Onderstepoort Journal of Veterinary Research,
Volume 28, Number 3, September, 1960.
The Government Printer, Pretoria.

BLOOD GROUPS IN BOVINES. I. PRODUCTION OF CATTLE ISO-IMMUNE SERA AND BLOOD GROUP REAGENTS

D. R. OSTERHOFF, Onderstepoort Laboratory

The study of the blood groups of cattle has made much progress during the past 30 years and it has become clear that blood group research has to investigate two distinct problems:—

(1) The immuno-serology of the blood groups, i.e. the occurrence and/or production of antibodies and the relation which exists between antibody and antigen.

(2) The genetics of the blood groups, i.e. the mode of inheritance of the red cell antigens and the relationship between the genes and the antigens.

This paper will only deal with the first problem, i.e. with the immunological results obtained by the Onderstepoort Blood Group Laboratory during the past two and a half years.

The most extensive reviews on blood groups in cattle are those given by Osterhoff & Rendel (1954), Irwin (1956) and Rendel (1957). Generally speaking, there have been two approaches to the problem of grouping cattle blood:—

(1) Following upon Landsteiner's (1901) discovery of the ABO group system in man, several workers have attempted to demonstrate antigenic differences between cattle by means of naturally occurring antibodies.

(2) Extensive studies, particularly by the American research workers, have demonstrated the presence of at least 40 different antigenic factors in cattle blood. This was done by means of antibodies artificially produced by hyper-immunization, i.e. by repeated transfusions of small quantities of blood.

These two lines of approach have led to the discovery of several identical blood factors. (Rendel *et al.*, 1957). The genetic side of the blood group research work has been investigated by Stormont *et al.*, during the past ten years. These workers were able to show that the antigenic structure of bovine erythrocytes is controlled by genes on at least 11 different loci, some of which carry multiple alleles.

A.—Terminology and Genetic Associations of Cellular Substances.

Antigens (abbreviation for anti-somato-gens) are substances usually containing proteins, lipoid-substances and polysaccharides, or combinations of these, e.g. bacteria, viruses, toxins and erythrocytes. The latter are the antigens used in blood group studies.

Received for publication on 11 November, 1959.—Editor.

Antibodies are specific substances, probably produced in the reticulo-endothelial system as preventive agents against foreign substances intoduced into the body. The chemical composition of antibodies is unknown but they are most probably euglobular and pseudoglobular proteins, which are very specific, i.e. they only react with the antigen by which they were produced. This characteristic is used to detect antigenic differences between red blood cells.

Antiserum is a serum containing antibodies. In blood group research, antisera are obtained by hyper-immunization with bovine erythrocytes, i.e. repeated transfusions of small quantities of blood.

Testserum or Reagent is an antiserum which has been rendered specific to a single antigenic factor. Each testserum is able to detect a corresponding antigenic factor on the erythrocytes.

Antigenic factors or blood factors are the specific entities on the erythrocytes which react with the different reagents (antisera). Thus, erythrocytes reacting with the A reagent (= anti-A) are said to carry the antigenic factor or blood factor A.

Blood group denotes a gene-determined unit, containing one or more antigenic factors.

Blood group system: Blood factors or blood groups which are determined by genes at the same locus and most probably on the same chromosome, are said to belong to the same blood group system.

Blood type of an animal is simply its complete antigenic formula, as determined with all available reagents in the blood group determination.

In the past it was believed that all antigenic factors were inherited independently from one another, and that each factor was controlled by a specific gene, each gene being located on a single chromosome. When it was found that the number of antigenic factors exceeded the number of chromosome pairs in cattle (determined as 30), it became obvious that some of the antigenic factors, and their controlling genes, must be genetically associated. Stormont *et al* (1951) were able to prove, by studies of the progeny of a number of bulls, that certain factors are inherited in groups or complexes which can be integrated into 11 different systems. The usual designation and arrangement of these systems are as follows: –

A H, B, C, F V, J, L, M, S U, Z, H′*, and Z′.

As in the case of human beings, blood factors are designated by letters, following the alphabet in the order in which they were discovered, i.e. A, B, C, etc. When all the letters of the alphabet had been so employed, the designations A′, B′, C′, etc. were used. It must, however, be pointed out that no relationship exists between A and A′, or B and B′; the similar designation is incidental.

*Since this article was written investigation have shown that blood factor H^1 belongs to the SU-locus and is therefore named S_2. Furthermore the factors E_1 and E_2 both belonging to the B-locus have been designated Y^1 and O^1 respectively by international agreement.

As stated, factors occur in several different genetically-determined combinations, some controlled by a great number of multiple alleles. Table 1 gives the relation between loci,—i.e. the points on the chromosomes where the systems are genetically placed—antigenic factors and alleles:—

TABLE 1.—*Genetic Systems of Cattle Blood.*

Locus	Factors	Alleles	Remarks
AH...	A, H.....	A, H, AH, a*.....	—
B.....	B, G, I, K, O_1, O_2, O_3, P Q, T_1, T_2, Y_1, Y_2, A′, D′ E'_1, E'_2, E'_3, I′, J′, K′ E_1, E_2,	More than 90 alleles giving the different combinations of factors	—
C.....	C_1, C_2, R, W, X_1, X_2, L′..	24 different alleles.....	—
FV....	F, V.....	F, V.....	Determined by two genes only.
J.....	J.....	J^{sc} = presence of J substance on the cells and in the serum	—
		J^s = presence of J substance in the serum only	—
		J^a = no J substance	
L.....	L.....	L, 1.....	—
M.....	M.....	M, m.....	—
SU....	S, U_1, U_2.....	S, U_1, U_2SU_2, s.....	—
Z.....	Z.....	Z, z.....	It is possible to differentiate between homo- and heterozygotes by means of intensity of reaction.
H′....	H′.....	H′, h′.....	—
Z′.....	Z′.....	?.....	Not yet clarified.
D.....	D.....	?.....	Not yet clarified.
?.....	SA_1, SA_2, SA_3.....	?.....	Not yet clarified, factors found in South Africa.

* The small letters indicate the absence of a recognizable antigenic factor.

It is not yet known where these loci are placed on the chromosomes, but the heredity of blood groups has been developed to a point where it may be profitably applied to a great diversity of practical problems.

B.—MATERIAL AND METHODS.

The first blood group tests in South Africa were conducted on 108 Afrikaner, 122 Friesian, and 101 different crossbred cattle.

The haemolytic test, as described by Stormont & Cumley (1943), was employed. For the lytic reactions, a combination is required of antigenic substances on the surface of the red cells, antibodies corresponding with these, and a third component called a complement. Fresh rabbit serum was used as a source of this complement.

For the experiments, about 50 c.c. of blood were taken from the ear veins of rabbits. The blood was left at room temperature and the serum separated from the clot by centrifugation. A preliminary test was done in each case to ensure that the rabbit serum did not contain any antibodies for cattle erythrocytes. The serum was stored in 15 c.c. tubes at — 79° C. Just before use, it was diluted with aliquots of physiological saline solution.

For the determination of blood factors, two drops (0·1 c.c.) of appropriately diluted test serum and one drop (0·05 c.c.) of erythrocyte suspension were mixed in a test tube. The cell suspension contained 2·5 per cent of cells, and was prepared with isotonic saline (0·92 per cent), using thoroughly washed erythrocytes. The blood samples from which the cell suspensions were made, were uncoagulated, an anticoagulant citrate solution being used (sodium citrate 2·0 gm., sodium chloride 0·5 gm. and dist. water to 100 c.c.).

After 15 minutes of incubation, one drop of complement was added and the mixture shaken. The degree of haemolysis was recorded after two and four hours. Complete lysis was indicated by a score of 4, while 3, 2, 1, trace and 0+ were used for incomplete reactions, showing decreasing degrees of haemolysis. The trace and 0+ reactions were not considered as positive reactions.

The blood used for immunization was drawn from the jugular vein into 600 c.c. bottles containing 60 c.c. isotonic citrate solution. These bottles were convenient, especially where the blood from one donor was used for more than one recipient. Frequently, however, 50 c.c. syringes, containing 5 c.c. anticoagulant, were used to draw the blood and to inject it immediately into the jugular vein of the recipient. The first injection in an immunization series always consisted of 100 c.c. citrated blood, and the second and subsequent injections of 50 c.c., in order to reduce anaphylactic shock. An immunization series consisted of five to ten injections per animal, given twice weekly. The antibody concentration was controlled by taking and testing a blood sample from a recipient before each new inoculation. One week after the last injection the recipients, which had developed antibodies in a sufficient quantity, were bled three to four litres, into preservation jars (800 c.c.). To obtain the maximal amount of serum, about 300 c.c. blood was collected in each jar, the jars were placed for one hour in a water bath at 37° C and then left overnight in an inclined position, so that the surface area of the clot was as large as possible. The untreated anti-sera were kept in a deep-freeze cabinet, at −79° C.

The antisera obtained in this way usually contained antibodies for more than one antigenic factor. For fractionation of the sera and isolation of the blood group reagents or specific test sera, the following absorption method was employed. The antiserum was mixed with an equal amount of washed and packed erythrocytes, the corresponding antibody of which was to be absorbed; the dilution of the antiserum depended entirely on the concentration of the antibodies present. The mixture was incubated for 30 minutes at room temperature (22 to 26° C), and centrifuged for half-an-hour at 2,500 r.p.m. The antiserum was then drawn off and again mixed with an equal amount of washed and packed cells of the same type. The mixture was then left for 30 minutes in the refrigerator (2 to 4° C). Deviations from this standard technique were sometimes necessary. After a second centrifugation, the serum (now referred to as " absorbed " serum) was stored in a deep-freeze cabinet. If necessary, further absorptions and further tests for purity were undertaken before the testserum (or reagent) against one specific antigenic factor was ready.

C.—Production of Iso-immune Sera

Following the preliminary tests, suitable donor-recipient combinations were chosen: the donor's blood had to contain only one or two factors which did not occur in the recipient in order to obtain antisera containing hemolysins for only these antigenic factors. Later on, immunizations were conducted with donor blood containing three to seven different factors not occurring in the recipient's blood, to obtain a better response from the recipient to antibody production against some of these antigenic factors.

In order to produce the necessary blood group reagents, a total of 121 immunization series, amounting to 827 injections (6·8 injections per animal), were performed; 81 series were conducted on Friesian cattle, the rest on Afrikaner and on crossbred cattle.

Production of antibodies was observed in 70 recipients, while 51 animals failed to yield antibodies in satisfactory quantities in spite of the fact that they received an average of 6·3 injections. Included among these were three animals that died, due to the transfer of pathogenic micro-organisms from donor to recipient (i.e. " heartwater " in two cases and " redwater " in the third*).

In order to determine whether significant differences exist between breeds in their antibody response, the Chi square method (χ^2) of Snedecor (1953) was employed.

TABLE 2.—*The differences between breeds in antibody production*

Breed of recipients	Number of recipients which		
	formed antibodies	did not form antibodies	Total
Friesian	41	40	81
Afrikaner	17	3	20
Crossbreds	12	8	20
	70	51	121

$\chi^2 = 3{\cdot}399$; degrees of freedom = 2; $P = 0{\cdot}20$.

In spite of the fact that the Afrikaner breed gave an antibody response of 85 per cent in comparison with 55 per cent for the Friesians, the difference was not statistically significant.

The same χ^2 test was also applied to investigate significant seasonal differences.

TABLE 3.—*The seasonal variations in antibody production*

Season	Number of recipients which—		
	formed antibodies	did not form antibodies	Total
Spring	16	9	25
Summer	15	3	18
Autumn	23	30	53
Winter	16	9	25
TOTAL	70	51	121

$\chi^2 = 4{\cdot}875$; degrees of freedom = 3; $P = 0{\cdot}20$.

* " heartwater " = bovine rickettsiosis.
" redwater " = bovine piroplasmosis.

There were no statistically significant differences between seasons in antibody production. In other words, good immunization results can be obtained throughout the year.

In animals no exact measurements of functional fitness are possible. The body weight is not necessarily a good indication of such fitness. It would appear, however, that animals in good bodily condition give a better antibody response than animals in poor condition.

An attempt was also made to find out whether the age of the recipient plays a role in antibody response to erythrocyte injections.

TABLE 4.—*The effect of age on antibody production*

Age	Number of recipients which—		
	formed antibodies	did not form antibodies	Total
Up to 2 years	6	14	20
3 to 4 years	14	7	21
5 to 6 years	20	20	40
7 to 8 years	13	7	20
9 to 10 years	4	1	5
Over 16 years	13	2	15
TOTAL	70	51	121

$\chi^2 = 5{\cdot}662$; degrees of freedom = 5; $0.50 < P < 0{\cdot}30$.

There were no statistically significant differences in antibody production between different age groups. From an analysis of the different sources of variation in the production of antibodies against the different antigenic factors, it may be concluded that it is impossible to give detailed directives for their production. Experience has, however, shown that if an immunization fails to give satisfactory results, a re-immunization, six to ten months later, with blood from the same donor, may be successful. Results obtained in this manner are not included in this article.

In connection with the injections, slight symptoms of anaphylactic shock, that is lassitude, coughing, salivation and sometimes muscular trembling, were observed immediately after them, on several occasions. In all such cases, the symptoms disappeared rapidly. In the 827 injections of small quantities of uninfected blood into bovines in the present series of hyperimmunizations, not a single animal was lost from anaphylactic shock.

The method of producing antibodies, described above, is called iso-immunization. One can also produce antibodies by hetero-immunization in animals of different species, e.g., by injecting cattle blood into rabbits. In all, 62 injections of bovine erythrocytes into rabbits were performed. In each case 2 c.c. of a 50 per cent suspension of cells, washed and packed three times, were used. The first injection was given intraperitoneally, and the second and subsequent injections intravenously into the ear vein every second day. Antibodies developed in all the rabbits and the titres were extremely high, in some cases as high as 1/4,000. For this reason, the test sera could be diluted considerably and used for the detection of antigenic factors in a large number of cattle.

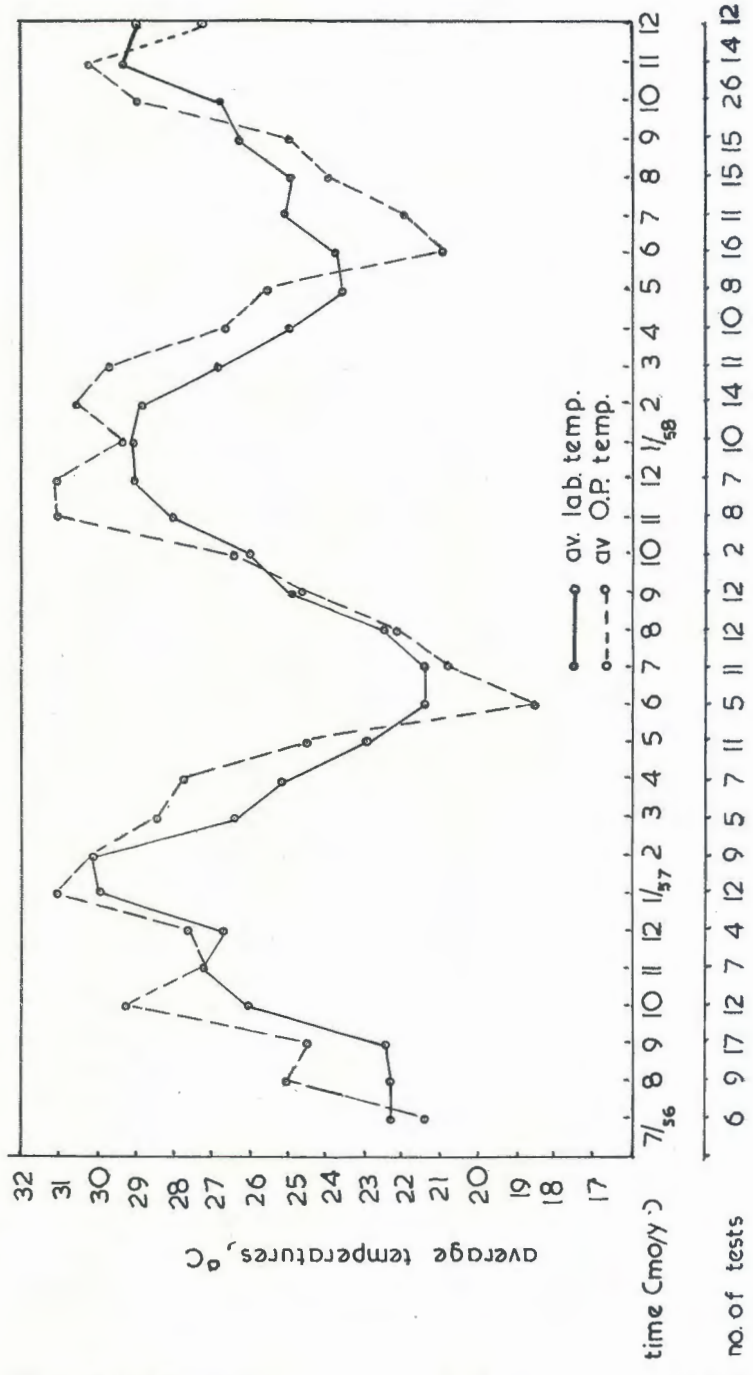

FIG. 1.—The average test-temperatures compared with the average maximum Onderstepoort temperatures

As previously mentioned, blood samples were taken from the recipients and tested for antibody titre at various stages during the immunization process. The antiserum was diluted 1/2 to 1/1,028 with isotonic saline solution and tested against 30 different erythrocyte types of which the antigenic factors were known. The usual test included 1,200 tubes, i.e. 40 different antisera, or dilutions of antisera, tested with 30 different samples of blood.

It soon became clear that, under South African climatic conditions, a serious problem had to be faced. The standard method of testing takes place at room temperature, i.e. between 22 and 24° C. In the laboratory, the temperature during the two-and-a-half years beginning July, 1956, varied between 19 and 32° C. In Figure 1, the average laboratory temperatures on test days are given for successive months, and these are compared with the average maximum temperatures at Onderstepoort; the number of tests performed each month is also given.

Extra heaters were installed. The effect can be seen in the average test temperatures, which were higher during the winter months (May to August) of 1958 than during those of 1956 and 1957. The test temperatures for 1958 varied between 23·5° C and 29·5° C. A method had to be found to adjust the standard testing procedure to the prevailing high temperatures.

Setting up the tests at 4.30 a.m. with the last test-readings at 12.30 p.m., did not solve the difficulty, neither did the changing of the time interval between the setting up and the reading of the test.

It is apparent, therefore, that temperature fluctuations under South African conditions are responsible for considerable errors in the determinations. The magnitude and influence of these cannot be easily estimated. The only way to overcome this problem is to carry out all blood work in an air-conditioned laboratory.

D.—Fractionation of immune sera (production of blood group reagents)

The fractionation of the antisera was done by absorptions of small quantities at a time, with cells from animals of different blood groups. Such cells had to contain some of the antigenic factors for which the antibodies were produced. Very often double absorptions were necessary to achieve the required fractionation of the immune sera into one or more specific test sera or reagents. Sometimes marked difficulties were encountered when hemolysins were found in antisera produced by weak antigenic substances on the donor's erythrocytes. These antigenic substances are usually very difficult to determine because no appropriate reagents are available. The hemolysins mentioned often caused trace reactions in later tests for specificity and purity.

The polyvalent antisera were fractionated to yield different single or monovalent reagents. Each antibody fraction appeared to be homogeneous, was tested with erythrocytes, usually of 30 cattle whose blood types were known (compare Table 5).

TABLE 5.—*Blood types of* 15 *head of cattle of the experimental herd at Onderstepoort*

No.	Blood types
1706	A — — — — I — — Q — — — — — — — — C_1 — — — F — — — — — — H′ —
2133	— B — — — — O_1 — — — Y_1 — D′ — — — — — — — F — — L M — — — —
3570	— B E_2 G K — — — Q — Y_2 A′ — — — — — C_1 — — X_2 — V — — — — — H′ —
3572	A B E_2 G K — — — — — Y_2 A′ — — — — — C_1 — — X_2 — V — — — — Z H′ —
3574	— — — — — — — — — — — — — — E'_3 — — — C_1 W — X_2 F — J L M — — — H′ —
4447	A B E_2 G K — O_1 — — Y_2 A′ — — — — — — — X_2 F — — L — S — — H′ —
5586	A — — — — — O_2 — Q — Y_1 — D′ — I′ — — C_1 W — X_2 F V — L — — — — H′ —
5852	A — — — — I O_3 — Q — — A′ — E'_2 I′ J′ K′ — W — — F — — — — S — Z — —
6302	A — E_2 G — — — — — — Y_1 — — — — I′ — — — W — — F V — — — — — Z H′ Z′
6317	A — — — — I O_3 — — — — A′ — E'_1 — — K′ — — — — F V J L — — — Z H′ Z′
6348	A — E_2 G — I O_3 — — — — A′ — E'_1 — — K′ — W — — — V — — — — U_2 Z H′ —
6433	A — — — — — — — — T_1 — A′ — E'_3 I′ — — — W — — — F — — L — S — Z H′ —
6655	A — — G — — — — — — Y_2 — — E'_1 — — — — — — — F — — L — — — — — —
6829	A — E_1 — — — O_2 P — — Y_1 — D′ E'_2 I′ — K′ — W — X_1 F — J — — S — Z H′ —
7500	A — — — — — — — — — — — E'_2 — — — C_1 W R — F — — L M — — — H′ —

The results of such tests were usually sufficiently conclusive to determine whether a reagent had a reasonable resemblance to the one already in use. When an antiserum was purified to such an extent that absorption with each positively reacting cell type resulted in a complete exhaustion of all hemolysins, the absorbed antiserum was referred to as a reagent.

Such a reagent was then subjected to a trial test including 80 to 100 blood samples of known blood type. If the reagent gave reactions with all erythrocytes having the corresponding factor and failed to react with the others, a large scale absorption was undertaken (at least 250 c.c. antiserum) and the resulting reagent was freeze-dried for storing. Depending on the different degrees of titration, the quantity of a specific reagent obtained from 250 c.c. antiserum could be used for blood group determinations of 10,000 (using dilution 1/4) to 30,000 animals (dilution 1/12).

It is impossible to describe the production of every reagent in detail. All test sera produced are, therefore, presented in Table 6, which lists the donor, the recipient, and the expected antibodies produced in the recipients by injection of the donor's blood, as well as the number of injections required to produce antibodies. The quantity of antibodies produced is indicated by the maximal titre, i.e. the highest titre against the cells of the donor. The isolated antibodies are also given, and the number of absorptions necessary to produce them is shown in the last column.

During the production of antisera it was found that, for several antigenic factors, the antibodies were easy to prepare and that in such cases the fractionation was relatively simple. Only a few absorptions were necessary to obtain pure reagents for certain antigenic factors, e.g.,A, G, P, R, or Z. Other antibodies, B, A′, O or H′ were difficult to produce and often very difficult to isolate.

The following example illustrates the point. Ten immunization series were performed in an effort to produce an anti-H′-serum; seven of these failed completely and the other three gave mixed H′ and other antibodies. It proved impossible to isolate anti-H′ bodies from these complex antisera, in spite of the fact that many single and combined absorptions were performed. So far, in routine blood group determinations for parentage, breed differentiation, etc., the anti-H′ reagent used has been that kindly supplied by the German Blood Group Laboratory of Göttingen.

It is impossible to present the data on the fractionation of all the test sera given in Table 6. It may be mentioned, however, that anti-L′reagent could not be produced because the experimental animals' blood could not be tested against L′ antibodies as, at that time, they were not available. Furthermore, only a few animals were injected with J′ and K′ cells, but here the production of specific antibodies failed completely.

TABLE 6.—*Antibodies, from which South African reagents have been isolated (for explanations see text)*

Donor	Recipient	Expected Antibodies	No. of Immunization	Maximum Titre	Antibodies Isolated	No. of Absorption Required
7934	7884	E′ L Z H′.....	7	128	Z.....	19
7832	7829	E_1 Z.....	8	32	Z.....	2
7678	7664	B I′.....	8	64	I′.....	19
6648	2796	Y.....	7	64	Y_2.....	17
6315	7619	I A′ F.....	8	64	I.....	14
6315	4447	I W Z.....	7	256	I.....	20
6315	6701	I V.....	7	32	I V.....	24
5783	7626	G K.....	7	32	G.....	9
5783	7620	B K.....	9	8	B K.....	18
2931	5519	G Q L.....	8	16	G.....	13
2133	4601	D′ L M.....	7	512	D′ L.....	22
2133	5461	B L.....	9	32	B.....	40
2133	2480	B D′ V L.....	9	32	V.....	29
2133	4091	B L M.....	10	32	M.....	12
3559	5060	A I′.....	6	64	A.....	6
3569	3358	A E′.....	6	32	A.....	4
6302	6315	Y I′ Z′.....	10	8	Y_1 Y_2.....	17
6302	5818	Y V Z′.....	8	128	Y_1 Y_2 V.....	30
2660	2322	V L S.....	7	64	V.....	11
2665	2707	W V L U.....	9	64	V U_2.....	37
2665	2667	L U.....	10	8	U_2.....	5
5064	4066	A E′.....	7	64	A.....	23
1355	3527	I K′ M S.....	6	32	S.....	11
5063	2664	C.....	8	16	C_1 C_2.....	9
5531	5692	D′ X.....	7	32	D′.....	73
4652	5694	W U H′.....	9	32	U_2.....	12
5558	6316	C L U Z′.....	9	8	Z_1.....	6
5558	5585	Y L U Z′.....	8	32	Z′.....	40
3574	6355	X F J L M.....	8	16	J.....	12
5502	5787	T I′.....	7	64	I′.....	10
5530	5560	P.....	9	16	P.....	32
5530	5512	P.....	9	64	A_1.....	10
7844	1892	O T K′.....	9	32	T_1 T_2.....	44
7844	6386	A K T A′ E′ K′ X	8	16	?.....	33
5502	2931	B E_2 T E′ W Z H′	8	256	B E_2 W.....	68
5502	6673	T W.....	8	16	SA_2.....	12
6395	2794	A′ Z′.....	9	16	Z′.....	7
6395	2661	A′ J′ Z′.....	7	32	J.....	5
6395	6302	O A′ J.....	7	32	J.....	6
6648	6344	O Y.....	7	64	O_1 O_2 O_3.....	12
6648	5095	O.....	10	16	?.....	5
6648	6878	O.....	8	8	?.....	5
7500	6644	E′ W R M.....	5	64	W.....	20
3018	3857	B X.....	6	64	SA_3.....	58
3018	2726	B X.....	6	128	B.....	78
7832	7836	E_1 F J Z.....	8	32	J.....	—
5063	5062	A′.....	9	8	A'_2.....	67
7994	8406	Q K′.....	6	16	Q.....	61
7773	2480	E′ X L.....	7	64	L.....	28
5530	5095	P I′.....	8	64	I′.....	49
5753	2133	K′ X S H′.....	6	64	S.....	26
Gr. V....	Vr. VII	B E_2 K A′ X....	7	32	B K X_1 X_2....	84
M.M.L. II	Paul. III	A E_1 A′ R L S..	5	128	A S.....	49
M.M.L. II	Gr. III.	E_1 A′ W R F S.	6	256	W S.....	33
M.M.L. II	M.A. III	E^1 Y A′ W R L.	7	128	W.....	48

TABLE 6 (*continued*)

Donor	Recipient	Expected Antibodies	No. of Immuni-zation	Maximum Titre	Antibodies Isolated	No. of Absorption Required
Di. I.....	Vr. V...	C F.....	5	256	F.....	22
U.A.A. III	Vr. II...	F E'.....	6	256	E'_1 E'_2 F.....	71
U.A.A. III	Bo. XI..	B E_2 K E' V....	6	512	V.....	17
U.A.A. III	V. III...	E' F.....	6	512	V.....	17
U.A.A. III	V. III...	E' F.....	6	512	F.....	52
Di. III....	Bo. XII.	P C L M.....	5	128	M.....	8
Di. III....	Bo. XVI	P L M.....	7	32	M.....	15
Bea.....	Rit. II...	W R.....	5	64	R.....	4
Bea.....	Rit. IV..	W R M.....	7	64	R M.....	26
Elsa.....	Ju. IV...	B X V I'.....	6	128	I' V.....	32
Elsa.....	E.B.....	B V.....	5	128	V.....	6
Vr. VI....	Bea II...	I' J H'.....	5	64	J.....	31
5768	7843	A' S.....	5	64	S.....	23
5768	5753	G A' E'C.....	5	32	E'_2 E'_3.....	35
7499	8498	A' C X L.....	4	64	L.....	36
7499	8502	A'.....	7	8	?.....	37
	Rabbits:					
5768	K I.....	G Y E' A' C F S H'	8	4,000	E'_1.....	28
4161	K 3.....	A' E' X F Z H'	8	4,000	Z.....	39
4161	K 4.....	A' E' X F Z H'	8	4,000	Z.....	46
7829	K 5.....	G Y E' X F V H'	7	4,000	V E''_1.....	43
7829	K 6.....	G Y E' X F V H'	8	4,000	E'_1.....	41
5586	K 8. ..	A. O QYD' I' CWX FC L H'	8	4,000	?.....	42

Table 6 shows that it was extremely difficult to prepare a pure A' reagent. It is known that the factor A' is closely related to many other factors in the B complex. Several attempts were made to produce anti-A' bodies, but most of the immunization series failed. The antiserum 5063 (5062) reacted with all A' positive cells, but also gave reactions with some A' negative cells. In all, 68 absorptions, both weak and strong, single and combined, were performed, but it was impossible to obtain reactions identical with those of the Scandinavian A' reagent.

At the Blood Grouping Conference in Wageningen, Holland, the writer had the opportunity to compare all the reagents prepared by him with the European reagents. It was found that the South African A' reagent is identical with the Dutch A' reagent, but not with the Scandinavian reagent. The reason probably is that the A' reagent of South Africa and that of Holland have been produced in Friesian cattle, and probably constitute a subgroup of A'. It should, therefore, be designated A'_2.

It must be mentioned that the isolation of these subgroups (e.g. C_1 and C_2 or or X_1 and X_2 or O_1, O_2 and O_3) was very difficult because they are strongly related. Test sera for serological subgroups are generally obtained by successive absorptions of an antiserum giving reactions with all sub-groups (e.g. O_1 and O_2 and O_3). It must also be pointed out that the above method of designating serological reactions of erythrocytes is an abbreviated one. For example, the subgroups C_1 and C_2 may be written $C_1 + {}_2$ and C_2 respectively, since both sub-groups have a factor in common but only sub-group C_1 has factor 1. The primary reason for the use of subgroups is the belief that reactions of this nature are indicative of an overlapping relationship (cross reactions) among the serologically reactive substances of bovine erythrocytes (Stormont, 1950).

The following example illustrates the phenomenon:—

TABLE 7.—*Sub-groups of blood group factor C*

Sub-groups of C	Reactions with C_2 reagent	Unabsorbed C_1 reagent	Reactions with C_2 reagent after absorption with—	
			C_2 cells	C_1 cells
C_1	4*	2	2	0
C_2	2	0	0	0
C†	0	0	0	0

* For an explanation of the scoring system used—see under " methods ".
† Indicates cells which do not react with either of the C reagents.

The isolation of these subgroups proved difficult, as indicated by the number of absorptions required (see Table No. 6). The use of weak absorptions and a very careful technique were necessary to avoid over-absorptions, which may exhaust the serum entirely.

In various laboratories in Scandinavia and Holland, several new blood group factors have been established (Rendel, 1958; Neimann Sørensen, 1958). During the Blood Group Conference in Wageningen a proposal was adopted that new factors should take their names from the initial letters of the country of their discovery, as far as such letters are not already in use. In Table No. 6 three new types of antibodies are listed corresponding to three new antigenic factors, SA_1, SA_2 and SA_3, found in South Africa and produced in Afrikaner cattle recipients. The value of these additional factors has already been confirmed in routine tests. After establishing to which genetic system they belong, factors should be internationally recognized.

E.— SUMMARY

A short introduction is given to the field of bovine blood group research. Some of the immunological terms used in immuno-genetics are explained. The genetic aspects are briefly dealt with.

The methods used for the production of iso-immune sera are described. An analysis is made of the different sources of genetical and environmental variation in antibody production, and from this it is concluded that it is impossible to give detailed directives for their production. The difficulties of testing sera under high temperature conditions are dealt with. It is concluded that these difficulties can be overcome in South Africa only by carrying out all blood group work in an air-conditioned laboratory.

The fractionation of different antisera is presented in Table No. 6. As a result of basic investigations in South Africa, three new types of antibodies have been isolated in Afrikaner cattle, which react with three so far unknown antigenic blood factors.

Only a few test sera could not be produced. Through international co-operation between blood group laboratories and particularly through exchanges of test sera, the South African laboratory was able to start routine blood group determinations for different practical purposes and genetical studies, in 1958.

REFERENCES

IRWIN, M. R. (1956). Blood Grouping and its utilization in animal beeding. *Proc. VII Int. Congr. Anim. Husb. Section*. Vol. 2; pp. 7–32.

LANDSTEINER, K. (1901). Ueber Agglutinationserscheinungen normalen menschlichen Blutes. *Wien. Klin. Wschr.*, Vol. 14, pp. 1132–1134.

NEIMANN SØRENSEN, A. (1958). Blood groups of cattle. Immunogenetic studies on Danish cattle breeds. 177 pp. A/S Carl. Fr. Mortensen, Copenhagen.

OSTERHOFF, D. R. & RENDEL, J. (1954). Die Bedeutung und praktische Anwendung der Immunogenetik. *Z. f. Tierzüchtg. u. Züchtgsbiol.*, Vol. 63, pp. 1–20.

RENDEL, J. (1957). Blood groups of farm animals. *Anim. Breed. Abstr.*, Vol. 25, pp. 223–238.

RENDEL, J. (1958). Studies of cattle blood groups. *Acta Agric. Scand.*, Vol. 8, No. 1, pp. 38 61.

RENDEL, J., TOLLE, A. & NEIMANN SØRENSEN, A. (1957). Blutgruppenuntersuchungen beim Rind. *Z. f. Tierzüchtg. u. Züchtgsbiol.*, Vol. 70, pp. 21 28.

SNEDECOR, G. W. (1953). Statistical Methods. 5th ed., 485 pp., Iowa St. Coll. Press.

STORMONT, C. (1950). Additional gene-controlled antigenic factors in the bovine erythrocytes. *Genetics*, Vol. 35, pp. 76 94.

STORMONT, C. (1952). The FV and Z systems of bovine blood groups. *Genetics*, Vol. 37, pp. 39–48.

STORMONT, C. (1955). Linked genes, pseudoalleles and blood groups. *Am. Naturalist*, Vol. 89, pp. 105–116.

STORMONT, C. & CUMLEY, R. W. (1943). Cellular antigens in cattle blood. *J. Hered.*, Vol. 34, pp. 34–41.

STORMONT, C., OWEN, R. D. & IRWIN, M. R. (1951). The B and C systems of bovine blood groups. *Genetics*, Vol. 36, pp. 134–161.