BLOOD GROUPS AND THE TAXONOMIC STATUS OF AMERICAN BUFFALO AND DOMESTIC CATTLE

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The generally accepted taxonomic position of the American buffalo (*Bison bison*) and domestic cattle (specifically, *Bos taurus*) is that of separate genera in the subfamily Bovinae. That the two species might very well occupy positions in the same genus is suggested by one of several alternative taxonomic designations for the American buffalo, notably, *Bos americanus* (see Roe, 1951). The two species are apparently fully cross-fertile, but only the females among the F_1 hybrids seem to be fertile (Deakin et al., 1942). Attempts to differentiate the two species by means of the classical precipitin technique have not been uniformly successful (Wolfe, 1939) and even when turbidimetric methods of measuring precipitates are used, the two species always stand closely side by side (Boyden et al., 1951). In this connection the question arises whether more extreme differences in precipitin values might be found between and perhaps even within breeds of domestic cattle. Likewise, attempts to demonstrate a clearly definitive difference between the two species by means of cross-absorbing antisera produced in rabbits against both bison and cattle red cells yielded equivocal results (Owen et al., 1958). In the case of each antiserum, absorption with a pool of red cells from the heterologous species succeeded in removing the greater portion and sometimes all of the antibodies directed against the red cells of the homologous species. Such observations suggest that the antigenic structure of the red cells of bison and domestic cattle must be very similar, and this was borne out to a considerable degree by blood typing bison with a rather large battery of blood-typing antibodies (reagents) which had been developed specifically for the purpose of exploring individual differences in cattle. For example, Owen et al. observed antigenic factors in bison blood which appeared to be indistinguishable from the bovine blood factors named **A**, **C**, **J**, **S**, **W**, **X**, and **Z**. Nevertheless, some rather marked differences were noted between the blood types of the two species, and those differences appeared to be significantly greater than any of the between-breed differences encountered in blood typing domestic cattle. For example, it appeared that the complex B locus of cattle is either not represented in bison or, if present, is occupied solely by alleles whose products (phenogroups) are essentially non-reactive with any of the 21 B system reagents used in that study.

The data reported by Owen et al. (1958) were actually collected during the years 1945–47. Since then, we have had the opportunity to develop in this laboratory an entirely new battery of cattle blood-typing reagents which derive from antisera independent of those used as sources of reagents by Owen et al. or by Miller and Stone (1955) in a further study of bison blood types. In connection with another study (Stormont and Suzuki, 1958), we had the opportunity to type three bison with our reagents. The results of those tests provided evidence which tended to narrow the magnitude of the blood-type differences between the two species. Further inquiry was therefore prompted and through the courtesy of Mr. Julian A. Howard, Refuge Manager of the Wichita Mountains Wild Life Refuge, Cache, Oklahoma, and Dr. D. C. Bostwick, we received 56 samples of citrated bison blood on October 29, 1959. (This was the same herd from which Owen et al. obtained 44 of the 69 samples used in their study, and it was also the same herd from which

TABLE 1. *The 41 classified "facsimile" reagents used in typing the 56 bison blood samples*[1]
(Information is given on the number of replicas of each reagent, as shown within parentheses; the letters a, b, c, . . . etc. . . . m indicate the sources of the antisera from which the reagents were obtained, and the decode for these letters is given as follows: a = cattle isoimmune serum; b = rabbit anti-cattle serum; c = sheep isoimmune serum; d = rabbit anti-bison serum; e = rabbit anti-bactrian camel serum; f = goat anti-cattle serum; g = sheep anti-cattle serum; h = rabbit anti-human A serum; i = cattle anti-human A serum; j = normal cattle serum; k = normal sheep serum; l = normal pronghorn antelope serum; m = cattle anti-bison serum.)

Reagents by systems	Sources and replicas	Reagents by systems	Sources and replicas	Reagents by systems	Sources and replicas
A system		*B system*		*F–V system*	
A_1	a (1)	A′	a (1)	F	b (2); d (1); e (1);
A_2	b (1)	D′	a (1)		f (1); k (1); l (2)
H	a (2)	E_1'	a (1); b (1)	V	a (3); b (1); g (1);
B system		E_2'	a (1)		m (1)
B	a (1)	E_3'	a (1); c (1)	*J system*	
G	a (5)	I′	a (2)	J	h (3); i (1); j (2)
I	a (1); c (1)	J′	a (2)	*L system*	
K	a (2)	K′	a (3)	L	a (1)
O_1	a (1)	*C system*		*S system*	
O_3	a (1)	C_1	a (2)	S	a (2)
P	a (1)	C_2	a (1)	U_1	a (1); b (1)
Q	a (1)	R	a (3); c (1)	U_2	a (1)
T_1	a (1)	W	a (2)	U′	a (1)
T_2	a (1)	X_1	a (1); d (1)	H′	a (2)
Y_1	a (1)	X_2	a (1)	*Z system*	
Y_2	a (2)	L′	a (6)	Z	a (2); b (1)
				Z′ system	
				Z′	a (1)

[1] Facsimile reagents are simply reagents which in blood typing cattle are known to be replicas or near-replicas of cattle reagents which have been previously described in the literature.

Miller and Stone obtained 193 samples for their study.) The present report is concerned with the results of blood typing those 56 samples. Data are also presented on the occurrence of certain natural antibodies and soluble blood group substances in the plasma of the bison blood samples.

MATERIALS AND METHODS

Hemolytic techniques, as described in previous reports cited by Owen et al. (1958), were used in this as well as the two previous studies. The only deviation in technique of the present study concerned sources of complement. Heretofore, fresh frozen rabbit sera has been used exclusively as complement in cattle and bison blood typing. We have observed, however, that a few of our reagents, particularly our anti-J reagents prepared from rabbit antisera engendered by immunizing with human red cells of type A (Stormont and Suzuki, 1960), have little or no lytic activity when complemented with fresh rabbit sera but do promote specific hemolysis when activated by fresh guinea pig sera used diluted in a range of 1:15 to 1:20.

In table 1 we have listed 41 cattle blood-typing reagents used in the present study which are known to be facsimiles or near-facsimiles in tests of cattle of the vast majority of the reagents used in the two previous studies. We also have noted in table 1 the source of each antiserum from which the various reagents were derived and the number of replicas of each reagent.

In table 2 are listed the new reagents, a total of 25, whose reactions with bison red cells have not previously been described. As in table 1, we have indicated in table 2 the sources of antisera from which the new reagents were derived and also the number of replicas of each reagent.

TABLE 2. *The 25 classified "new" reagents used in typing the 56 bison bloods*

(The number of replicas of each reagent is shown within parentheses. The decode of the sources of antisera is as follows: a = cattle isoimmune serum; b = rabbit anti-cattle serum; c = sheep isoimmune serum; d = normal cattle serum.)

Reagents by systems	Sources and replicas
A system	
D	b (7)
B system	
B_2	a (1)
I_2	a (2)
O_x	a (1)
P_x	a (1)
A_2'	a (1)
B′ or NF6	a (2)
E_x'	a (4)
F′	a (1)
G′	a (2)
O′ or NF8	a (1)
Y′ or NF4	a (1)
NF7	a (1)
S2	c (1)
S5	c (1)
S26	c (1)
S27	c (1)
S34	c (1)
C system	
E	a (2)
NF12	a (1)
J system	
Oc	d (1)
M system	
M_1	a (1)
M_2	a (1)
S system	
S + H′	a (2)
U_1 + H′	a (1); b(1)

All the reagents listed in tables 1 and 2 are classified reagents. That is, they have been classified with respect to the known genetic systems of blood groups in cattle in which they react (see Stormont, 1958). Attention is called to the reclassification of H′ reagent, which in the two previous studies had been regarded as being reactive in a system (the H′ system) independent of the 10 systems listed in tables 1 and 2. We have shown that H′ reagent reacts in the S system of bovine blood groups. Likewise, attention is called to a reclassification of the new D reagent (table 2) which was previously believed (Stormont and Suzuki, 1956) to be reactive in a new system (the D system) independent of the others. As pointed out in a brief note published elsewhere (Stormont et al., 1960), it was the results of typing the present sample of 56 bison bloods which provided the first clue (see table 3) relating the reactions of D antibodies to the A system of cattle.

RESULTS

Bona fide or true cross reactions, as determined by appropriate absorptions, were obtained in typing bison red cells with the facsimile reagents A_1, A_2, C_1, C_2, F, J, L, S, V, W, X_1, X_2, Z, and E_3' of table 1 and also with the new reagents D, M_2, P_x, S + H′, one of the two U_1 + H′, E_x' and NF (new factor) 12 of table 2. All the remaining reagents (27 in table 1 and 18 in table 2) produced no bona fide cross reactions with the red cells of bison. In this connection, however, we should mention that one of the five replicated G reagents (table 1) produced lysis of all 56 bison bloods but that the reactions, as readily demonstrated by absorbing the particular reagent with both bison red cells and with G-positive cattle cells, could not be attributed to the specific G antibodies in the reagent. Rather, they were due to certain heterophile antibodies which were probably present in the normal serum of the cow which produced the antiserum. At any rate, absorption of this particular G reagent with G-positive cattle red cells provided us with a testing fluid which could be used to distinguish bison red cells from those obtained from domestic cattle.

Summarized in table 3 are the reactions of the 15 reagents which distinguished individual differences in the 56 bison bloods. By means of appropriate symbols (see table 3), we have attempted to differentiate partial or incomplete cross reactions from complete cross reactions believed to be fully equivalent to the reactions obtained with the particular reagents in typing animals of the homologous species. Likewise, dosage reactions (indicative of two doses of a given blood factor) are differentiated

TABLE 3. *Summary of the reactions of 56 bison blood samples with 15 blood-typing reagents which disclosed individual differences*

(The dot [·] symbol indicates no hemolysis; the plus [+] symbol indicates hemolysis equivalent to that seen in typing cattle bloods; the plus–minus [±] symbol indicates a "partial" cross reaction in which hemolysis was not as intense as that regularly encountered in typing cattle; the letter d attached to the symbols ± and + indicates dosage reactions. In the J system, the symbol +1 indicates strong hemolysis with all J reagents; +2 indicates strong lysis with certain J reagents from rabbit anti-human A serum but somewhat weaker lysis with other J reagents; +3 indicates weaker lysis with strong J reagents and considerably weaker or even no hemolysis with other J reagents.)

			Reagents by Systems														
Bison	Sex	Age	A			B			C			J	M	S			Z
			A_1	A_2	D	P_x	E_8'	E_x'	C_1	C_2	W	J	M_2	S	S+H′	U_1+H′	Z
26	M	4	·	·	·	±	±	+	·	·	·	+1	·	+	+	±	+d
27	M	2	·	·	·	±	±	+	·	·	+	+1	·	+	+	±	·
28	M	2	·	·	·	·	·	·	·	·	·	+1	·	·	·	·	+d
29	M	4	·	·	·	·	·	·	·	·	+	+2	+	·	·	·	+
30	M	2	·	·	·	±	±	+	·	·	+	+1	+	·	·	·	+
31	M	2	·	·	·	·	·	·	·	·	+	+1	·	+	+	±d	+
32	M	2	+	+	±	·	·	·	·	·	·	+1	·	+	+	±	+
33	M	2	+	+	±	·	·	·	·	+	+	+1	·	·	·	·	·
34	M	3	+	+	±	±d	±d	+	·	·	·	+1	·	·	·	·	+d
35	M	4	+	+	±	±	±	+	·	·	·	·	·	·	·	·	+
36	M	2	·	·	·	·	·	·	·	+	+	+3	·	+	+	±	+
37	M	2	+	+	±	·	·	·	·	·	·	+1	·	·	·	·	+d
38	M	2	·	·	·	·	·	·	·	·	·	+1	·	·	·	·	+d
39	M	2	+	+	±	±	±	+	·	+	+	+1	·	·	·	·	+d
40	F	8	+	+	±	·	·	·	·	·	·	+2	·	·	·	·	+d
41	F	4	·	·	·	·	·	·	·	+	+	+1	·	+	+	±	·
42	F	4	·	·	·	·	·	·	·	·	+	+3	·	·	·	·	·
43	M	2	+	+	±	·	·	·	·	+	+	+1	·	+	+	±	·
44	M	10	+	+	±	±	±	+	·	+	+	+1	+ *	·	·	·	+
45	M	3	·	·	·	·	·	·	·	·	+	+1	·	+	+	±	+
46	M	3	+	+	±	·	·	·	·	·	·	·	·	·	·	·	+d
47	M	2	+	+	±	±	±	+	±d	+d	+	+1	·	+	+	±	+
48	M	3	+	+	±	·	·	·	·	·	·	+1	·	·	·	·	+d
49	M	2	·	·	·	±	±	+	·	·	·	+3	·	·	·	·	+d
50	M	2	·	·	·	±d	±d	+	·	·	·	+2	·	+	+	±	+
51*	?	?	·	·	·	±	±	+	·	·	+	+1	·	·	·	·	+
52	M	2	+	+	±	±d	±d	+	·	+	+	+1	·	·	·	·	+
53	M	2	+	+	±	±	±	+	·	·	·	+1	·	+	+	±	+d
54	M	2	·	·	·	±	±	+	·	·	·	+1	·	·	·	·	+d
55	M	2	·	·	·	±	±	+	·	·	+	+1	·	·	·	·	+d
56	M	2	·	·	·	±	±	+	·	·	·	+1	·	·	·	·	+
57	M	2	+	+	±	·	·	·	·	·	+	+1	·	+	+	±	+d
58	M	2	·	·	·	±	±	+	·	·	·	·	·	·	·	·	+
59*	?	?	+	+	±	·	·	·	·	·	+	+1	·	+	+	±d	+
60	F	3	·	·	·	·	·	·	·	·	+	·	·	·	·	·	+d
61*	?	?	+	+	±	±d	±d	+	·	·	·	+2	·	·	·	·	+
62	M	2	·	·	·	·	·	·	·	+	+	+2	·	·	·	·	+
63*	?	?	·	·	·	·	·	·	·	·	+	·	·	+	+	±	+
64	M	3	·	·	·	·	·	·	·	·	·	·	+	+	+	±	·
65	M	3	+	+	±	·	·	·	±d	+d	+	+2	·	+	+	±	+d
66	F	7	·	·	·	±	±	+	·	·	+	+1	·	·	·	·	+d
67	F	6	·	·	·	·	·	·	·	·	+	+1	·	·	·	·	+
68	F	3	+	+	±	±	±	+	·	·	+	+1	·	+	+	±	+
69	F	5	+	+	±	·	·	·	·	·	·	·	·	·	·	·	+d
70	F	6	·	·	·	·	·	·	·	·	+	+1	·	·	·	·	+
71	M	10	·	·	·	±	±	+	·	·	+	+1	+	·	·	·	+d
72	F	14	·	·	·	·	·	·	·	+	+	+1	·	+	+	±	+d
73	F	9	+	+	±	·	·	·	·	+	+	·	·	·	·	·	+d
74	F	3	·	·	·	·	·	·	·	·	+	·	·	·	·	·	+
75	F	4	·	·	·	·	·	·	·	·	+	+1	·	·	·	·	+
76	M	3	+	+	±	·	·	·	·	·	·	+1	·	·	·	·	+
77	M	3	·	·	·	±	±	+	·	·	+	+1	·	·	·	·	+d
78	F	3	·	·	·	±	±	+	·	·	+	+1	·	+	+	±	·
79	F	4	·	·	·	·	·	·	·	·	·	+1	·	+	+	±	+d
80	M	3	·	·	·	±	±	+	·	·	·	·	·	+	+	±	·
81	M	?	·	·	·	·	·	·	·	·	·	·	+	·	·	·	+

* Samples which lost their labels in transit. There were three males and one female.

and so, also, are the four main categories of J reactions. Note that the following repairs and trios had identical blood types: 28–38, 37–48, 46–69, 55–66–77, and 67–70–75.

The A System in Bison

Three of the four A system reagents used in typing cattle cross reacted with bison red cells, and all three distinguished individual differences. These were the reagents A_1, A_2, and D. However, each of these reagents produced the same reactions in the parallel tests, as shown in table 3. Consequently, a single pair of bison phenogroups A_1D and "–" (the negative alternative) is all that is required to explain the reactions. The phenogroup A_1D cross reacted with A_1, A_2, and D antibodies whereas the "–" alternative was non-reactive with all of the A system reagents presently available. Estimates of the frequencies of the two alleles which control these phenogroups in the Oklahoma or Wichita population of bison are given in table 4.

The question arises whether the phenogroups A_1D and "–" of bison have their equivalents in domestic cattle. At the time these data were collected we could not be certain, since, as already noted, it was the present results which first called our attention to the fact that reagent D must be reacting not only in the A system of bison but also in the A system of cattle. Reanalysis of the A system of cattle has indicated (Stormont et al., 1960) that a minimum of seven phenogroups is required to explain the reactions of reagents A_1, A_2, D, and H with cattle bloods. Since all seven of these phenogroups exhibit positive reactions with one or more of the A system reagents, there is as yet no evidence for a phenogroup equivalent to the "–" alternative in bison. We have, however, observed an A system phenogroup in cattle which exhibits the same reactions as A_1D of bison. We have not had the opportunity to compare it with bison A_1D in absorptions with D reagent. As indicated in table 3, the D reactions of bison blood were not fully equivalent to those of cattle blood. When bison red cells of phenotype A_1D were used to absorb D reagents not all of the D antibodies for cattle were exhausted. However, in those tests there were no cattle which possessed phenogroup A_1D to the exclusion of other D-positive phenogroups. Further study is therefore required in order to decide whether A_1D of bison is equivalent to A_1D of cattle in the extent to which it will absorb D antibodies.

The B System in Bison

As indicated by the large number of B system reagents (tables 1 and 2) used in typing cattle, the B system of cattle is extremely complex. This is further indicated by the fact that we presently recognize, in our laboratory alone, 251 B phenogroups and their controlling alleles. A list of some of the B phenogroups of cattle and estimates of the frequencies of their alleles in several breeds of cattle are given elsewhere (Stormont, 1958).

Only three of the 37 B system reagents (tables 1 and 2) cross reacted with bison red cells. These were the reagents E_3' (facsimiles of the E_3' reagent used in two previous bison studies), P_x and E_x'. All three of these reagents are known to produce related patterns of cross reactions with cattle red cells. Each of the three reagents distinguished individual differences in bison, but, as shown in table 3, they produced the same reactions in the parallel tests.

As in the A system of bison, these results can be explained by postulating a single pair of bison phenogroups which we designate as E_x' and "–". Phenogroup E_x', as indicated by the results summarized in table 3, shows partial cross reactions with P_x and E_3' reagents but exhibits essentially complete cross reaction with the E_x' fraction of antibodies in the serologically complex E_x' subtyping reagents. As indicated in table 4, the "–" alternative in the bison B system has its homologue in domestic cattle, and that homologue occurs rather frequently in certain breeds such as Herefords and Shorthorns (see Stormont, 1958). There is no evidence so far for a B phenogroup in cattle which is equivalent to E_x' of bison. The closest resemblance to it is probably O_xE_3' of cattle. This phenogroup differs from E_x' of bison primarily by the fact that it cross reacts with O_x antibodies and also reacts more completely with E_3' and P_x antibodies than does phenogroup E_x' of bison. Estimates of the frequencies of the alleles which control phenogroups E_x' and "–" in the Oklahoma population of bison are given in table 4.

The C System in Bison

The C system is the second most complex system of blood groups recognized in cattle. Over 30 C phenogroups and their controlling alleles are presently known.

All the C system reagents (table 1 and table 2) except R, L′, and E produced bonafide cross reactions with bison red cells. The two facsimile reagents X_1 and X_2, and the new reagent designated NF12 (table 2) cross reacted intensely with all 56 bison bloods. The cross reaction of NF12 antibodies with all 56 bison bloods was fully expected in view of the fact that NF12 reacts with all C phenogroups in cattle which are characterized by either or both blood factors **R** and **X**.

The reactions of the three C system reagents, notably C_1, C_2, and W, which distinguished individual differences in bison are summarized in table 3.

The present results with C system reagents, we believe, can be attributed to a minimum of three bison phenogroups designated X_1, WX_1, and C_2WX_1. A fourth C phenogroup, designated C_1WX_1, might be indicated by the weak or partial cross reactions obtained with our two C_1 reagents in tests of the red cells of bison numbered 47 and 65 in table 3. We have, however, for the purpose of the present report, taken the position that bison 47 and 65 were probably homozygous for phenogroup C_2WX_1 and that the weak reactions of their red cells with C_1 reagents resulted from a double dose of blood factor $\mathbf{C_2}$.

Estimates of the frequencies of the three alleles controlling phenogroups X_1, WX_1, and C_2WX_1 in the Oklahoma population of bison are given in table 4. Also, as noted in table 4, we have indicated that phenogroups X_1 and WX_1 have known homologues in domestic cattle but that, so far, there is no known homologue of C_2WX_1.

The F-V System in Bison

The F-V system in domestic cattle is typified by a single pair of phenogroups F and V which are controlled by a pair of alleles such that three phenotypes F (or F/F), FV (or F/V), and V (or V/V) are recognized. Recent observations in our laboratory suggest that the regular V phenogroup of cattle is divisible into two subgroups. Atypical variants of blood factor **V** have also been noted in this and other laboratories in typing *Bos indicus*, and there is suggestive evidence that variants of **F** also exist. In the present study, however, the reactions of bison red cells with F and V reagents were compared only with the regular F and V phenogroups of *Bos taurus*.

Partial cross reactions of all bison bloods of the present study were obtained with all of our F and V reagents (table 1) except in the case of the one F reagent obtained from a rabbit antiserum against the red cells of bison and the one V reagent obtained from a cattle antiserum against bison red cells. The reactions of the latter reagents were, of course, fully equivalent to those obtained with cattle red cells since, in these instances, bison rather than cattle represented the homologous species. However, two of the F reagents and three of the V reagents failed to produce visible hemolysis with bison red cells at the dilutions at which these reagents are ordinarily used in typing cattle. Nevertheless, absorption of these reagents as well as the other "partially" cross-reactive F and V reagents had a pronounced effect on the antibody populations. In each instance, the titer and degree of reactivity with cattle red cells were considerably reduced.

We propose that these results can be attributed to a single phenogroup in the F-V system of bison. We have designated this phenogroup as FV_b and note in table 4 that it does not presently have a known homologue in domestic cattle. Comparison of F-V variants in cattle with phenogroup FV_b of bison is a subject for further study.

That phenogroup FV_b of bison can be used to engender both F and V antibodies which will distinguish the three phenotypes F, FV, and V of domestic cattle is indicated by the sources of one of the F and one of the V reagents (table 1) used in the present study.

The J System in Bison

In the study of Owen et al., only one J reagent, a normal serum obtained from cow 17Z, was used. Nevertheless, it was possible, as in typing cattle, to distinguish different degrees of reactivity with J-positive bison red cells. In the present study we employed three J reagents obtained by immunizing J-negative rabbits with human red cells of type A, one J reagent obtained by immunizing a J-negative cow with human red cells of type A, and two normal cattle serums which contained rather strong J isolysins (see table 1). With these reagents, as explained in the note to table 3 and as indicated by appropriate symbols in table 3, we were able to distinguish four main categories of J reactions which were typical of the four main categories of J reactions distinguished by these reagents in typing cattle bloods.

Although we propose that a minimum of four J alleles, with homologues in cattle, are required to explain these four categories, we made no attempt to distinguish between these alleles in estimating the gene frequencies given in table 4.

The blood plasmas of each of the 56 bison were examined for the presence of natural isolysins and heterolysins, in particular any heterolysins which might serve to distinguish cattle from bison. No antibodies of the latter specificity were observed.

All J-negative bison (table 3) possessed natural isolysins for J of bison and these also reacted with J of cattle. The activity of these natural J antibodies, as in J-negative cattle, varied from extremely weak to very strong from individual to individual.

In addition to the occurrence of natural anti-J, some of the bison (nos. 48, 59, and 57 in table 3) possessed natural lysins for cattle bloods of subtype U_1. Such antibodies are occasionally encountered in the normal serum of cattle (Stone and Miller, 1961).

The bison plasmas were also examined for the presence of heterolysins for the red cells of domestic sheep (*Ovis aries*) and for lysins which might distinguish group R from group O sheep. Ten of the J-negative bison and six of the J-positive bison possessed heterolysins for the red cells of all sheep. None of the bison possessed lysins specific for the red cells of group O sheep but all J-negative bison, as expected, possessed lysins specific for the red cells of group R sheep. (The R population of antibodies always includes anti-J, and both the J and R fractions are readily absorbed by sheep red cells of group R as well as by human red cells of groups A and AB.) The results were typical of those we have obtained in screening normal cattle serums or plasmas for anti-J, anti-R, anti-sheep O, and anti-human A, except in bison sera that we encountered no antibodies specific for the red cells of group O sheep. However, as pointed out by Sprague (1958b), natural antibodies specific for the red cells of group O sheep have so far been found only in one breed of cattle, the Hereford.

Some of the bison plasmas were examined not only for soluble J substance but also for the recently discovered soluble Oc substance (Sprague, 1958a). Three J-Oc phenotypes indistinguishable from three of the four types described by Sprague (1959a) in studies of cattle were encountered. These were the types J (soluble J but no Oc), JOc (soluble J and Oc), and Oc (soluble Oc but no J). None of the plasmas that were examined lacked both J and Oc activity. As in the case of JOc-positive and Oc-positive cattle, the red cells of JOc-positive and Oc-postive bison were not lysed by Oc antibody (specifically, natural bovine anti-sheep O).

The L System in Bison

The L system in domestic cattle is presently characterized by two phenogroups: L and its negative alternative "–", distinguished by tests with L reagent.

In the present and previous studies, all bison bloods were L-positive and the reactions were fully equivalent to those obtained in typing domestic cattle, thereby indicating, as shown in table 4, that the allele for L must be at or near fixation in the Oklahoma population of bison. However, as noted in the study of Owen et al., the red cells of one of 9 F_1 hybrids (cattalo) were L-negative thereby indicating

TABLE 4. *Phenogroups in the A, B, C, F–V, J, L, M, S, Z, and Z′ systems of bison blood groups and estimates of their gene frequencies in the Oklahoma population*

Genetic systems	Phenogroups	Gene frequencies	Known homologues in cattle	Known blood factors associated with each phenogroup
A	A_1D	0.22	Yes?[1]	A_1, A_2 and D[2]
	"–"	0.78	No	None
B	E_x'	0.25	No	P_x,[2] E_3'[2] and E_x'
	"–"	0.75	Yes	None
C	X_1	0.64	Yes	X_1, X_2 and NF12
	WX_1	0.22	Yes	W, X_1, X_2 and NF12
	C_2WX_1	0.14	No	C_2, W, X_1, X_2 and NF12
F–V	FV_b	1.00	No	F[2] and V[2]
J	J	0.55	Yes	J
	"–"	0.45	Yes	None
L	L	1.00	Yes	L
M	M_2	0.05	Yes	M_2
	"–"	0.95	Yes	None
S	S_b	0.21	No	S
	"–"	0.79	Yes	None
Z	Z	0.63	Yes	Z
	"–"	0.37	Yes	None
Z′	"–"	1.00	Yes	None (see Addendum)

[1] Homologues have not been compared in absorption tests.
[2] "Partial" cross reactions only with the respective cattle reagents.

that the allele for the negative alternative occurs in some bison.

The M System in Bison

As shown in table 3, five bison bloods cross reacted with antibodies in the M_2 reagent and the reactions were fully equivalent to those obtained in testing the bloods of domestic cattle of subtype M_2. As in cattle, the M_2 reactions of bison red cells (table 3) appeared to be independent of those obtained in the systems A, B, C, J, S, and Z.

It is concluded, therefore, that two alleles which control the phenogroups M_2 and the "–" alternative can account for the results obtained in typing these 56 bison with cattle M reagents. Estimates of the frequencies of the two alleles in the Oklahoma population are given in table 4.

The S System in Bison

The five reagents, S, U_1, U_2, U′, and H′ are presently used in typing the S system of domestic cattle and with these five reagents it has been possible to distinguish five phenogroups SH′, U_1H′, U_2, H′ and a negative alternative designated "–". As may be noted by the symbolism used in designating these cattle phenogroups, the S-positive phenogroup (SH′) and the U_1-positive phenogroup (U_1H′) are characterized in part by the fact that they both react with H′ antibodies. That is to say, we have yet to find in typing domestic cattle any S-positive or U_1-positive or SU_1-positive bloods which are negative with H′ antibodies.

In view of the association of blood factors **S** and **U_1** with blood factor **H′** in domestic cattle, we were particularly anxious to re-examine S-positive bison bloods with all available H′ reagents, since in the two previous studies there was no indication of an **H′** or **H′**-like blood factor in bison. For this reason, we used not only our pure H′ reagents but also incorporated the regular appearance of this blood factor with high frequency in equivalent samples of various breeds of cattle. Although this observation was reaffirmed by the data of Stone and Miller (1955) and by the present data, the significance of the point has been considerably reduced following recent observations made in this laboratory which have shown that **H′** is a member of the S system of cattle rather than a separate system by itself. It is certain that the S locus is represented in bison, in spite of the fact that there is no evidence in that species for blood factors related to $\mathbf{U}_1$, $\mathbf{U}_2$, **U′**, and **H′** of cattle. Even though no phenogroup equivalent to S_b of bison has as yet been detected in cattle, the fact that such a phenogroup occurs in bison makes us hopeful that its homologue may eventually be found in cattle.

The third point made by Owen et al. was the observation that all phenogroups in the C system of bison cross reacted strongly with reagents $\mathbf{X}_1$ and $\mathbf{X}_2$, while in cattle there are numerous C phenogroups which do not cross react with these reagents. The present data in no way detract from this salient feature; rather they add to it by the inclusion of still another reagent, namely, NF12, which cross reacted with all bison C phenogroups. However, in contrast with the two previous studies which reported positive reactions of all bison bloods with L′ reagent, none of six replicated L′ reagents of the present study produced reactions with bison red cells. The present results substantiate the opinion of Owen et al. that the previous L′ reactions were not of the nature of true cross reactions.

The fourth and final point made by Owen et al. was the indication of an allele F^{V_2}, controlling a phenogroup designated V_2, which would appear to be fixed in bison but has so far not been recognized in domestic cattle. We now know that the phenogroup produced by the bison F-V allele is partially cross reactive not only with V antibodies but also with F antibodies of various origin. Furthermore, we have been able to show that this particular bison phenogroup, designated FV_b in the present study, is capable of engendering antibodies which parallel the reactions of both F and V reagents in the cattle blood-typing tests. These differences between the F-V phenogroups in bison and cattle still stand as perhaps the most definitive differences between the two species. The question is, however, just how much longer will they stand? As already intimated elsewhere in this report, there is evidence for more than two alleles at the F-V locus of cattle and the product of one of the additional alleles might possibly turn out to be serologically indistinguishable from FV_b.

Actually, one of the most striking differences between the two species is probably not so much the allelic divergence at the various blood-group loci but rather the lack of heterogeneity in blood group alleles in bison, particularly at the B locus, as contrasted with the great heterogeneity in cattle. Several explanations could be advanced to account not only for the lack of diversity in bison but also to account for the extensive diversity in cattle but only one needs to be emphasized here, namely, the fact that our present populations of American buffalo have all been developed in modern times from foundation stocks of only a few animals (see discussion by Owen et al., 1958). We can never know precisely what the picture of bison blood groups was when literally millions of buffalo roamed the plains of North America, but better estimates can be made when we have more information not only on other herds of bison but also on blood-group heterogeneity in other wild species as contrasted with domestic forms that have arisen from them or have been closely associated with them in derivation. Hence, there is need to pursue much further the studies of blood groups in the subfamilies Bovinae, Caprinae, and Ovinae which are so richly represented by both wild and domestic species. Reed (1959) has expressed the opinion that comprehensive studies of blood groups in both wild and domestic on the serologic complexity of the phenogroups.

The question has arisen whether bison might possess one or more blood group systems not detectable by cattle reagents. All we can say at present is that there is little indication of such systems from the results of using cattle red cells in cross-absorbing both rabbit and cattle antisera against bison red cells. The antibodies in the rather low-titered "bison specific" reagents which have been developed from rabbit anti-bison serum can, for example, be attributed to phenogroup FV_b.

We believe that the answers to some of these questions will be forthcoming. In the meantime, it is established that at least 9 of the 10 blood group systems now recognized in domestic cattle are represented in bison. This observation, in conjunction with the observation that most of the blood-group alleles in bison (table 4) seem to have close homologues if not precise homologues in cattle, suggests that the two species are very closely related in evolutionary derivation, indeed, close enough in our opinion to justify their inclusion in the same genus.

In connection with another study (Stormont and Suzuki, 1958), we had the opportunity to test our cattle reagents with the red cells of one or more representatives of some other species (*Bubalus bubalis, Syncerus caffer, Anoa depressicornis, Boselaphus tragocamelis*, and *Taurotragus oryx*) in the subfamily Bovinae. However, in no instance was there any indication that any of these species stand near bison in the degree of blood-group relationship to cattle.

The only extensive comparisons of blood groups in cattle with those in a species other than bison, we believe, are the comparisons being made in this laboratory between the blood groups in domestic sheep (*Ovis aries*) and cattle. Serologic relationships have been established between the complex B and C systems of the two species and between the S system of cattle and the M system of sheep (Rasmusen et al., 1958; Rasmusen, 1960; Rasmusen et al., 1960; and certain unpublished data), and these relationships are in addition to the earlier known relationship between the J system of cattle and the R-O system of sheep (see Sorensen et al., 1954). But, in contrast with the bison-cattle comparisons, the sheep-cattle relationships extend only to the loci and not to the alleles. All the known phenogroups of the B, C, M, and R-O systems in sheep are clearly distinguishable from those of the equivalent systems in cattle. This also applies to the blood factors of the two species even though, for example, reagents which are identical in specificity in the cattle tests to such reagents as I, R, E_3', and NF6 (or B') have been prepared from sheep isoimmune antisera. Another blood-type distinction, which has some taxonomic significance in artiodactyls (see Stormont and Suzuki, 1958), is that sheep are Forssman-positive whereas bison and cattle are Forssman-negative.

SUMMARY

Data are presented on the results of blood typing 56 American buffalo (*Bison bison*) with 66 different cattle blood-typing reagents.

Evidence is presented which indicates that at least nine genetic systems of blood groups now recognized in cattle have their homologues in bison. These are the systems A, B, C, F-V, J, L, M, S, and Z. Comparisons are drawn between blood factors common to bison and cattle, and between the phenogroups in each of the systems of the two species. It is shown that most of the bison phenogroups and, by implication, their controlling alleles have closely similar or precise homologues in cattle. It is also shown that bison have soluble blood group substances serologically indistinguishable from the J and Oc properties of cattle. The same is true for the isohemolysins anti-J and anti-U_1 and certain heterohemolysins which occur naturally in the two species.

Certain differences between the two species, notably those encountered in typing the F-V and C systems, still persist and those differences are probably more ex-

treme than any so far encountered in comparing various breeds of domestic cattle. Furthermore, there is a marked reduction of heterogeneity in bison blood groups as contrasted with cattle. This is particularly true in the B system. Nevertheless, there are many striking resemblances that outweigh the few differences.

The over-all results indicate close taxonomic affinity of the two species. On the basis of the evidence on blood groups and on other observations mentioned in the introductory remarks of this report, further consideration should be given to the proposition that the American buffalo and domestic cattle deserve to be recognized as members of the same genus.

ADDENDUM

Since the manuscript was submitted, it has been observed in this laboratory that the rare bovine blood factor named Z′ actually belongs to the A system of bovine blood groups. This observation obviates the need to consider Z′ as representing a separate blood-group locus as was done in this paper. The blood-group loci which should be considered are A, B, C, F-V, J, L, M, S, and Z. On this basis it is clear that each of the 9 blood-group systems presently recognized in *Bos taurus* has a known homologue in American buffalo, thereby narrowing somewhat more the differences between the two species.

Literature Cited

Boyden, A., R. De-Falco, and D. Gemeroy. 1951. Parallelism in serological correspondence. Bull. Serol. Museum, **6**: 6–7.

Deakin, A., G. W. Muir, A. G. Smith, and A. S. MacLellan. 1942. Hybridization of domestic cattle and buffalo (*Bison americanus*). Progress report of the Wainwright experiment 1935–41. Exp. Farms Service, Dept. Agr., Dominion of Canada. 10 pp. (Mimeo.).

Miller, W. J., and W. H. Stone. 1955. Blood typing of the erythrocytes of bison with reagents for antigenic factors of cattle. (Abstract). Genetics, **40**: 586.

Owen, R. D., C. Stormont, and M. R. Irwin. 1958. Studies on blood groups in the American buffalo (bison). Evolution, **12**: 102–110.

Rasmusen, B. A. 1958. Blood groups in sheep. I. The X-Z system. Genetics, **43**: 814–821.

——. 1960. Blood groups in sheep. II. The B system. Genetics, **45**: 1405–1417.

——, C. Stormont, and Y. Suzuki. 1958. Blood groups in sheep. (Abstract). Proc. X Int. Cong. Genetics, **II**: 227.

——, C. Stormont, and Y. Suzuki. 1960. Blood groups in sheep. III. The A, C, D, and M systems. Genetics, **45**: 1595–1603.

Reed, C. A. 1959. Animal domestication in the prehistoric Near East. Science, **130**: 1629–1639.

Roe, F. G. 1951. The North American Buffalo. University of Toronto Press.

Sprague, L. M. 1958a. On the recognition and inheritance of the soluble blood-group property "Oc" of cattle. Genetics, **43**: 906–912.

——. 1958b. On the distribution and inheritance of a natural antibody in cattle. Genetics, **43**: 913–918.

Sorensen, A. N., J. Rendel, and W. H. Stone. 1954. The J substance of cattle. II. A comparison of normal antibodies and antigens in sheep, cattle and man. J. Immunology, **73**: 407–414.

Stone, W. H., and W. J. Miller. 1961. Naturally occurring isoantibodies of the S blood group system in cattle. J. Immunology, **86**: 165–169.

Stormont, C. 1958. On the application of blood groups in animal breeding. Proc. X Int. Cong. Genetics, **I**: 206–224.

——, W. J. Miller, and Y. Suzuki. 1960. The convergence of the A-H and D systems of bovine blood groups. (Abstract). Genetics, **45**: 1013.

—— and Y. Suzuki. 1956. The "D" system of bovine blood groups. (Abstract). J. Animal Science, **15**: 1208–1209.

—— and ——. 1958. The distribution of Forssman blood factors in individuals of various artiodactyl species. J. Immunology, **81**: 276–284.

—— and ——. 1960. On the J classification of rabbits and the production of anti-J in J-negative rabbits. Proc. Soc. Exp. Biol. Med., **105**: 123–126.

Wolfe, H. R. 1939. Serologic relationships among Bovidae and Cervidae. Zoologica, **24**: 309–321.