DIFFERENCES IN SPECIFICITY OF THE ANTIGENIC PRODUCTS OF A SERIES OF ALLELES IN THE CHICKEN[1]

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THE ability of normal bovine serum to agglutinate differentially the erythrocytes of chickens was first reported by LANDSTEINER and LEVINE (1932). Later, using an inbred strain of Rhode Island Red chickens, OLSON (1943) concluded that these differences in reactivity were due to a single pair of allelic genes. He proposed that one allele produced a strongly reactive agglutinogen while the other had little or no antigenic effect. A dosage effect was displayed in that the cells of birds homozygous for the allele effecting the antigen were more reactive and had greater absorptive power than the cells of heterozygotes.

The purpose of the present study was to investigate further the inheritance of the agglutinability of chicken erythrocytes with normal bovine serum and to determine the genetic relationships of such agglutinogens with those then known to exist in the chicken (BRILES, MCGIBBON and IRWIN 1950).

MATERIALS AND METHODS

The chickens used in this study were from a group maintained by the Wisconsin Agricultural Experiment Station for the study of cellular antigens. They were second and third generation crosses of birds derived from Rhode Island Reds, Single Comb White Leghorns and Barred Plymouth Rocks.

Blood was taken from the chickens by making a small cut in a wing vein and allowing the blood to run into tubes containing an isotonic solution of sodium citrate (2.00 percent) and sodium chloride (0.42 percent). The cells were washed three times with saline solution (0.75 percent sodium chloride) before they were tested. The bovine sera were obtained from blood taken from the jugular vein. These sera and the chicken isoimmune reagents employed in this study were stored at -18 to -23°C.

The normal bovine sera were inactivated for 20 minutes at 56°C in a water bath before they were used in testing cells for agglutination. Three different bovine sera were tested in serial dilutions (1 : 1, 1 : 2, 1 : 4, 1 : 8, etc.) against erythrocytes of the various chickens. It was found that the sera were alike

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except for minor differences in titer. One of the sera was arbitrarily selected for use in this study; however, as a check, erythrocytes from various birds were occasionally tested with one of the other two sera. For the most part, the erythrocytes of each chicken were tested for agglutination with the selected bovine serum at two dilutions, 1:2 and 1:8, which were found to give the most effective differentiation of the types of cells.

Tests for agglutination were performed in small test tubes by adding one drop (0.05 ml) of a two percent cell suspension to two drops of diluted cattle serum. This mixture was shaken at intervals of 15 minutes. After 45 minutes at room temperature the tubes were observed macroscopically for agglutination. A second observation was made after a total incubation period of two hours.

The erythrocytes of the chickens used in this study were also tested with reagents specific for the A and B series of agglutinogens recently discovered in the fowl. The preparation of these reagents and the genetic classification of the genes producing these agglutinogens have been presented in detail by BRILES, MCGIBBON and IRWIN (1950). Of the nine alleles of the *A* locus (A^{23}, A^{123}, A^{1236}, A^{2346}, A^{23456}, A^{3456}, A^{237}, A^{8} and *a*), four were present in the families used in this study; viz., A^{23}, A^{23456}, A^{237} and A^{8}. Of the five alleles of the *B* locus (B^{15}, B^{25}, B^{3}, B^{4} and *b*) three were present in the birds under study—B^{15}, B^{3} and *b*. In each series the locus is designated by an italicized capital letter. The digits in the superscripts following the letters indicate the ability of the antigenic substance determined by the respective alleles to react with reagents having presumably homologous components. The letters *a* and *b* indicate alleles for which no antigenic effects have been detected. In general, reagents used to detect each antigen of the A and B groups are prepared by absorbing isoimmune sera with the red cells of selected chickens until only components specific for particular antigenic factors remain. The resulting reagents are designated by capital letters followed by digits; the capital letter indicates the locus of the allele producing the homologous antigen or antigenic substance and the digit or digits represent the antibody fraction or fractions present in the reagents. Thus, the four *A* alleles (A^{23}, A^{23456}, A^{237} and A^{8}) possessed by the birds used in this study were identified by the reagents A2, A3, A456, A7 and A8. The antigen determined by each allele is given a subscript designation corresponding to the superscript designation of the causative gene. Thus, the antigens produced by the *A* alleles above are designated A_{23}, A_{23456}, A_{237} and A_{8}, respectively.

The agglutination tests with the A and B reagents were performed in the same manner as with normal cattle serum, except that the first reading was made after incubation for one and one-half hours at room temperature and confirmatory readings were made following overnight incubation in the refrigerator.

EXPERIMENTAL RESULTS

Upon testing adult chickens from several families it was found that the birds could be divided into three general classes on the basis of the agglutination of

their erythrocytes with unabsorbed normal bovine serum and on their capacity to absorb bovine serum. The finding of these three classes is in agreement with the earlier work of OLSON (1943). These three classes will be referred to as " weak," " moderate " or " strong " reactors. The weak reactor class includes all birds whose cells either failed to agglutinate when tested with unabsorbed normal bovine serum at a 1 : 2 dilution, or else reacted only weakly at that dilution; none of these weakly reactive cells were agglutinated at a 1 : 4 dilution. The cells of the strong reactors were definitely agglutinated by a 1 : 16 dilution and to some extent by a 1 : 32 dilution of normal bovine serum. The moderate reactors were between these two extremes; the cells of some of these birds reacted strongly at a 1 : 2 dilution but only slightly at a 1 : 4 dilution, while those of others gave a fair reaction at a dilution of 1 : 8 or even 1 : 16. These classifications were made on the basis of readings made after the serum-cell mixtures had been incubated at 35°C for two hours. If tests were held longer than two hours, the difference between the moderate and strong reactors was less apparent as both tended to become completely agglutinated. Thus, the main difference between the moderate and strong reactors appeared to be in their relative rates of reaction.

To check further the above classification, normal bovine serum was absorbed separately with the three types of cells. A given quantity of the serum was diluted with an equal part of saline and placed in a water bath at 56°C for 20 minutes before the absorptions were made. The number of absorptions and the quantities of the three kinds of cells used to exhaust the serum of " homologous " agglutinins were different for each of the three kinds of cells. The erythrocytes from weak reactors were employed in a ratio of one part of cells to one part of the diluted serum. The cells were divided into two equal portions. The serum was mixed with one portion of the cells and allowed to incubate, with frequent agitation, at room temperature for 45 minutes; the serum was drawn off following centrifugation, mixed with the second portion of cells, and the mixture was allowed to stand at room temperature for one hour. After this second absorption the supernatant was tested for reactivity with each of the three types of cells. It was observed that the cells of weak reactors failed to react with the absorbed fluid while the cells of the moderate and strong reactors were readily agglutinated. A few absorptions of bovine serum were made employing the cells of weak reactors in a ratio of two to three parts of cells to one part of serum. It was found that the degree of reaction of the cells of moderate and strong reactors to bovine sera so absorbed was not significantly reduced. Thus, it appeared that absorbing bovine serum with the cells of weak reactors did not remove to any appreciable extent the agglutinins for moderate or strong reactors.

To effect complete absorption with the cells of moderate reactors, it was usually necessary to use two volumes of cells to one volume of diluted serum and to divide the cells equally into three successive absorptions. These absorptions were carried out as described above, except that the third absorption was stored overnight in the refrigerator after it had been incubated at room temperature for one hour. Test-fluids thus prepared were then tested with the

cells of several birds representative of the three types of reactors. These tests demonstrated that the antibodies capable of agglutinating the cells of weak reactors had been removed, but agglutinins for the cells of strong reactors still remained in serum exhausted of agglutinins for moderate reactors. The moderate reactors all had similar but not duplicate absorptive capacities, especially on the basis of readings made after the tests had been stored overnight in the refrigerator, then allowed to stand one hour at room temperature (approximately 26°). Weak reactions for certain of the moderate group thus observed indicated that the moderate reactors differed in their affinities for bovine serum. Highly specific test-fluids which might have been used to detect these differences were not obtained although differential absorptions were attempted. The failure to obtain such test-fluids was possibly due to the rather broad specificity of antibodies in bovine serum exhibited towards the cells of the moderate and strong reactors.

When the cells of the strong reactors were used in absorbing bovine serum, three volumes of cells were used to absorb one volume of serum dilution. The absorption of agglutinins was more likely to be complete if the cells were divided into four equal parts and used in four successive absorptions—three at room temperature for three-fourths to one hour each and one, usually the last, for about 18 hours in the refrigerator in addition to one hour at room temperature. The cells of the strong reactors removed antibodies for themselves and also for the moderate and weak reactors.

In general, the above results confirm the finding of OLSON (1943) that there were three reactive classes into which the cells of various chickens could be grouped on the basis of their reactions to bovine serum. However, in the present study the somewhat variable results obtained following absorption with the cells of different moderate reactors suggested that the moderate reactors were not all alike with respect to their serological specificity.

In the hope of obtaining highly specific reagents capable of identifying the antigenic differences not clearly revealed by absorbed bovine serum, it was decided to attempt to produce test-fluids from isoimmune sera. Several immunizations were made between chickens whose red cells differed in reactivity to normal cattle serum. The method used in analyzing the resulting antisera has been reported in a previous paper (BRILES, MCGIBBON and IRWIN 1950). Analysis by this method showed that the isoagglutinins produced in such immunizations were of two kinds. One was found to be reactive with an agglutinogen which was also reactive with previously prepared reagents A2 and A3; this new agglutinin was assigned the symbol A7 and the agglutinogen was designated A_{237}. The second isoagglutinin was reactive with an agglutinogen not reactive with any of the previously prepared A or B reagents. However, tests for genetic segregation showed that this new agglutinogen was determined by a gene belonging to the *A* series of alleles. This agglutinogen and the reagent used to identify it were assigned the symbols A_8 and A8, respectively.

The identification of these two new agglutinogens, A_{237} and A_8, made it possible to mate birds of appropriate blood types so that families could be

produced in which the genotype at the *A* locus could be completely determined by direct agglutination test. On the basis of their reaction to the isoimmune reagents used to detect the A series of alleles, the three general classes of chicken cells detected by testing with bovine serum were found to fall into ten genotypic classes; the weak reactors were found to be of three genotypic classes, the moderate reactors fell into four classes, and the strong reactors were found to consist of three classes.

The family given in table 1 is from the mating of parents (both classified as moderate reactors to bovine serum) which possessed no allele in common

TABLE 1

*Agglutination of the red blood cells of members of family R426 × R450 with various dilutions of normal bovine serum.**

Members of family		Serum dilutions 1:2	1:4	1:8	1:16	Type of cell	Genotype at *A* locus	Genotype at *B* locus
Sire	R426	+++	+++	++	0	M**	A^{23456}/A^{8}	B^{15}/B^{3}
Dam	R450	++++	+++	0	0	M	A^{237}/A^{23}	b/b
F_1	C610	0	0	0	0	W	A^{23456}/A^{237}	B^{15}/b
	C612	+++	++	++	+	M	A^{23456}/A^{23}	b/B^{3}
	C613	++++	++++	++++	++	S	A^{23}/A^{8}	b/B^{3}
	C614	++++	++++	++++	++++	S	A^{23}/A^{8}	B^{15}/b
	C615	++++	+++	++	0	M	A^{237}/A^{8}	B^{15}/b
	C616	++++	+++	++	+	M	A^{23456}/A^{23}	b/B^{3}
	C617	++++	++++	++++	++++	S	A^{23}/A^{8}	B^{15}/b
	C618	±	0	0	0	W	A^{23456}/A^{237}	b/B^{3}
	C619	±	0	0	0	W	A^{23456}/A^{237}	B^{15}/b
	C620	++++	++++	++++	++++	S	A^{23}/A^{8}	b/B^{3}
	C622	+	0	0	0	W	A^{23456}/A^{237}	b/B^{3}
	C623	+++	++	±	0	M	A^{237}/A^{8}	B^{15}/b
	C624	±	0	0	0	W	A^{23456}/A^{237}	B^{15}/b
	C635	+++	+++	+	0	M	A^{237}/A^{8}	b/B^{3}
	C2043	++++	++++	++++	++++	S	A^{23}/A^{8}	b/B^{3}
	C2649	++++	+++	++	+	M	A^{23456}/A^{23}	b/B^{3}
	C3351	±	0	0	0	W	A^{23456}/A^{237}	B^{15}/b
	C3352	++++	++	0	0	M	A^{237}/A^{8}	B^{15}/b
	C3353	±	0	0	0	W	A^{23456}/A^{237}	b/B^{3}

*Readings made after two hours of incubation.

**The letters W, M and S indicate "weak," "moderate" and "strong" reactors, respectively.

Symbols: 0 = no agglutination; ± = very weak agglutination; +, ++, +++ and ++++ indicate increasing degrees of agglutination.

at either the *A* or *B* locus. The genotypes of the nineteen offspring of this mating could be directly determined by test with appropriate A and B reagents. The genotype of the sire, R426, was $A^{23456}/A^{8}\ B^{15}/B^{3}$ while that of the dam, R450, was $A^{237}/A^{23}\ b/b$. As had been found in other matings between moderate reactors, their offspring were classified as strong, moderate or weak reactors with bovine serum. It was also noticed (table 1) that all offspring classified as strong reactors—C613, C614, C617, C620 and C2043—were of the genotype A^{23}/A^{8}. All offspring classified as moderate reactors—C612, C615, C616, C623, C635, C2649 and C3352—possessed only one of the two alleles comprising the genotype of the strong reactors, A^{23} or A^{8}, in combina-

tion with A^{23456} or A^{237}, respectively (*i.e.*, A^{23456}/A^{23} or A^{237}/A^{8}). The offspring classified as weak reactors—C610, C618, C619, C622, C624, C3351 and C3353—were of the genotype A^{23456}/A^{237}. Since the alleles A^{23} and A^{8}, together or in combination with either A^{237} or A^{23456}, were present in the genotype of all offspring whose red cells were agglutinated very strongly by the bovine serum, it was tentatively concluded that A^{23} and A^{8} were primarily responsible for the ability of the cells of certain members of this family to react with normal bovine serum. The antigens determined by the alleles of the *B* locus did not appear to be associated in any way with the agglutination of the cells of the members of this family by bovine serum. The B_3 antigen was distributed among the strong, moderate and weak reactors in a ratio of 3:4:3. The B_{15} antigen appeared among the strong, moderate and weak reactors in a ratio of 2:3:4. These ratios undoubtedly represent chance distributions.

TABLE 2

*Agglutination of the red blood cells of members of family A1543 × R635 with various dilutions of normal bovine serum.**

Members of family		Serum dilutions			Type of cell	Genotype at *A* locus	Genotype at *B* locus
		1:2	1:4	1:8			
Sire	A1543	++++	++++	++++	S	A^{23}/A^{8}	B^{15}/b
Dam	R635	+++	0	0	M	A^{237}/A^{8}	B^{3}/b
F_1	C545	++++	++++	++++	S	A^{23}/A^{8}	B^{15}/b
	C546	++	0	0	M	A^{237}/A^{23}	B^{15}/b
	C547	++++	++++	++++	S	A^{23}/A^{8}	B^{15}/b
	C548	+++	0	0	M	A^{237}/A^{23}	b/b
	C549	+++	0	0	M	A^{237}/A^{8}	B^{15}/B^{3}
	C550	++++	++++	++++	S	A^{23}/A^{8}	B^{3}/b
	C551	++++	++++	+++	S	A^{8}/A^{8}	B^{15}/B^{3}
	C991	+++	++	0	M	A^{237}/A^{23}	B^{3}/b
	C993	++++	++++	+++	S	A^{8}/A^{8}	B^{15}/b
	C994	++++	++	0	M	A^{237}/A^{23}	B^{15}/B^{3}
	C995	±	0	0	W?	A^{237}/A^{8}	B^{3}/b
	C2992	++++	+	0	M	A^{237}/A^{23}	B^{15}/b
	C2993	++	0	0	M	A^{237}/A^{8}	B^{3}/b

*Readings made after two hours of incubation.
For explanation of symbols, see table 1.

The reactivities to normal bovine serum of the red blood cells of 15 members of another family and the genotypes of the various members at the *A* and *B* loci are given in table 2. The sire of this family was classified as a strong reactor with bovine serum, and with respect to the *A* and *B* loci was of the genotype $A^{23}/A^{8}\ B^{15}/b$; the dam was classified as a moderate reactor and was of the genotype $A^{237}/A^{8}\ B^{3}/b$. The cells of the offspring of the genotype A^{23}/A^{8} (C545, C547 and C550) and A^{8}/A^{8} (C551 and C993) reacted strongly with bovine serum, even at a dilution of 1:8. The eleven remaining progeny possessed the allele A^{237} along with either A^{23} or A^{8}. The offspring of the genotype A^{237}/A^{23} (C546, C548, C991, C994 and C2992) were classed as moderate reactors since their cells clumped fairly strongly at a dilution of 1:2 of bovine serum, and three were reactive at a dilution of 1:4. Those of the

genotype A^{237}/A^{8} (C549, C995 and C2993) were reactive only at a dilution of 1:2 and the cells of one of the birds, C995, were so weakly reactive with bovine serum that on the basis of the single test performed they fell into the weak reactor class, those of the other two were placed in the moderate class. The weak reaction shown by the cells of this bird is probably accounted for by the somewhat variable nature of serological cross reactions; although, in general, the repeatability of the agglutination tests with normal bovine serum was found to be remarkably high throughout this study. As with the previous family, there was no apparent relationship between the reactions obtained with bovine serum and the genotypes at the *B* locus.

The results obtained with the above two families indicated that the alleles A^{23} and A^{8} produced agglutinogens strongly reactive with normal antibodies of bovine serum and that the alleles A^{23456} and A^{237} produced agglutinogens showing only slight affinity for normal bovine serum. Furthermore, a dosage

TABLE 3

Number of chickens of each genotype showing the various degrees of agglutination with normal bovine serum.

Genotype at *A* locus	Number of chickens tested	Type of cell	Agglutination of red cells with bovine serum									
			Serum dil. 1:2					Serum dil. 1:8				
			++++	+++	++	+*	0	++++	+++	++	+	0
A^{23}/A^{23}	1	S	1	0	0	0	0	1	0	0	0	0
A^{8}/A^{8}	2	S	2	0	0	0	0	0	2	0	0	0
A^{23}/A^{8}	16	S	16	0	0	0	0	8	7	1	0	0
A^{8}/A^{237}	16	M	8	4	3	1	0	0	0	0	1	15
A^{8}/A^{23456}	10	M	4	6	0	0	0	0	0	3	1	6
A^{23}/A^{237}	35	M	6	19	9	1	0	0	0	0	1	34
A^{23}/A^{23456}	22	M	11	11	0	0	0	0	1	2	2	17
A^{237}/A^{23456}	26	W	0	0	0	6	20	0	0	0	0	26
A^{237}/A^{237}	3	W	0	0	0	0	3	0	0	0	0	3
A^{23456}/A^{23456}	3	W	0	0	0	0	3	0	0	0	0	3

*Very weak reactions (±) are summarized above under the degree of reaction +.

effect was apparent in that the cells of birds possessing both A^{23} and A^{8} were more reactive than the cells of those possessing one of these alleles along with A^{23456} or A^{237}. To test this hypothesis, the parents and offspring of ten additional families were tested with appropriate A reagents and with 1:2 and 1:8 dilutions of bovine serum. A summary of these tests, including the data on the two families already presented, is given in table 3. In summarizing these data very weak reactions of the degree ± were included among the weak reactions indicated by the symbol +. Of the 134 birds included in this table, 19 belonged to the strong reactor class. These birds belonged to one of three genotypes—A^{23}/A^{23}, A^{8}/A^{8} or A^{23}/A^{8}. Thus, it appears as though A^{23} and A^{8} together are able to elicit approximately the same quantitative reaction with bovine serum as a double dose of either.

The reactions obtained at a 1:8 dilution of bovine serum served to differentiate readily the moderate from the strong reactors. In general, the moder-

ates reacted relatively weakly, if at all, while the strong reactors were usually strongly clumped at a 1:8 dilution. Even at a 1:2 dilution the moderate reactors usually failed to react with the strength characteristic of the strong reactors. Among the birds used in this study all moderate reactors possessed one of four genotypes—A^8/A^{237}, A^8/A^{23456}, A^{23}/A^{237} or A^{23}/A^{23456}. Each of these birds with moderately reactive cells thus possessed one and only one allele in common with those possessed by the strongly reactive birds, that is A^{23} or A^8. It is also apparent that each bird having moderately reactive cells possessed either the allele A^{23456} or A^{237}. The weak or non-reactive class of birds, except for bird C995 discussed earlier, belonged to one of three genotypes—A^{237}/A^{23456}, A^{237}/A^{237} or A^{23456}/A^{23456}.

DISCUSSION

The evidence presented indicates that the agglutinability of chicken erythrocytes by bovine serum was primarily, if not completely, determined in the population under study by four alleles of the *A* locus. Agglutination tests with isoimmune reagents clearly indicate that the antigenic product of each of the *A* alleles is biochemically distinct from that produced by the other members of the allelic series (BRILES, MCGIBBON and IRWIN 1950). Although the antigenic product of each of the four alleles has its own particular serological characteristics, the effects of their respective antigens on the agglutinability of erythrocytes by normal bovine serum appear to fall in one or the other of two categories in that they produce antigens having either very strong or very weak affinity for bovine serum. The antigenic products of the alleles A^{23} and A^8 are strongly reactive, while the antigens produced by the alleles A^{23456} and A^{237} are very weakly reactive, if at all, with bovine serum. (Although the assumption is made that these causative genes are members of an allelic series, the possibility of pseudo-allelism cannot be excluded.)

The over-all effect of the *A* locus on the agglutinability of chicken erythrocytes by bovine serum appears to be quantitative and determined by the total affinity contributed by the independently acting *A* alleles. For example, when antigen A_{23} was found in cells strongly reactive with bovine serum, it was present in *double dose* in the homozygote A^{23}/A^{23} or accompanied by the antigen A_8 in the heterozygote A^{23}/A^8; when antigen A_{23} was found in moderately reactive cells, it was accompanied by antigens A_{237} or A_{23456}, having negligible affinity for bovine serum. It is quite possible that the antigens A_{23} and A_8 are different in their affinity for bovine serum; it may be that their affinities are so similar that the quantitative agglutination test employed was inadequate to disclose definite antigenic differences, which are clearly demonstrable by qualitatively different isoimmune reagents.

Through the use of bovine serum alone, OLSON (1943) identified an antigenic locus to which he assigned the symbol *A*. On the basis of his work on bovine serum he concluded that at this locus probably two alleles were present which determined the antigenic affinity of chicken erythrocytes for bovine serum: *AA* being the genotype of the strong reactors, *Aa* the genotype of the

moderate reactors, and *aa* the genotype of those individuals whose cells showed negligible affinity for bovine serum. This locus may be identical with the first blood group locus (*A*) discovered independently by BRILES, MCGIBBON and IRWIN (1950) with isoimmune agglutinins. (The assigning of the symbol, *A*, to the locus was coincidental.) The latter work disclosed the existence of at least nine alleles at this locus. The affinity for bovine serum of the antigenic products of four of these alleles—A^{23}, A^{23456}, A^{237} and A^{8}—has been presented in this study. Unfortunately, time did not permit the testing of the affinity for bovine serum of the antigens of the remaining *A* alleles. It is possible that only two alleles determining antigenic affinity of the red cells for bovine serum were present in the inbred population investigated by OLSON; however, in view of the results reported in this paper, more than two alleles could have existed in the population studied by OLSON since the affinity for bovine serum of the antigenic products of different *A* alleles may be similar or perhaps identical.

Antigenic differences somewhat analogous to those demonstrated in the present study for chicken cells have been detected with normal bovine serum in human red blood cells by STORMONT (1949). He found that a normally occurring antibody, called anti-J, agglutinated human cells possessing the A antigen. This antibody is present only in the serum of certain cattle and presumably is due to a single gene. The bovine sera used in these studies agglutinated chicken red cells equally well irrespective of whether or not the antibody for J was present. Further, in recent tests of the sera of over 20 individual cattle, no difference in titer for chicken cells was found in cattle sera possessing the antibody for J as compared to those lacking the antibody (IRWIN, unpublished). However, it appears quite unlikely that the antibody in cattle sera reactive with human A_2 or O is related to the antibody specific for chicken cells.

Saline extracts from certain seeds have been shown by RENKONEN (1948) and BOYD and REGUERA (1949) to agglutinate human cells possessing certain antigens of the A–B–O blood group. For example, BOYD and REGUERA found that extracts from certain varieties of beans agglutinated only cells possessing antigen A. It appears reasonable to propose that further specificities in gene products may be demonstrated by naturally occurring antibodies.

SUMMARY

The agglutination of chicken erythrocytes by normal bovine serum appears to be determined by the alleles of the *A* series. The agglutinogens effected by these alleles were identified through the use of isoimmune reagents. Of the nine known alleles of the *A* group, four were utilized in this study. The agglutinogens produced by two of these alleles, A^{23} and A^{8}, reacted strongly with normal bovine serum. The antigenic products of the other two alleles, A^{237} and A^{23456}, reacted only very weakly, if at all, with bovine serum.

There was a distinct dosage effect of the reactive alleles A^{23} and A^{8}. The cells of individuals having a single dose of a reactive allele (for example, A^{23}/A^{237} or A^{8}/A^{237}) were only moderately reactive, while the cells of indi-

viduals possessing a *double dose* of the reactive alleles (A^{23}/A^{23}, A^{8}/A^{8} or A^{23}/A^{8}) were very strongly reactive.

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