that is, they acted as lysins and produced subthreshold reactions in the direct tests with certain bloods possessing the respective blood factors. We also note that the presumptive linked combinations of the five blood factors studied by FRANKS were so tightly linked that they segregated as the products of alleles. Although it seems unnecessary to implicate linked genes in such situations, we fully realize that our present data on the Q system could be interpreted, if one so desires, on the basis of closely or absolutely linked genes, but to do so would leave many questions unanswered.

To explain the differences in expressivity of blood factors 1, 2 and 5, FRANKS proposed that antigen 1 but apparently not 2 becomes reduced in amount whenever the hypothetical linked genes for 1 and 2 are in the *trans* position. Contrariwise, when in the *cis* position, antigen 2 becomes reduced in amount. Similarly, the *trans* position for 1 and 5 is said to cause the reduction of the amount of 1,

whereas in the *cis* position both antigens are fully expressed. Although FRANKS stated that genes 8 and 10, regardless of position, had no effect on the expressivity of antigens 1, 2 and 5, he failed to mention anything about the expressivity of antigens 2 and 5 with respect to the positions of their controlling genes. Obviously, before such an explanation can be taken seriously some evidence for recombination is necessary. In the experience of the authors, all degrees of expressivity become apparent when dosage reactions are compounded with subtyping effects. Thus, such problems are primarily serological rather than genetic, but the correct genetic theory is always helpful in their resolution.

The authors are particularly indebted to F. W. KOESTER of the California Thoroughbred Breeders Association, to C. F. OSBORN and W. R. BURNS of the American Shetland Pony Club, to MARSHALL CASSIDY and DR. M. A. GILMAN of the Jockey Club, and to the many breeders (too numerous to mention) who supplied us with blood samples and pedigrees of their horses. We are also indebted to numerous veterinarians, in particular DR. E. A. RHODE, for their assistance in collecting the blood samples.

SUMMARY

Data are presented on the inheritance and distribution of 16 equine blood factors named A_1, A_2, A′, C, D, H, J, K, P_1, P_2, P′, Q, R, S, T and U. It is shown that blood factors A_1, A_2, A′ and H belong to a genetic system controlled by a minimum of five alleles designated, a, a^{A_1}, $a^{A'}$ a^H and $a^{A'H}$, that D and J belong to a second system controlled by a minimum of three alleles designated d, d^D and d^J, that P_1, P_2 and P′ belong to a third system controlled by a minimum of three alleles designated p, p^{P_1} and $p^{P'}$, that Q, R and S belong to a fourth system controlled by a minimum of six alleles designated q, q^Q, q^R, q^S, q^{QR} and q^{RS}, that C, K, T and U belong to as many separate systems and that at present each of those systems involves but a single pair of alleles. Thus, in all, eight loci are now implicated in the control of blood groups in horses. There was no evidence for sex linkage and there was no evidence for linkage between any of the eight loci. This, of course, does not exclude the possibility that some of the loci could be linked.

Data are also presented on the frequencies of the alleles in the various systems, and it is shown that two breeds (Shetland Ponies and Thoroughbreds) are markedly distinct with respect to those frequencies.

The genetic theory of multiple alleles for each of the systems A, D, P and Q is examined by means of chi-square tests for goodness of fit. With certain exceptions in the Q system, the observed phenotype frequencies were in good agreement with expected, thereby confirming the genetic theories.

The present results are contrasted with those of contemporaneous investigators engaged in genetic studies of equine blood groups.

LITERATURE CITED

BRAEND, M., and C. STORMONT, 1964 Studies on hemoglobin and transferrin types of horses. Nord. Vet. Med. **16**: 31–37.

COTTERMAN, C. W., 1954 Estimation of gene frequencies in nonexperimental populations. Chap. 35. *Statistics and Mathematics in Biology*. Edited by O. KEMPTHORNE, T. A. BANCROFT, J. GOWEN and J. LUSH. Iowa State College Press, Ames.

FRANKS, D., 1962 Horse blood groups and hemolytic disease of the newborn foal. Ann. N.Y. Acad. Sci. **97:** 235–250.

NEIMANN-SØRENSEN, A., 1958 Blood groups of cattle. Thesis (177 pp.) A/S CARL FR. MORTENSEN, Copenhagen, Denmark.

PODLIACHOUK, L., 1957 Les antigèns de groupes sanguins des equidés et leur transmission héréditaire. Thesis, Univ. Paris.

PODLIACHOUK, L., 1958 Les groupes sanguins des equidés (cheval, mulet, ane). Ann. Inst. Pasteur **95:** 7–22.

PODLIACHOUK, L., A. KACZMAREK, and J. ZWOLINSKI, 1962 Les groupes sanguins de chevaux de six races de Pologne. Ann. Inst. Pasteur **103:** 943–949.

STORMONT, C., 1961 Serological criteria for allelism in blood-typing studies. Tenth Pac. Sci. Congr. (Abstracts): 186–187.

STORMONT, C., Y. SUZUKI, and E. A. RHODE, 1964 Serology of horse blood groups. Cornell Vet. **54:** 439–452.