BLOOD GROUPS IN SHEEP. II. THE B SYSTEM¹

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EXAMINATION of the cross-reactions of numerous bovine and ovine isoimmune antisera with red cells of cattle and sheep, reported by STORMONT, SUZUKI and RASMUSEN (1957), has provided serological evidence for a system of blood groups in sheep homologous to the complex B system of bovine blood groups described by STORMONT, OWEN and IRWIN (1951). This report presents further evidence for the B system of sheep. It is primarily concerned with the genetic elucidation of B phenogroups in sheep and a description of their cross-reactions with diagnostic reagents prepared from isoimmune and heteroimmune antisera.

MATERIALS AND METHODS

Bovine and ovine isoimmune antisera and heteroimmune antisera produced in sheep against red cells of cattle were provided by Dr. CLYDE STORMONT for these studies. The procedure followed in the production of bovine isoimmune antisera is described by STORMONT (1950) and of ovine isoimmune antisera by RASMUSEN, STORMONT and SUZUKI (1960). The heteroimmune antisera were produced by immunizing sheep with pooled erythrocytes from three cows, XX, 88 and 2758. Their phenogroup formulas in the B system were known to be, respectively, $IE'_1/O_xE'_3$, $BGKO_2Y_1A'E'_3K'4,6,8/BGKO_2Y_1A'E'_3K'4,6,8$ and $BGKE'_2,7,8/O_xD'E'_3$. Eighteen sheep were immunized by two 30-ml injections made three days apart, and antisera were collected nine days after the last injection. While some of these heteroimmune antisera contained antibodies which cross reacted with the red cells of individual sheep, the cross-reactions were generally too weak to be of use in blood typing of sheep. Consequently, approximately four months later the sheep were given an additional injection of 30 ml of red cells from the same donor cows, and antisera were collected nine days later. Seven of these antisera proved to be of use as a source of blood-typing reagents for the present study.

Absorptions with erythrocytes of individual sheep were performed on each heteroimmune and isoimmune antiserum that contained antibodies reactive with the red cells of sheep in order to separate any of the populations of antibodies

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which might be present in them. In some cases, trial absorptions showed that an antiserum contained but a single population of antibodies so that no absorptions were necessary for the preparation of a reagent. The antisera were then titrated to determine an appropriate dilution for testing. The titrations were set up as a series of doubling dilutions, and the greatest dilution which resulted in complete or nearly complete lysis with most of the reactive cells at the end of $2\frac{1}{2}$ to 3 hours was chosen as the dilution for testing. The dilution chosen was usually one fourth. Frequently, natural anti-R was present in antisera from group O sheep. These interfering, natural antibodies were routinely removed by two successive absorptions (15 minutes and 30 minutes) of the undiluted serum with washed, human erythrocytes of group A, using a ratio of cells to serum of 1:4 per absorption.

When an antiserum was found by trial absorptions to contain mixed populations of antibodies or subfractions of what appeared to be a single population, the tests of the individual absorptions were compared, and appropriate cells were selected in order to prepare reagents containing but a single population or fraction of antibodies. In many cases, trial absorptions were performed with a combination of absorbing bloods to produce such reagents.

In most cases, it was possible to exhaust all of the antibodies for the absorbing bloods by means of two successive absorptions (15 minutes and 30 minutes) when a ratio per absorption of 1:2 of cells to diluted serum was used. After trial tests and titrations, appropriate dilutions of the reagents were selected for use in the blood-typing tests. Usually the antiserum was diluted one half for absorption and one half for testing, making a final dilution of one fourth of the original antiserum.

Reagents were named by letter for convenience. Those which closely paralleled any previously prepared reagent in reactivity were designated by the same letter as the original reagent.

The procedure followed in the blood-typing tests was described by RASMUSEN (1958). Briefly, two drops (0.10 ml) of diluted antiserum and one drop (0.05 ml) of a two to three percent suspension of washed erythrocytes were mixed in a 10×75 mm glass tube. After a few minutes one drop (0.05 ml) of complement was added, the tubes were again shaken, and the test was read at approximately one half, $2\frac{1}{2}$ and $4\frac{1}{2}$ hours later. The tubes were shaken after each of the first two readings. Rabbit complement, absorbed at 4°C to remove natural heterohemolysins for sheep, was found to be satisfactory for use with all of the reagents reacting in the B system.

EXPERIMENTAL RESULTS

Family studies: Materials for the genetic studies consisted of blood samples from 214 ewes, their 255 lambs and the 15 rams which sired the lambs. These were largely the same blood samples utilized in the genetic studies of the X-Z system (RASMUSEN 1958).

Although each of the blood samples was tested with 44 reagents in addition to the six reagents utilized in the tests for R-O and X-Z phenogroups, this discussion

is confined to the reactions obtained with those reagents shown to be reactive with phenogroups in the B system. These were the 12 reagents named B', E, E_x, E', E' + I', I₂, N, P₁, P_x, Q, S and U₂ prepared from ovine isoimmune antisera, the nine reagents B, B', I_x, I', O'₁, O'_x, P₂, S and Y prepared from bovine isoimmune antisera and the seven reagents E, I₁, T₁, T₂, T₃, U₁ and U₃ prepared from ovine heteroimmune antisera against cattle blood. Reagents with the same name which are designated with subscripts bear subtyping relationships to each other similar to those described by STORMONT (1950) in studies of bovine isoimmune antisera. In these cases, the more broadly cross-reactive reagent was designated by a subscript "x", or "3" or "2", so that the order from the least cross-reactive to the most cross-reactive reagent in such sets would be 1, 2, 3 . . . x. In two cases, two reagents were found to bear a linear subtyping relationship to a more widely cross-reactive reagent, but were not themselves linearly related. The reactions of reagents O'₁ and Q were always within those of O'_x. Similarly, reactions of I₂ and I' were within those of I_x, as were the reactions of I₁ which was simply a subtype of I₂.

The red cells of Shropshire ram 114 were lysed by reagents B, B', E, E_x, O'₁, O'_x, Q, T₃, U₁, U₂ and U₃. When the erythrocytes of any of the 51 lambs of ram 114 were lysed by any of these nine reagents which did not lyse the erythrocytes of the dams of these lambs, the reagents lysing the blood of the lamb fell into one of the two groups: B, B', E, E_x, O'₁, O'_x, T₃, U₁, U₂, and U₃, as opposed to O'_x and Q. None of the reactions of the red cells of the lambs of ram 114 fell outside these two groups.

On the basis of cross-reactions with the B-system reagents, the two B phenogroups of ram 114 may be written BB'EO'₁T₃U₁ and O'_xQ. Twenty-six of his lambs inherited BB'EO'₁T₃U₁ from him, 21 inherited O'_xQ, and four ewes and their lambs had the same B phenogroups as the ram so that his contribution to these lambs could not be determined.

Table 1 shows the B phenogroups contributed by ram 114 to 20 of his lambs: ten to which he contributed BB'EO'₁T₃U₁, and ten to which he contributed O'_xQ. The probable phenogroups of the dams of these lambs are also listed. The lambs were selected to show the various combinations of phenogroups observed in blood samples from this sire family, as well as the inheritance of the phenogroups.

Table 2 shows the inheritance of the two B phenogroups in 20 selected progeny of a Targhee ram, 9501T, whose cells were lysed by reagents B, B', E, E_x, I₁, I₂, I_x, I', P₁, P₂, S, T₁, T₂, T₃ and Y. Eighteen of his lambs inherited phenogroup EE'I_xI'P₁T₁ from him and 12 inherited BB'E_xI₁SY. Thus the reagents E', I₁, I₂, I_x, I', P₁, P₂, P_x, S, T₁, T₂ and Y, in addition to B, B', E, E_x, N, O'₁, O'_x, Q, T₃, U₁, U₂ and U₃ in the family of ram 114 were clearly shown to react in the B system. The families of rams 114 and 9501T were selected for illustration since the B phenogroups of the two rams reacted with all the B reagents of the present study with but one exception, reagent N. The N reagent was shown to be reacting in the B system in the family of ram 114 (Table 1) as well as in the families of rams 5020T, 53035T, 2435XW and 396XW (Table 3).

All of the sire families were studied in a manner similar to the studies of the

TABLE 1

Inheritance of B phenogroups of ram 114 (Shropshire) by 20 of his offspring

Mating number	Dam's number	Probable phenogroups of dam		Offspring's number	Phenogroups of offspring from sire from dam	
1	739	O _x Q	/BNY	401	O _x Q	/O _x Q
2	731	O _x Q	/BB'EO' ₁ T ₃ U ₁	417	O _x Q	/O _x Q
3	227	EO' ₁ Y	/BNY	423	O _x Q	/EO' ₁ Y
4	181	BNY	/O _x Q	409	O _x Q	/BNY
5	23	BNY	/BEE'P ₂ T ₁ U ₁ Y	436	O _x Q	/BNY
6	21	BEE'P ₂ T ₁ U ₁ Y	/BNY	477	O _x Q	/BEE'P ₂ T ₁ U ₁ Y
7	231	BB'EO' ₁ T ₃ U ₁	/BB'EO' ₁ T ₃ U ₁	420	O _x Q	/BB'EO' ₁ T ₃ U ₁
8	101	BB'EO' ₁ T ₃ U ₁	/BEE'P ₂ T ₁ U ₁ Y	391	O _x Q	/BB'EO' ₁ T ₃ U ₁
9	167	NY	/BNY	421	O _x Q	/NY
10	578	NY	/BEE'P ₂ T ₁ U ₁ Y	482	O _x Q	/NY
10	578	BEE'P ₂ T ₁ U ₁ Y	/NY	481	BB'EO' ₁ T ₃ U ₁	/BEE'P ₂ T ₁ U ₁ Y
11	743	BEE'P ₂ T ₁ U ₁ Y	/BB'EO' ₁ T ₃ U ₁	455	BB'EO' ₁ T ₃ U ₁	/BEE'P ₂ T ₁ U ₁ Y
12	222	BB'EO' ₁ T ₃ U ₁	/O _x Q	444	BB'EO' ₁ T ₃ U ₁	/BB'EO' ₁ T ₃ U ₁
13	111	E'P ₂ T ₂ U ₃ Y	/BNY	474	BB'EO' ₁ T ₃ U ₁	/E'P ₂ T ₂ U ₃ Y
14	108	O _x Q	/BNY	392	BB'EO' ₁ T ₃ U ₁	/O _x Q
15	182	EO' ₁ Y	/BNY	422	BB'EO' ₁ T ₃ U ₁	/EO' ₁ Y
16	662	EO' ₁ Y	/NY	476	BB'EO' ₁ T ₃ U ₁	/EO' ₁ Y
17	206	BNY	/BB'EO' ₁ T ₃ U ₁	473	BB'EO' ₁ T ₃ U ₁	/BNY
18	826	BNY	/BNY	479	BB'EO' ₁ T ₃ U ₁	/BNY
19	96	O _x QY	/BNY	475	BB'EO' ₁ T ₃ U ₁	/O _x QY

TABLE 2

Inheritance of B phenogroups of ram 9501T (Targhee) by 20 of his offspring

Mating number	Dam's number	Probable phenogroups of dam		Offspring's number	Phenogroups of offspring from sire from dam	
1	7477T	NY	/BEO' _x QY	1589GT	EE'I _x I'P ₁ T ₁	/NY
2	7287T	NY	/BB'O' ₁ Q	1678GT	EE'I _x I'P ₁ T ₁	/NY
3	8946T	BB'EO' _x T ₃ U ₂	/EE'I _x I'P ₁ T ₁	1658GT	EE'I _x I'P ₁ T ₁	/BB'EO' _x T ₃ U ₂
4	8904T	EE'I _x I'P ₁ T ₁	/BB'E _x I ₁ S	1877GT	EE'I _x I'P ₁ T ₁	/EE'I _x I'P ₁ T ₁
5	9910T	BB'O' _x	/BB'E _x I ₁ SY	2068GT	EE'I _x I'P ₁ T ₁	/BB'O' _x
6	7817T	BB'O' _x	/NY	2107GT	EE'I _x I'P ₁ T ₁	/BB'O' _x
7	7641T	BB'E _x I ₁ S	/NY	2122GT	EE'I _x I'P ₁ T ₁	/BB'E _x I ₁ S
8	9387T	BB'E _x I ₁ S	/EE'I _x I'P ₁ T ₁	1641GT	EE'I _x I'P ₁ T ₁	/BB'E _x I ₁ S
9	127GT	BB'E _x I ₁ SY	/NY	1461GT	EE'I _x I'P ₁ T ₁	/BB'E _x I ₁ SY
10	9116T	BB'E _x I ₁ SY	/BEO' _x QY	2241GT	EE'I _x I'P ₁ T ₁	/BB'E _x I ₁ SY
10	9116T	BB'E _x I ₁ SY	/BEO' _x QY	2240GT	BB'E _x I ₁ SY	/BB'E _x I ₁ SY
11	7941T	BB'E _x I ₁ SY	/NY	1594GT	BB'E _x I ₁ SY	/BB'E _x I ₁ SY
12	8864T	BB'E _x I ₁ SY	/BB'EO' _x T ₃ U ₂	2261GT	BB'E _x I ₁ SY	/BB'E _x I ₁ SY
13	6246T	BB'E _x I ₁ SY	/BB'E _x I ₁ SY	2179GT	BB'E _x I ₁ SY	/BB'E _x I ₁ SY
14	6517T	BB'E _x I ₁ S	/BB'O' ₁ Q	1687GT	BB'E _x I ₁ SY	/BB'E _x I ₁ S
15	8659T	BB'O' _x	/EE'I _x I'P ₁ T ₁	2043GT	BB'E _x I ₁ SY	/BB'O' _x
16	9475T	BB'EO' _x T ₃ U ₂	/BB'E _x I ₁ SY	1607GT	BB'E _x I ₁ SY	/BB'EO' _x T ₃ U ₂
17	8809T	BY	/EE'I _x I'P ₁ T ₁	1431GT	BB'E _x I ₁ SY	/BY
18	9570T	EE'I _x I'P ₁ T ₁	/BB'ENO' _x Q	1783GT	BB'E _x I ₁ SY	/EE'I _x I'P ₁ T ₁
19	8687T	EE'I _x I'P ₁ T ₁	/BEO' _x QY	2007GT	BB'E _x I ₁ SY	/EE'I _x I'P ₁ T ₁

TABLE 3

B phenogroups of 15 rams

Breed	Ram no.	Phenogroup formula	Number of lambs inheriting each phenogroup
Shropshire	114	BB'EO' ₁ T ₃ U ₁ /O' _x Q	26/21
Shropshire	30	BB'EO' ₁ T ₃ U ₁ /EO' ₁ Y	24/24
Suffolk	669-0	O' _x Q /O' ₁	7/1
Suffolk	76650	O' _x Q /O' ₁	3/1
Suffolk	105334	BB'E _x I ₁ Y /BO' _x Y	7/5
Grade Suffolk	21	B /E'P ₂ ST ₁	9/12
Corriedale	412	BO' ₁ Y /BEO' _x QY	11/11
Targhee	5020T	BB'ENO' _x Q /BB'E _x I ₁ Y	10/3
Targhee	53035T	BB'ENO' _x Q /BEY	8/8
Targhee	9501T	BB'E _x I ₁ SY /EE'I _x I'P ₁ T ₁	12/18
Rambouillet	458RW	BB'E _x O' _x QS /BB'E _x O' _x QS	10
Rambouillet	2516XW	BB'E _x O' _x QS /B'O' ₁ Y	2/1
Rambouillet	4375RW	BB'EO' ₁ P ₁ Q /B'O' _x S	6/8
Rambouillet	2435XW	E'NP ₃ T ₂ Y /E'NP ₃ T ₂ Y	9
Rambouillet	396XW	BB'E _x I ₁ S /E'NP ₃ T ₂ Y	3/2

families of Shropshire ram 114 and Targhee ram 9501T. In each family an attempt was made to postulate as few phenogroups as possible. Nevertheless, postulation of a total of 52 different B phenogroups was required to account for the cross-reactions observed in comparing the reactions of the red cells of the lambs and of their parents. This number is a minimum for the flocks studied; more phenogroups might have been diagnosed if more reagents had been available and if the matings had been suitable for revealing all of the phenogroups in each flock. Table 4 lists the cross-reactions of the 52 B phenogroups which were postulated. Code designations, which will be discussed elsewhere in this report, are also given in Table 4.

Cross-reactions of the B phenogroups: Shropshire ram 30 was used in the same flock as ram 114. He contributed BB'EO'₁T₃U₁, already identified in ram 114, to ten of his lambs, and EO'₁Y to 24 lambs (Table 3). The three phenogroups BB'EO'₁T₃U₁, O'_xQ and EO'₁Y, together with a fourth, BNY, comprised over 80 percent of the phenogroups in the flock in which these two rams were used. Phenogroup BNY was deduced from serological evidence as well as from a study of phenogroups inherited by the lambs from their dams. Bloods from this flock which were lysed by reagent N were invariably lysed by reagent Y, known to be reacting in the B system, as evidenced by phenogroup EO'₁Y which appeared as the genetic alternative to BB'EO'₁T₃U₁ in the progeny of ram 30. Most of the bloods lysed by reagents N and Y were also lysed by reagent B (the only exceptions were those whose two B phenogroups were NY plus any other phenogroup which did not result in lysis by the B reagent). Phenogroup BNY could be frequently identified, and was most easily seen in bloods of the formula BNY/O'_xQ.

The B phenogroups were characterized not only by the reagents with which they reacted, but also by the intensity or degree of cross-reactions of the reagents.

TABLE 4

Cross-reactions of phenogroups of the B system of sheep

Code no.	1. The E' set	Code no.	2. The I set	Code no.	3. The Q set
E' 1	BEE'P ₂ T ₁ U ₁ Y	I 1	BB'E _x I ₁ N	Q 1	BB'ENO _x Q
E' 2	BEE'P ₂ T ₂ U ₂	I 2	BB'E _x I ₁ NS	Q 2	BB'EO ₁ P ₁ Q
E' 3	EE'I _x I'P ₁ T ₁	I 3	BB'E _x I ₁ S	Q 3	BB'EO _x QST ₂ U ₂
E' 4	E'NP ₃ T ₂ Y	I 4	BB'E _x I ₁ SY	Q 4	BB'E _x O _x QS
E' 5	E'P ₂ ST ₁	I 5	BB'E _x I ₁ Y	Q 5	BB'O ₁ Q
E' 6	E'P ₂ T ₂ U ₃ Y	I 6	BEI _x I'ST ₂ U ₁	Q 6	BB'O _x QSY
		I 7	BI _x Y	Q 7	BEO _x QY
		I 8	I ₁ Y	Q 8	B'O _x Q
		I 9	I _x Y	Q 9	O _x Q
				Q 10	O _x QY
Code no.	4. The O' set	Code no.	4. The O' set	Code no.	5. The B set (residual)
O' 1	BB'EO _x SY	O' 10	BO ₁ Y	B 1	— (no cross-reactions)
O' 2	BB'EO ₁ T ₃ U ₁	O' 11	BO _x Y		
O' 3	BB'EO ₁ U ₃	O' 12	B'EO _x U ₃ Y	B 2	B
O' 4	BB'EO _x T ₃ U ₂	O' 13	B'NO ₁	B 3	BE
O' 5	BB'O ₁	O' 14	B'O ₁	B 4	BENY
O' 6	BB'O _x	O' 15	B'O ₁ Y	B 5	BEY
O' 7	BENO ₁	O' 16	B'O _x S	B 6	BNY
O' 8	BEO ₁ T ₂ U ₂	O' 17	EO ₁ Y	B 7	BY
O' 9	BO ₁	O' 18	O ₁	B 8	NY
		O' 19	O ₁ S		

Table 5 summarizes the tests of the bloods in which phenogroups BB'EO₁T₃U₁, EO₁Y, O_xQ and BNY were present in various combinations in this flock. Degrees of hemolysis are listed as 0, 1, 2, 3 and 4 (complete hemolysis). Although some variability in degrees of hemolysis of bloods of the same phenogroup formula was observed, it was never marked. This table summarizes the modal degrees of hemolysis for bloods of each formula with pertinent reagents. It shows only the degree of hemolysis seen five hours after the tests were set up and, therefore, does not take into account the rate of hemolysis with individual reagents. Nevertheless, even here differences are apparent; the O_x reagent, for example, lysed cells of three of the four phenogroups, but to differing degrees. Bloods with phenogroup O_xQ were characterized by rapid, complete hemolysis with the O_x reagent, those with phenogroup EO₁Y by moderate hemolysis, and those with BB'EO₁T₃U₁ by very slow, slight hemolysis. Differences between homozygotes and heterozygotes in rates and degrees of reaction with many of the reagents was observed throughout this study. Such dosage effects were also observed in some cases between certain phenogroups in heterozygous combinations (especially when the reagent showing dosage reacted with each of the two phenogroups so combined) but also occasionally when this was not the case. For example, dosage was observed with the O₁, O_x, U₁ and U₂ reagents so that bloods of the phenogroup formula BB'EO₁T₃U₁/BB'EO₁T₃U₁ were lysed more completely by each of these four reagents than were bloods of formula BB'EO₁T₃U₁/BNY, as

TABLE 5

Summary of the cross reactions of four B phenogroups of Shropshire sheep

Phenogroup formulas	No. of bloods	Reagents and antiserum numbers*														
		B	B'	B'	E	E	E _x	N	O' ₁	O' _x	Q	T ₃	U ₁	U ₂	U ₃	Y
		C	C	S	S	SC	S	S	C	C	S	SC	SC	S	SC	C
O' _x Q	/O' _x Q	2	0	0	0	0	0	0	0	4	4	0	0	0	0	0
O' _x Q	/BB'EO' ₁ T ₃ U ₁	23	4	4	4	4	4	0	3	4	4	3	1	3	4	0
O' _x Q	/EO' ₁ Y	5	0	1	0	3	3	0	4	4	4	0	0	0	0	4
O' _x Q	/BNY	17	4	0	0	0	0	0	4	0	4	0	0	0	0	4
BB'EO' ₁ T ₃ U ₁ /BB'EO' ₁ T ₃ U ₁		4	4	4	4	4	4	0	4	2	0	3	3	4	4	0
BB'EO' ₁ T ₃ U ₁ /EO' ₁ Y		5	4	4	4	4	4	0	4	3	0	3	1	3	4	4
BB'EO' ₁ T ₃ U ₁ /BNY		13	4	4	4	4	4	4	1	1	0	2	1	3	4	4
EO' ₁ Y	/EO' ₁ Y	1	0	1	0	3	3	3	0	4	3	0	0	0	0	4
EO' ₁ Y	/BNY	20	4	1	0	3	3	3	4	3	0	0	0	0	0	4
BNY	/BNY	4	4	0	0	0	0	4	0	0	0	0	0	0	0	4

* Bovine isoimmune antisera are coded C, ovine isoimmune antisera are coded S and ovine heteroimmune antisera against cattle red cells are coded SC. Bloods of the phenogroup formulas listed in this table did not react with reagents E' (S21), I' + E' (S56), I₁ (SC38), I₂ (S63), I_x (C31), I' (C10), P₁ (S18), P₂ (C47), P_x (S26), S (S35), S (C27), T₁ (SC55) and T₂ (SC44).

may be seen in Table 5. Similarly, cells of formula O'_xQ/BB'EO'₁T₃U₁ were lysed more completely by the O'₁ reagent than were those of formula BNY/BB'EO'₁T₃U₁. Observations of this kind were very helpful in diagnosing phenogroups, as the reagents were quite consistent in their reactions with bloods of each phenogroup formula.

Phenogroup EO'₁Y was characterized in part by partial hemolysis with the B' reagent prepared from a bovine isoimmune antiserum, C71. However, absorption of this reagent with bloods of formula EO'₁Y/O'_xQ or EO'₁Y/BNY removed this weakly cross-reactive fraction without removing the principal fraction which paralleled the reactions of the B' reagent prepared from an ovine isoimmune antiserum, S4. Minor differences in reagents of this order often proved helpful in the diagnosis of the phenogroups. Not all such information was incorporated into the phenogroup formulas.

The results of the tests of the Targhees showed numerous examples of graded cross reactions, just as did those of the Shropshires. For example, the phenogroups listed as BB'E_xI₁S and BB'E_xI₁SY in Tables 2, 3 and 4 appear to be very similar on the basis of a comparison of their phenogroup formulas, but cells with the phenogroup BB'E_xI₁S were lysed much more slowly by the B reagent, more rapidly by the E_x reagent and more slowly by the I₁ and I₂ reagents than those with BB'E_xI₁SY, so that in addition to the reaction with the Y reagent the two phenogroups were clearly distinguishable.

The effects of dosage were observed in many cases in addition to those listed in Table 5. Comparison of the cross-reactions of the reagents with bloods with a specific phenogroup in one dose and in two doses revealed differences in reactivity in most cases in which the reagent did not completely lyse the erythrocytes of the heterozygote. Ram 4458RW was homozygous for BB'E_xO'_xQS, and ram

2516XW was heterozygous for this phenogroup; dosage was observed with the B and Q reagents, and the E reagent prepared from a heteroimmune antiserum (SC48) slightly lysed the cells of the homozygous ram. Ram 2435XW was homozygous for phenogroup E'NP₃T₂Y, and ram 396XW was heterozygous for this phenogroup. Dosage was observed with one E' reagent and with the N reagent. Reagents E (SC48) and I₁ also lysed the erythrocytes of the homozygote, apparently due to residual antibodies in these reagents which were comparable to those of the E' reagent, and which were of sufficient concentration to lyse erythrocytes of the homozygote E'NP₃T₂Y but not those of heterozygotes.

As a general rule, reagents prepared from bovine isoimmune antisera produced more rapid and complete hemolysis, or more uniformly lysed reactive cells than many of those of ovine origin, so that cells lysed by these reagents were usually very clearly distinguished from those not lysed. Nevertheless, they were actually less useful in identifying individual phenogroups on the basis of degrees of cross-reactions than were the reagents which produced different degrees of hemolysis with reactive bloods of different phenogroup formulas.

In cases where the reactions were of a doubtful nature (e.g., very slow or very weak hemolysis) absorption tests were performed, when sufficient quantities of blood were available, to determine whether the antibodies could be absorbed from the reagent by erythrocytes with the phenogroup in question. If the antibodies were completely absorbed, the reagent name was included in the formula of cross reactions for that phenogroup. Absorptions sometimes failed to give clear-cut results, so that in some cases antibodies might be partially absorbed, and in others, completely so. Therefore, designating cross-reactions in the phenogroup formulas was occasionally somewhat arbitrary.

Coding the phenogroups: In view of the fact that reagents for B-system phenogroups of sheep differed widely in the cross reactions they exhibited, and the possibility that another set of reagents could be developed which for the most part might be quite unlike those used in this study, it was considered advisable to code the 52 B phenogroups in order to avoid undue emphasis on the battery of reagents used to diagnose them. The coded phenogroups (Table 4) have been arranged in sets based on the reactions with certain key reagents which are likely to be reproduced in future studies. These sets are similar in arrangement to those proposed by STORMONT (1955) for B phenogroups of cattle.

(1) The E' set of B phenogroups (Table 4) includes all phenogroups which reacted to some degree with E' reagents. (2) The I set (Table 4) includes all phenogroups which reacted with the I_x reagent excepting those included in the E' set. Of the phenogroups observed, only one in the E' set, (coded E' 3) cross-reacted with the I_x reagent. (3) The Q set (Table 4) includes the phenogroups which reacted with the Q reagent. (4) The O' set (Table 4) includes the phenogroups which reacted with the O'₁ and/or the O'_x reagents, excepting those already assigned to the Q set. (5) The B set (Table 4) is the residual set and includes all phenogroups not in the foregoing sets.

The "key" E' reagents were prepared from ovine isoimmune antisera, coded S21 and S56, which have also been used in this laboratory as sources of I and E'.

reagents for cattle blood-typing tests. Three of the other four key reagents (I_x , O'_1 and O'_x) were prepared from bovine isoimmune antisera, coded C31, C71 and C65, which have served, respectively, as sources of the cattle reagents named I , O_1 and O_x . Thus, four of five key reagents in the sheep tests were from antisera which have served as important sources of B-system reagents in the cattle tests.

Table 6 lists the breeds of sheep studied and the phenogroups found in each breed. As can be seen from the table, some phenogroups were found in more than one breed. The asterisks which designate the phenogroups which were relatively

TABLE 6

Distribution by breed of B phenogroups of sheep

Rambouillet	Targhee	Corriedale	Shropshire	Suffolk
E' 4*	E' 3*	I 3	E' 1	E' 1
I 1	I 3*	Q 7	E' 6	I 5
I 2	I 4*	Q 8	Q 9*	I 7
I 3*	I 5	O' 10	Q 10	I 8
I 4	Q 1	O' 11	O' 2*	I 9
I 5	Q 5	O' 13	O' 3	Q 8
Q 1*	Q 7	B 1	O' 12	Q 9*
Q 2*	O' 4		O' 17*	Q 10
Q 4	O' 6		O' 18	O' 5
O' 4	B 5		B 4	O' 9
O' 15	B 7		B 6*	O' 11
O' 16	B 8*		B 8	O' 14
O' 18				O' 18*
O' 19				B 2

* Phenogroups of relatively high frequency.

TABLE 7

The inheritance of B, R-O and X-Z phenogroups of ram 5020T (Targhee) by 13 of his offspring

Offspring number	Phenogroups from sire in blood-group system		
	B	R-O	X-Z
6	Q1	*	X
11	Q1	*	X
14	Q1	O	Z
15	Q1	R	Z
19	Q1	R	Z
27	Q1	R	X
38	Q1	O	Z
39	Q1	R	X
49	Q1	O	X
67	Q1	O	*
33	I5	O	X
50	I5	O	*
66	I5	R	*

* Contribution of sire not determined.

frequent in the flocks studied may not necessarily designate those which are frequent in the breed as a whole inasmuch as the blood samples which were tested were collected from a small number of flocks.

Tests for independence of the blood-group systems B, R-O and X-Z: Table 7 shows the B phenogroups, I 5 and Q 1, the R-O phenogroups, R and O (STORMONT 1951; RENDEL, NEIMANN-SØRENSEN and IRWIN 1954), and the X-Z phenogroups, X and Z, which 13 lambs inherited from their sire, Targhee ram 5020T. (The asterisks in Table 7 indicate that the contribution of ram 5020T to his offspring could not be determined. This was the case if in the R-O system, the sire, dam and lamb were all of group R, and in the X-Z system, if the lamb and its parents were all of type XZ.) These and other data indicate that the B, R-O and X-Z blood groups represent three different systems. While the genes controlling these three systems appear to assort independently, the possibility of loose linkage is not excluded by the present data.

DISCUSSION

Observations of tests of cattle red cells with ovine isoimmune antisera (STORMONT, SUZUKI and RASMUSEN 1957) provided serological evidence for the existence in sheep (*Ovis aries*) of a blood-group system comparable to the complex B system of cattle (*Bos taurus*). Twenty-nine of 47 ovine isoimmune antisera tested contained antibodies which distinguished individual differences in cattle. From analysis of the cross-reactions of 20 of these 29 antisera, it was clearly evident that the reactions of 18 of the 20 antisera could be ascribed solely to phenogroups in the B system of cattle. Furthermore, it was possible to prepare reagents from some of these antisera which produced reactions in the cattle tests that paralleled the reactions of such previously known cattle reagents as I and E'₃. (Many of the cross-reactions of ovine isoimmune antibodies with B phenogroups of cattle were, however, unique patterns not previously encountered in the cattle studies). Also, as already noted, nine of the 26 reagents used in characterizing phenogroups in the B system of sheep were derived by absorbing bovine isoimmune antisera with selected sheep bloods. The specific antibodies in these nine reagents used for typing sheep red cells were known to have been engendered by B phenogroups in cattle. In addition the linear and nonlinear subtyping relationships observed in tests with the various reagents, regardless of their source and derivation (bovine or ovine isoimmune antisera or ovine heteroimmune antisera against cattle red cells), indicate that these reagents must be reacting in a single system in sheep.

The genetic data presented in this report provide confirmation of the earlier serological evidence for a blood-group system in sheep analogous to or homologous with the B system of cattle. Furthermore, the present evidence indicates that the B system of sheep may be fully as complex as its homologue in cattle. The question remains whether the demonstrated serological homology between B phenogroups in the two species extends to the level of the gene. It seems likely that it does, but in the absence of any authentic reports of the existence of viable hybrids between the two species, no means of obtaining direct evidence is presently available.

In genetic studies based on reactions of blood-typing antibodies, it may seem desirable to have reagents which react to produce rapid, complete hemolysis with all bloods with which they react. With such reagents there would be little question in differentiating negative from positive reactions. A reagent which produces a graded series of reactions from rapid, complete hemolysis through varying degrees of hemolysis to no hemolysis presents serious problems in interpretation in the absence of any knowledge of the phenogroups with which it cross reacts. Is the reaction due to a single population of antibodies in the reagent, or is it not? Resort to absorption with the red cells showing dubious reactions often leads to dubious results; manipulation of the ratio of cells to serum, the dilution of the serum, the number and length of absorptions and the temperature at which they are performed may result in removal of the antibodies or lowering their titer so that the reagent fails to react. Or, the antibodies may be only partially removed, and some cross-reactions are observed with the absorbed reagent. Indeed, many of the reagents, especially those prepared from ovine antisera, both isoimmune and heteroimmune, had low titers of antibodies, exhibited graded series of cross-reactions, and absorptions sometimes yielded dubious results. Nevertheless, it was just such reagents which ultimately proved to be most useful in diagnosing B phenogroups. The degree of cross-reaction with red cells of the same phenogroup formula was remarkably constant; the main difficulty became one of terminology.

Studies of the specificity of antigens and antibodies (reviewed by LANDSTEINER 1945) have shown that a single antigen may stimulate the production of multiple antibodies differing in specificity and in degree of affinity with the homologous antigen and closely related chemical substances. Such studies provide models for the cross-reactions in the B system of cattle (STORMONT *et al.* 1951), in the B system of sheep presented in this report, and for the cross-reactions of ovine and bovine isoimmune antisera in these systems (STORMONT *et al.* 1957). They have also been used as models for cross-reactions in complex blood-group systems in man (WIENER and WEXLER 1952) and in chickens (BRILES, IRWIN and MCGIBBON 1950). In the author's opinion, any attempt to describe the reactions of B phenogroups of sheep in terms of models involving discrete blood factors controlled by closely linked genes, subgenes, pseudoalleles or other hypothetical entities, rather than in terms of cross-reactions exhibited by a series of closely related antigens controlled by the alleles at a single complex locus would not only be a misrepresentation of the observations but would also serve to complicate interpretation of the reactions observed.

A reagent may show clear-cut individual differences in reactions with certain B phenogroups, but may only slowly and partially lyse bloods of other B phenogroups. The use of letters to signify cross-reactions with the individual reagents is helpful to visualize the cross-reactions of the phenogroups, but does involve the possibility of conveying the impression of an all-or-none situation, which is not the case in the B system of sheep or any system of comparable complexity in other species. Only by a consideration of the reactions with the entire battery of B-system reagents can the pattern of cross-reactions of the phenogroups be

determined. The reagents used in this study are certainly not the only B-system reagents which could be developed. In addition to the named reagents, other reagents clearly reacted in the B system, but were not given letter designations since including them in the pattern of cross-reactions would only serve to complicate an already complex nomenclature and would not further differentiate the 52 B phenogroups of this study. They were sometimes of use, however, in differentiating between certain combinations of B phenogroups, just as certain reagents of ovine isoimmune origin are of use in B phenogrouping in cattle (STORMONT 1958).

The decision to include or not include the name of any given reagent in the pattern of cross-reactions was sometimes difficult and arbitrary. A table of cross-reactions such as Table 4 in this report is especially desirable in detecting new phenogroups and as a guide in recognizing those already known. It is readily expandable; a new reagent may be designated with a new letter and its reactions then added to the table. The most serious difficulty which such a method presents as a basis for the nomenclature of a blood-group system is that it cannot be used readily by other investigators using a different battery of reagents. The coding system proposed in this report provides a system for naming phenogroups which is based on a few key reagents. New phenogroups can be readily fit into this nomenclature, and the sets of phenogroups, though somewhat arbitrary, appear to be reasonably logical groupings which can be expanded if necessary.

The coding system is less cumbersome than the use of a complex pattern of cross-reactions, and provides a simpler nomenclature with which to identify the phenogroups. Neither system is without defects, but difficulties which are likely to be encountered in duplicating many of the B-system reagents make exclusive use of the complete pattern of cross-reactions undesirable as the sole basis for describing the B system of sheep.

The coding system may also be used as a basis for naming the alleles in the B series, on the assumption that each phenogroup is the product of an individual allele. *B* is the base symbol for the locus, and the alleles are designated by appropriate superscripts: $B^{E'1}$ through $B^{E'6}$, B^{I1} through B^{I9} , B^{Q1} through B^{Q10} , $B^{O'1}$ through $B^{O'19}$ and B^{B1} through B^{B8} , making a total of 52 alleles.

SUMMARY

Evidence is presented for a blood-group system in sheep, the B system, which is serologically homologous to the complex B system in cattle and resembles the cattle system in its genetic features. It seems probable that the homology between the B systems in cattle and in sheep extends to the level of the gene or locus. Of a total of 50 reagents used in blood typing of sheep, 33 reacted in the B system. Tests with these reagents combined with genetic data indicate a minimum of 52 alleles in the B series of sheep.

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