Inheritance of Human Leukocyte Antigens*

ROSE PAYNE[1] AND EMANUEL HACKEL[2]

WITH THE TECHNICAL ASSISTANCE OF MISS MILLIE TRIPP

INTRODUCTION

INTENSIVE INVESTIGATION into the immunology of both leukocytes and erythrocytes began at the end of the nineteenth century. Prior to the 1950's, however, studies on leukocyte immunology were limited for the most part to whether or not these antigens were tissue or species specific. Our knowledge of the nature of leukocyte antigens lagged far behind that of the red blood cells. The genetics and immunochemistry of erythrocyte antigens, by virtue of easily observed agglutination reactions, were more readily clarified. The summation of cumulative studies since 1900 slowly established the existence of leukocyte antigens which were clearly different from those of the platelets and red blood cells. Bedson in 1921 decisively demonstrated that an anti-guinea-pig-leukocyte serum prepared in rabbits would not agglutinate guinea pig platelets and conversely that a similarly prepared anti-platelet serum would not agglutinate the leukocytes. The presence of an antigenic difference between leukocytes and erythrocytes was confirmed by Chew, Stephens, and Lawrence (1936) who produced an anti-guinea-pig-leukocyte serum in rabbits which was free of hemolytic and hemagglutinating capacity. Working with another animal species, Amos (1954) found antigens in the leukocytes of four inbred strains of mice that were lacking in the corresponding erythrocytes.

Little material is available on differing leukocyte antigens within a single species, i.e., isoantigens. Almost all present information is confined to the isoantigens of human leukocytes. The first studies on leukocyte isoantigens attempted to detect in them the presence of A and B antigens with the aid of isohemagglutinins (Doan, 1926; Wichels and Lampe, 1928). The quest much later for naturally occurring white cell agglutinins comparable to anti-A and anti-B proved unsuccessful (Butler, 1956; Moeschlin and Schmid, 1954). It was not until 1953 that agglutinins for leukocytes were repeatedly observed in human sera (Dausset and Nenna, 1953). Leukoagglutinins were shown to be immune rather than naturally occurring isoantibodies which were capable of reacting with human leukocytes (Payne, 1957; Brittingham, 1957; van Loghem

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[1] *Department of Medicine, Stanford University, School of Medicine, Palo Alto, California.*

[2] *Office of the Dean, University College, Michigan State University, East Lansing, Michigan.*

et al., 1957). The stimuli for their formation appeared to be either multiple transfusions or repeated pregnancies (Payne and Rolfs, 1958; van Rood *et al.*, 1959). At the present time, a number of specific leukoagglutinins which detect different leukocyte antigens may be found in immunized individuals. However, the grouping of human leukocyte antigens into systems by corresponding immune agglutinins has not been attained.

Although investigators turned their attention to the inheritance of human leukocyte antigens soon after the discovery of leukoagglutinins, this subject is still in the exploratory period. Dausset and Brecy (1957) using 28 leukoagglutinating sera compared the reaction of the leukocytes in pairs of monozygotic and dizygotic twins. The three pairs of monozygotic twins reacted similarly while the single pair of dizygotic twins did not. These findings in twins were later confirmed by Lalezari and Spaet (1959). In 1958 Payne and Rolfs demonstrated the inheritance of leukocyte antigens from the father in ten newborn infants. Six more extensive pedigrees were presented in which the family members' leukocytes had been tested with leukoagglutinins. In one family the inheritance of a specific leukocyte antigen(s) through three generations was shown. These families illustrated that the genes determining the different leukocyte antigens expressed themselves in the heterozygous state. As with classic red blood cell inheritance, an antigen present in the offspring was always present in at least one of the parents, and in no instance were antigens detected in the offspring that were lacking in both parents. Working independently in Holland, van Rood, van Leeuwen, and Eernisse (1959) reported similar results. Using nine leukoagglutinating sera, they established the incidence of corresponding leukocyte antigens in several hundred persons selected at random. The frequency of the leukocyte antigens so identified in the population varied from 14 to 85 per cent.

This paper presents the results of family studies carried out with a number of leukocyte antisera from multiparous women.

METHODS

Leukocyte antigens were identified by observing the agglutination of leukocytes with specific leukoagglutinating sera. The technique was similar to that employed with red blood cells. The method has been described in detail in a previous publication (Payne and Rolfs, 1958).

A brief resume of the procedure follows: To prepare a suspension rich in leukocytes and relatively poor in erythrocytes, defibrinated blood was mixed with a sedimenting agent (4 parts of defibrinated blood to 1 part of 4% polyvinylpyrrolidone in physiologic saline). This mixture was allowed to settle for approximately one hour after which time the supernatant containing about equal proportions of red and white blood cells was removed. This leukocyte suspension was standardized to contain about 4000 leukocytes per mm^3. If dilution was necessary, cell free serum was employed. The leukocyte suspensions were prepared daily since spontaneous agglutination develops on prolonged standing.

Antisera containing leukoagglutinins were obtained from nontransfused

women with a history of recent pregnancy. These sera were stored in the frozen state. Before use the sera were inactivated for 30 minutes at 56°C. In the test, each serum was mixed with the leukocyte suspension in the proportion of 0.15 ml. of serum to 0.05 ml. of leukocyte suspension. The mixture was then incubated for 90 minutes at 37°C. After incubation the red cells were lysed with 0.1 ml. of 3% acetic acid. The sedimented leukocytes were taken up with a Pasteur pipette, placed on a glass slide and examined microscopically at 100x magnification for agglutination. All tests were run in duplicate. Prior to testing sera with cells of incompatible ABO blood groups, the anti-A and anti-B red cell isoagglutinins were removed by absorption with group A and/or B washed red cells.

RESULTS

In the course of the investigation, antisera from 34 different women were used. Comparison of their reactions with a panel of leukocytes showed that only 19 different kinds of serum were involved. This paper is concerned with the eight sera for which more complete data were obtained.

It seems inappropriate, at this time, to propose a rigid system of terminology. This report considers a number of leukocyte antigens, each presumably defined by a single antiserum. The term "antigen" is used here in the sense of antigenic determinant, i.e., that structure or series of structures responsible for the agglutination of leukocytes by a given leukocyte antiserum. For the present, numerals will be used to distinguish among the antigens and the corresponding antisera.

Families were tested with the various antisera, and were classified on the basis of the parents' types. For purposes of analysis, the data for each type were pooled, and these are presented in table 1.

Gene frequencies were computed from the results of the reactions of the various antisera with leukocytes of unrelated individuals. In the analysis of the

TABLE 1. SUMMARY OF FAMILIES TESTED WITH EIGHT DIFFERENT LEUKOAGGLUTININS

Leukocyte Antigen	Total Families	Matings								
		Neg. × Neg.			Pos. × Neg.			Pos. × Pos.		
		Families	Offspring		Families	Offspring		Families	Offspring	
			+*	−*		+	−		+	−
1	30	5	0	11	17	28	17	8	22	4
2	26	7	0	20	17	20	20	2	5	0
3	23	2	0	3	10	19	9	11	23	1
4	19	4	0	8	8	16	7	7	11	5
5	16	2	0	8	9	12	8	5	8	0
6	24	11	0	23	11	12	13	2	8	3
10	19	1	0	1	12	15	13	6	12	0
12	17	2	0	4	7	12	2	8	14	1

* + = offspring having leukocyte antigen.
− = offspring lacking leukocyte antigen.

TABLE 2. GENE FREQUENCIES BASED ON LEUKOAGGLUTININ REACTIONS AMONG UNRELATED INDIVIDUALS

Leukocyte Antigen	Number Tested	Pos.	Neg.	p	q
1	99	62	37	.389	.611
2	94	36	58	.215	.785
3	65	46	19	.460	.540
4	66	34	32	.304	.696
5	67	34	33	.298	.702
6	54	20	34	.206	.794
10	57	39	18	.438	.562
12	52	38	14	.481	.519

data for each of the antigens under consideration the following assumptions were made: (1) the inheritance of white cell antigens is analogous to that of red cell antigens; (2) a single pair of genes is involved in the inheritance of each antigen and those individuals who are positive may be either homozygous or heterozygous for the gene producing the antigen, but those who are negative must be homozygous for the allele; (3) the population is in equilibrium; and (4) the population is panmictic. On the basis of these assumptions positive individuals comprise $p^2 + 2pq$ of the population, and the negative individuals q^2 of the population. Here p is the frequency of the gene producing the antigen under consideration and q is the frequency of the allele which does not produce the antigen. The gene frequencies obtained are shown in table 2.

The probabilities that a positive person is either homozygous or heterozygous may be obtained from the following equations: If $p^2 + 2pq$ = that portion of the population which is positive, then

$$f_{AA} = \frac{p^2}{p^2 + 2pq} = \frac{p}{1 + q},$$

and

$$f_{Aa} = \frac{2pq}{p^2 + 2pq} = \frac{2q}{1 + q}.$$

Using these probabilities, a general expression for the expected frequency of the different phenotypes among the offspring from the various types of mating may be computed. These are shown in table 3.

The comparisons of the corresponding observed and expected values are presented in table 4. There is no entry for the negative x negative mating group since no positive offspring are expected or observed for these matings (table 1). These data were subjected to the χ^2 test for goodness of fit. Since the data are not continuous, Yates' correction for continuity was applied in all cases except where it would inconsequential (Cochran, 1952). The results of this analysis are included in table 4.

In addition to these family studies, the data on incidence of the eight leukocyte antigens in unrelated individuals (table 5) were analyzed for independence (table 6). This was done by use of 2×2 tables and the resultant χ^2 values are

TABLE 3. NATURE OF OFFSPRING EXPECTED IN EACH MATING GROUP

Mating	Expected Offspring	
	Positive	Negative
Positive × Positive	$N(1 + 2q)/(1 + q)^2$	$Nq^2/(1 + q)^2$
Positive × Negative	$N/(1 + q)$	$Nq/(1 + q)$
Negative × Negative	0	N

N = Number of observed offspring in each mating group

TABLE 4. COMPARISON OF OBSERVED WITH EXPECTED OFFSPRING

Leukocyte Antigen	Mating	Positive Offspring		Negative Offspring		χ^2	P (for D.F. = 2)
		Obs.	Exp.	Obs.	Exp.		
1	Pos × Neg*	28	27.92	17	17.08	0.0006	
	Pos × Pos	22	22.26	4	3.74	0.0180	
					Total χ^2 =	0.0186	.99
2	Pos × Neg	20	22.40	20	17.60	0.3662	
	Pos × Pos†	5	4.03	0	0.97	0.2825	
					Total χ^2 =	0.6487	.73
3	Pos × Neg	19	18.19	9	9.81	0.0151	
	Pos × Pos	23	21.05	1	2.95	0.8127	
					Total χ^2 =	0.8278	.67
4	Pos × Neg	16	13.56	7	9.44	0.6762	
	Pos × Pos	11	13.30	5	2.70	1.4436	
					Total χ^2 =	2.1198	.36
5	Pos × Neg*	12	11.75	8	8.25	0.0129	
	Pos × Pos	8	6.64	0	1.36	0.6551	
					Total χ^2 =	0.6680	.71
6	Pos × Neg	12	13.94	13	11.06	0.3363	
	Pos × Pos	8	8.85	3	2.15	0.0708	
					Total χ^2 =	0.4071	.81
10	Pos × Neg	15	17.92	13	10.08	0.9078	
	Pos × Pos	12	10.44	0	1.56	0.8279	
					Total χ^2 =	1.7357	.40
12	Pos × Neg	12	9.22	2	4.78	1.6514	
	Pos × Pos	14	13.25	1	1.75	0.0404	
					Total χ^2 =	1.6918	.44

* Yates' correction for continuity not applied.

† Contains expected value of less than 1 and is included for completeness. If this is omitted, the χ^2 value for antigen 2 is 0.3662 and the value of p (for DF = 1) is .55.

shown in table 6. The underscored entries in the table are values of χ^2 which indicate non-independence at the one per cent level. The asterisks indicate those pairs in this group which according to family studies (see Appendix table) are not closely linked.

TABLE 5. FREQUENCIES OF OCCURRENCE OF TWO LEUKOCYTE ANTIGENS IN UNRELATED INDIVIDUALS

No. Tested	Leukocyte Antigen							
	1	2	3	4	5	6	10	12
11	+	+						
24	+	−						
15	−	+						
12	−	−						
19	+		+					
24	+		−					
24	−		+					
11	−		−					
12	+			+				
13	+			−				
11	−			+				
13	−			−				
22	+				+			
19	+				−			
22	−				+			
17	−				−			
20	+					+		
16	+					−		
5	−					+		
25	−					−		
8	+						+	
10	+						−	
6	−						+	
9	−						−	
13	+							+
9	+							−
11	−							+
11	−							−
30		+	+					
2		+	−					
18		−	+					
31		−	−					
11		+		+				
9		+		−				
15		−		+				
15		−		−				
12		+			+			
15		+			−			
27		−			+			
20		−			−			
6		+				+		
14		+				−		
20		−				+		
25		−				−		
28		+					+	
1		+					−	
10		−					+	
30		−					−	
15		+						+
10		+						−
18		−						+
10		−						−
12			+	+				
14			+	−				
10			−	+				
9			−	−				

TABLE 5.—*Continued*

No. Tested	Leukocyte Antigen							
	1	2	3	4	5	6	10	12
19			+		+			
13			+		−			
14			−		+			
10			−		−			
11			+			+		
25			+			−		
11			−			+		
15			−			−		
30			+				+	
7			+				−	
6			−				+	
22			−				−	
14			+					+
10			+					−
8			−					+
7			−					−
11				+	+			
13				+	−			
18				−	+			
8				−	−			
8				+		+		
15				+		−		
11				−		+		
14				−		−		
12				+			+	
13				+			−	
12				−			+	
12				−			−	
10				+				+
3				+				−
10				−				+
9				−				−
10					+	+		
20					+	−		
11					−	+		
20					−	−		
13					+		+	
19					+		−	
20					−		+	
10					−		−	
11					+			+
10					+			−
12					−			+
11					−			−
7						+	+	
16						+	−	
23						−	+	
16						−	−	
9						+		+
16						+		−
14						−		+
14						−		−
15							+	+
13							+	−
15							−	+
6							−	−

TABLE 6. SUMMARY OF INDEPENDENCE ANALYSIS OF LEUKOCYTE ANTIGENS
χ^2 VALUES OBTAINED FROM 2 × 2 TABLES

Leukocyte Antigen	1	2	3	4	5	6	10
2	2.7202						
3	3.7523	*23.7569					
4	0.0180	0.0033	0.0130				
5	0.0005	0.6999	0.0061	1.9263			
6	*8.9293	0.6770	0.4697	0.1274	0.0086		
10	0.0093	*31.9540	20.6018	0.0196	3.2366	3.6449	
12	0.0876	0.0014	0.0006	1.0450	0.0001	0.0935	0.9474

——— = Non-independence at the 1% level
* = Not closely linked according to family studies

The Appendix table gives the phenotypes of all the families which were tested with more than one leukoagglutinin. The other families included in the study were, for a variety of reasons, tested with only one antiserum and are therefore not included in the table. With regard to genetic nomenclature, it is proposed that the symbol *W*, followed by the number of the antigen and the superscript *a*, be used for the gene producing the antigen. Thus the symbol for the gene producing antigen 1 is $W1^a$. The same symbol, with no superscript, is used for the allele or alleles which do not produce the antigen; the allele(s) of $W1^a$ is therefore $W1$. When more antigens belonging to any series are discovered, allelic genes of the series may be designated by additional superscripts, i.e., $W1^b$, $W1^c$, etc.

Several of the families included in the Appendix table show that the genes governing the production of some of the antigens are not alleles. In the Re family, the father's genotype, with regard to antigens 1 and 6 must be $W1^a/W1\ W6^a/W6$ and the mother's must be $W1/W1\ W6/W6$. Child 1, whose genotype is $W1^a/W1\ W6/W6$, could only have received the $W1^a\ W6$ combination from the father, while her brother, whose genotype is $W1/W1\ W6/W6$, must have received the $W1\ W6$ combination from the father. These two combinations indicate that the genes governing antigens 1 and 6 are not alleles and are not absolutely linked. A similar conclusion regarding antigens 2 and 3 may be drawn from the W family (see Appendix table). Here it is the mother who is the double-heterozygote-$W2^a/W2\ W3^a/W3$; the father who is negative for both antigens has the genotype $W2/W2\ W3/W3$. Since the father can only contribute the $W2\ W3$ combination, the daughter (genotype $W2/W2\ W3/W3$) must have received the $W2\ W3$ combination from her mother, too, and the son, whose genotype is $W2/W2\ W3^a/W3$, received the $W2\ W3^a$ combination from the mother. Hence, the genes for these two antigens are also not absolutely linked. The R_0 family shows assortment of the $W2^a/W2$ and $W10^a/W10$ genes demonstrating the lack of absolute linkage between antigens 2 and 10.

DISCUSSION

The results of these family studies show that leukocyte antigens are inherited (table 4). Their pattern of inheritance, however, is not so easily resolved. The

independence analysis indicated several pairs to be non-independent. Family studies rule out absolute linkage as the cause of non-independence on three of the cases: antigens 1 and 6 (Re family), antigens 2 and 3 (W family), and antigens 2 and 10 (Ro family). In each of these cases, as well as in the fourth case of non-independence, allelism (i.e., each of the non-independent antigens being produced by one member of an allelic pair) is not involved. If it were, the entry in the double-negative category for each pair would be zero. The presence of a sizeable number of individuals in this category (see table 5) leaves multiple antibodies or multiple alleles as the possible explanation for the non-independence. For antigens 3 and 10, absolute linkage has not been excluded, nor has the possibility that the association is due to the fact that both antigens are associated with antigen 2. The latter hypothesis is supported by the results of a simultaneous analysis of antigens 2, 3 and 10 which show that when those who are positive for antigen 2 are considered separately from those who are negative, antigens 3 and 10 are independent. (The non-independence derived earlier resulted when antigens 3 and 10 were analyzed without regard to the presence or absence of antigen 2.)

In working with leukoagglutinating antisera, a number of considerations must be kept in mind. The problem of multiple antibodies in these sera is perhaps the most serious. The likelihood of their occurrence in antisera from multitransfused patients precludes using such sera in genetic studies. Antisera produced solely as a result of immunization during pregnancy are less likely to contain as many different kinds of antibody. It should be emphasized, however, that even such sera may contain more than one kind of leukoagglutinin. Some of the evidence presented here (see tables 5 and 6) indicates that this may well be the case. In the analysis of the family data, a necessary assumption was that each of the antisera contained only one kind of antibody. While the likelihood of multiple antibodies was known to exist at the time, this had to be set aside to simplify the analysis. Evidence from this investigation as well as from other sources establishes the possibility that multiple antibodies exist in several of the antisera. For future work, these antisera will require absorption. Finally, it should be mentioned that nearly all leukoagglutinating sera tend to have low titers and therefore are used at full strength to ensure maximum reliability.

While the inheritance of leukocyte antigens is now clearly demonstrable, the questions of linkage and of allelism remain unresolved. Absorption studies now under way are expected to yield pure sera which will permit the solution of these problems. Considering the number of blood group systems in relation to the number of chromosomes, it is highly probable that some of the genes causing the production of some of the leukocyte antigens are carried on the same chromosomes as genes producing certain of the erythrocyte antigens. In the present limited investigation it has not been possible to show linkage with the ABO, MNSs, or Rh systems. However, the use of leukoagglutinins may prove to be a fruitful procedure in future work in human genetics. Leukocyte antigens considerably expand the number of hereditary traits available for study in a single sample of blood.

SUMMARY

The inheritance of human leukocyte antigens has been investigated employing eight different leukoagglutinating sera obtained from multiparous women. Population and family studies of the leukocyte antigens identified by these sera were carried out. Gene frequencies of the leukocyte antigens were calculated from tests on unrelated individuals. From these gene frequencies, the distribution of the expected offspring in various mating groups was computed and compared with those observed. The data were consistent with the hypothesis of genetic control of leukocyte antigens.

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APPENDIX TABLE

Family	Individual	Sex	Reaction with Leukoagglutinin								Erythrocyte Phenotypes		
			1	2	3	4	5	6	10	12	ABO	MN	Rh*
Reh	Fa		−	−	n	−	+	−	n	−	A	MN	R_2R_0
	Mo		−	−	n	−	−	−	n	+	A	MN	rr
	1	♂	−	−	n	−	+	−	n	+	A	N	R_0r
	2	♂	−	−	n	−	+	−	n	−	A	M	R_2r
W	Fa		+	−	−	n	n	+	n	n	A	M	R_1r
	Mo		−	+	+	n	n	−	n	n	O	M	rr
	1	♀	+	−	−	n	n	+	n	n	A	M	R_1r
	2	♂	−	−	+	n	n	−	n	n	A	M	R_1r
Ro	Fa		−	+	n	+	−	−	+	+	O	MN	R_2r
	Mo		+	−	n	−	+	+	−	+	A	N	R_1r
	1	♀	−	−	n	−	−	+	−	+	O	MN	R_1r
	2	♂	−	−	n	−	−	+	+	+	O	n	n
	3	♀	+	+	n	−	−	+	−	+	O	MN	R_1R_2
	4	♀	+	−	n	+	−	+	−	+	O	MN	R_1R_2
O	Fa		−	−	−	+	−	−	n	+	A	MN	R_2r
	Mo		−	+	+	−	−	−	n	−	O	MN	R_1r
	1	♀	−	−	−	+	−	−	n	+	A	MN	R_2r
	2	♀	−	−	−	+	−	−	n	+	O	N	rr
	3	♂	−	+	+	+	−	−	n	+	A	N	R_1R_2
	4	♂	−	+	+	−	−	−	n	+	O	M	R_1R_2
B	Fa		−	+	+	+	+	+	+	+	A	MN	R_1r
	Mo		−	+	+	+	−	−	+	+	A	MN	rr
	1	♂	−	+	+	−	+	−	+	+	A	M	rr
	2	♀	−	+	+	−	−	+	+	+	A	MN	R_1r
Re	Fa		+	+	n	−	n	+	+	+	O	MN	R_1R_1
	Mo		−	−	n	−	n	−	+	−	A	M	R_1r
	1	♀	+	+	n	−	n	−	+	−	A	MN	R_1r
	2	♂	−	+	n	−	n	−	+	+	O	MN	R_1r
Ba	Fa		+	−	+	+	−	−	+	+	A	M	R_1r
	Mo		−	+	+	+	+	−	+	+	O	MN	R_1r
	1	♀	−	+	+	+	+	−	+	+	A	MN	rr
Ba_2	Mo[1]												
	Fa		+	−	+	−	+	−	+	+	O	MN	R_2R_0
	1	♀	+	−	+	+	+	−	+	+	O	MN	R_0r
	2	♂	−	+	+	+	+	−	+	+	O	MN	R_0r
Wa	Fa		−	+	n	+	n	−	+	+	O	MN	rr
	Mo		−	−	n	+	n	−	−	+	O	N	R_2r
	1	♀	−	−	n	+	n	−	−	+	O	N	rr
Wa_2	Mo[1]												
	Fa		+	+	n	+	n	+	+	+	O	M	R_1R_1
	1	♂	+	−	n	+	n	+	+	+	O	MN	R_1r
	2	♀	−	+	n	+	n	−	+	+	O	MN	R_1r

APPENDIX TABLE—*Continued*

Family	Individual	Sex	Reaction with Leukoagglutinin								Erythrocyte Phenotypes		
			1	2	3	4	5	6	10	12	ABO	MN	Rh*
Ci	Fa		−	+	+	n	+	−	+	+	B	N	R_1r
	Mo		+	−	+	n	+	−	−	+	O	M	R_1r
	1	♀	−	−	+	n	+	−	−	+	B	MN	R_1r
Ci$_2$	Mo[1]												
	Fa		+	+	+	n	+	−	+	+	O	M	R_1r
	1	♀	−	−	−	n	+	−	−	+	O	M	R_1r
	2	♂	+	+	+	n	+	−	+	−	O	M	R_1r
Go	Fa		+	−	−	n	+	+	−	+	A	MN	$R'r$
	Mo		+	−	−	n	+	+	−	−	O	MN	R_1r
	1	♀	+	−	−	n	+	−	−	+	A	MN	R_1R'
Go$_2$	Mo[1]												
	Fa		+	−	−	n	+	+	+	−	A	M	R_1R_1
	1	♀	+	−	−	n	+	−	+	+	A	M	R_1R_1 or R_1R'
	2	♀	+	−	−	n	+	−	+	+	O	MN	R_1R_1 or R_1R'
By	Fa		+	n	n	+	n	−	−	n	O	MN	R_1R_2
	Mo[2]		+	n	n	+	n	+	+	n	A_2B	N	R_1r
	1	♀	+	n	n	−	n	−	−	n	B	MN	R_1R_1
	2	♀	+	n	n	−	n	−	+	n	B	MN	R_1R_1
Bl	Fa		+	n	−	n	n	n	n	n	A_1	N	R_2r
	Mo[2]		+	n	+	n	n	n	n	n	A_2B	N	R_1r
	1	♀	+	n	+	n	n	n	n	n	B	N	R_2r
	2	♂	+	n	−	n	n	n	n	n	A_2	N	R_1r
	3	♀	+	n	−	n	n	n	n	n	A_2	N	R_1r
	4	♂	+	n	−	n	n	n	n	n	B	N	R_2r
P	Fa		−	−	+	−	n	n	n	n	A	MN	R_1r
	Mo		−	+	+	−	n	n	n	n	O	N	R_1R_1
	1	♀	−	−	+	−	n	n	n	n	A	MN	R_1R_1
	2	♀	−	+	+	−	n	n	n	n	O	N	R_1R_1
S	Fa		+	−	n	n	n	+	−	n	A_1B	M	rr
	Mo		+	−	n	n	−	+	−	n	O	MN	R_1r
	1	♀	+	−	n	n	−	+	−	n	B	M	R_1r
	2	♀	+	−	n	n	−	+	−	n	A_1	MN	R_1r
	3	♂	+	−	n	n	−	+	−	n	B	MN	rr
	4	♀	−	−	n	n	−	−	−	n	B	M	R_1r
	5	♂	+	−	n	n	−	+	−	n	A_1	M	R_1r
	6	♂	+	−	n	n	−	+	−	n	A_1	M	R_1r
	7	♂	+	−	n	n	−	+	−	n	B	MN	rr
	8	♂	+	−	n	n	−	+	−	n	B	M	rr
	9	♂	+	−	n	n	−	+	−	n	A_1	M	R_1r
	10	♀	+	−	n	n	−	+	−	n	B	M	rr
	11	♀	−	−	n	n	−	−	−	n	A_1	MN	rr
	12	♂	+	−	n	n	−	+	−	n	A_1	MN	rr

APPENDIX TABLE—*Continued*

Family	Individual	Sex	Reaction with Leukoagglutinin								Erythrocyte Phenotypes		
			1	2	3	4	5	6	10	12	ABO	MN	Rh*
Cu	Fa		−	+	n	+	n	−	+	n	A_2	MN	R_2R_0
	Mo		+	−	n	+	n	−	−	n	O	N	R_2r
	1	♀	+	−	n	+	n	−	+	n	O	N	R_0r
	2	♀	+	−	n	−	n	−	n	n	O	N	R_2r or R_2R_0
	3	♀	+	−	n	+	n	−	−	n	A_2	N	R_2r or R_2R_0
	4	♂	+	−	n	+	n	−	−	n	O	MN	R_2R_2
	5	♂	+	+	n	+	n	−	−	n	A_2	N	R_0r
	6	♂	+	−	n	+	n	−	−	n	A_2	N	R_2r or R_2R_0
Hi	Fa		−	+	n	n	n	−	+	−	O	N	R_1r
	Mo		+	−	n	n	n	+	−	−	A_1	MN	rr
	1	♂	−	+	n	n	n	−	+	−	A_1	MN	rr
	2	♀	+	+	n	n	n	+	+	−	O	N	rr
Bs	Fa		−	+	+	n	n	−	+	−	A_1	M	R_1r
	Mo		+	−	−	n	n	+	−	+	O	MN	R_1r
	1	♂	+	+	+	n	n	+	+	+	O	MN	R_1R_1
	2	♂	−	+	+	n	n	−	+	+	O	MN	rr
Br	Fa		+	n	+	+	n	n	n	n	O	N	$R'r$
	Mo		−	−	+	−	+	−	+	n	A	MN	R_0r
	1	♀	n	−	+	+	+	−	−	n	A	N	R_0R'
	2	♂	n	−	+	−	+	−	−	n	O	MN	R_0R'
	3	♂	n	n	+	+	+	−	−	n	O	MN	R_0r
	4	♀	n	−	+	+	+	−	−	n	A	N	R_0R'
	5	♀	+	n	+	+	+	n	n	n	O	N	R_0r
U	Fa		−	n	+	n	n	−	n	n	B	M	R_1r
	Mo		+	n	−	n	n	+	n	n	A	N	R_1R_1
	1	♂	+	n	+	n	n	+	n	n	AB	MN	R_1R_1
	2	♀	+	n	+	n	n	−	n	n	A	MN	R_1r
	3	♂	+	n	+	n	n	−	n	n	O	MN	R_1R_1
	4	♂	n	n	+	n	n	n	n	n	A	MN	R_1R_1
Bo	Fa		+	n	−	n	n	n	n	n	O	M	R_1r
	Mo		+	n	+	n	n	n	n	n	A	M	$R'r$
	1	♂	+	n	+	n	n	n	n	n	O	M	R_1R'
	2	♀	+	n	+	n	n	n	n	n	A	M	rr
	3	♀	+	n	+	n	n	n	n	n	O	M	R_1r
	4	♀	−	n	+	n	n	n	n	n	O	M	R_1R'
	5	♂	−	n	+	n	n	n	n	n	O	M	R_1R'
Ja	Fa		+	−	+	n	n	−	n	n	B	N	R_0r
	Mo		+	−	−	n	n	−	n	n	A	M	R_0r
	1	♂	+	−	−	n	n	−	n	n	A	MN	R_0r
	2	♂	+	−	−	n	n	−	n	n	O	MN	R_0r

APPENDIX TABLE—*Concluded*

Family	Individual	Sex	Reaction with Leukoagglutinin 1	2	3	4	5	6	10	12	Erythrocyte Phenotypes ABO	MN	Rh*
Str	Fa		+	+	n	n	−	n	n	n	O	M	R_2r
	Mo		−	−	n	n	+	n	n	n	O	M	R_0r
	1	♀	−	+	n	n	+	n	n	n	O	M	R_0r
	2	♀	−	+	n	n	+	n	n	n	O	M	R_2r
	3	♀	−	−	n	n	−	n	n	n	O	M	R_2r
	4	♀	−	+	n	n	−	n	n	n	O	M	R_2r
	5	♂	+	−	n	n	+	n	n	n	O	M	R_0r
Gi	Fa		+	−	+	n	n	+	n	n	A	M	R_1R_1
	Mo		+	−	+	n	n	−	n	n	O	M	R_2r
	1	♀	+	n	n	n	n	+	n	n	O	M	R_2R_1
	2	♀	−	−	−	n	n	+	n	n	A	M	R_2r

* = tested with anti-D, -C, -E, -c, and (when necessary) with anti-e.
[1] Child of preceding family.
[2] Mrs. By and Mrs. Bl are twin sisters.
\+ = positive with antiserum
− = negative with antiserum
n = not tested with antiserum