ON MULTIPLE ALLELES EFFECTING CELLULAR ANTIGENS IN THE CHICKEN[1]

W. E. BRILES,[2] W. H. McGIBBON, AND M. R. IRWIN
University of Wisconsin, Madison, Wisconsin

Received March 27, 1950

COINCIDENTALLY with the beginning of modern genetics in 1900, antigenic differences in the erythrocytes of members of two species of animals were discovered. LANDSTEINER (1900, 1901) made his now famous discovery of the blood groups of man. EHRLICH and MORGENROTH (1900) demonstrated the existence of individual differences in the antigenic constitution of goat erythrocytes. Later, TODD and WHITE (1910) proposed on the basis of their work with the cellular antigens of cattle that the red cells of almost every individual could be distinguished from those of any other individual.

LANDSTEINER and MILLER (1924) were the first to detect individual differences in the antigenic constitution of the erythrocytes of chickens. By absorbing rabbit anti-chicken serum individually with the cells of various chickens and testing the resulting fluids with the cells of ten individuals, eight different cell types were recognized. Following the injection of the blood of one chicken into another (iso-immunization), TODD (1930) found that iso-agglutinins were produced against the cells of the donor as well as against the cells of many other individuals. By using highly polyvalent antisera, produced by pooling the sera from several chickens which had been previously injected with the pooled cells of as many as 22 other chickens, TODD was able to show that the cells of any chicken, with the exception of close relatives, could be differentiated from the cells of every other individual. Further, he demonstrated that any cellular antigen possessed by an individual was present in the cells of one or both of its parents. Later TODD (1931) reported that the cells of each chick in each of three families appeared to have different absorptive or reactive capacities from the cells of every other chick, although some were quite similar. In accounting for the probably genetic relationships of the genes responsible for these observed antigenic differences, WIENER (1934) hypothesized the existence of three or more alleles at some loci.

KOZELKA (1933), using absorbed rabbit anti-chicken sera, found marked similarity in the corpuscles of individuals belonging to different breeds. This led him to believe that the apparent multiplicity of antigenic differences previously reported in chickens by TODD could be explained "by a variable assortment of a limited number of factors".

[1] From the Departments of Genetics (No. 411) and Poultry Husbandry, Agricultural Experiment Station, UNIVERSITY OF WISCONSIN. This project was supported in part by the Research Committee of the Graduate School from funds supplied by the WISCONSIN ALUMNI RESEARCH FOUNDATION.

[2] Present address, Department of Poultry Husbandry, A. AND M. COLLEGE OF TEXAS, College Station, Texas.

Normal cattle serum was found by LANDSTEINER and LEVINE (1932) to agglutinate differentially the cells of various chickens; also, antisera prepared against the Forssman antigen allowed a second independent classification of the cells of individual chickens. It was reported in the same paper that one of the authors, working with L. C. DUNN, had used rabbit anti-chicken serum to detect a single agglutinogen. Studies on several families had indicated that the antigenic character was controlled by a single dominant gene.

That agglutinogens may result from the action of recessive genes or from the complementary effect of non-allelic genes was suggested by the work of THOMSEN (1934, 1936) and BOYD and ALLEY (1940) in which agglutination of the red cells of a few individuals was obtained with a specific test-fluid even though the cells of both parents failed to react.

This paper describes the serological and genetic identification in chickens of cellular antigens, seemingly effected by two independent autosomal series of alleles.

MATERIALS AND METHODS

Most of the chickens used in these studies were Barred Plymouth Rocks, although New Hampshires and Single Comb White Leghorns were utilized to a limited extent. The Barred Plymouth Rocks will be indicated by number only; otherwise the breed or breed derivation will be given.

Iso-immune sera were prepared by injecting citrated whole chicken blood or washed red blood cells into closely related individuals. Approximately one cc of whole blood or 50 percent cell suspension was injected intravenously at three to four day intervals until the antibody titer was satisfactory—usually three to four injections were necessary, although occasionally excellent antisera resulted from a single injection. In the beginning of this study, the antisera were stored in a refrigerator (2 to 6°C) after merthiolate solution (1:100) had been added in sufficient quantity to give a final concentration of 1:2000. More recently the antisera have been stored in a deep freeze box (−18 to −32°C) without the addition of a preservative.

To prepare test-fluids or reagents specific for each antigenic factor, an antiserum was diluted with saline (according to its titer) and mixed with a suitable quantity of washed cells (usually 1 cc of cells to 1.6 cc of diluted antiserum). The mixture was allowed to stand at room temperature (22 to 26°C) with frequent agitation for 20 to 30 minutes, after which it was centrifuged. The resulting supernatant was mixed with a second portion of cells, and further absorption of the antibodies was allowed to take place for a period of one to two hours. Each subsequent absorption was similarly made except that one of the absorptions, usually the last, was placed in the refrigerator (2 to 6°C) overnight. The absorptions were repeated until the supernatant ceased to produce agglutination with the cells used in absorption. When not used immediately, the reagents were stored at −18 to −23°C.

The agglutination tests were performed by adding one drop of a two percent cell suspension in saline to two drops (0.1 cc) of a reagent in appropriate dilutions, usually made serially. Each mixture of cells and reagent was observed

for the presence of agglutination after standing for one and one-half hours at room temperature; confirmatory readings were made after storage of the test overnight in the refrigerator. Doubtful readings and negative controls were checked microscopically.

TERMINOLOGY

To facilitate the presentation of results, it seems advisable at this point to indicate the terminology used in designating the antigenic characters, their causative genes, and the test-fluids used in characterizing them. The antigens so far detected appear to be determined by genes belonging to one or the other of two series of alleles. The type of action displayed by the alleles of each series indicates a complete lack of dominance, so that each allele always produces its particular antigenic substance, regardless of the other alleles present. One series has been given the locus designation *A* and consists of nine alleles tentatively designated as follows: A^{123}, A^{1236}, A^{23}, A^{237}, A^{2346}, A^{23456}, A^{3456}, A^{8} and *a*. The letter *a* indicates an allele for which no antigenic effect has been detected with the reagents employed. Each digit in the superscript indicates the ability of the antigenic substance determined by that allele to react with the respective reagents designated by digits preceded by a standard capital letter indicating the locus of the gene producing the homologous antigen or antigenic factor. Thus, the reagents employed to designate the A series of antigens are A1, A2, A3, A5, A45, A456, A7 and A8. The reagents are simply test-fluids containing agglutinins whose specificities have been found to be limited to the antigenic component or components indicated by the digits following the capital letter. The antigenic unit determined by each allele is given a subscript designation in contrast to the superscript symbol assigned to the gene. The antigens produced by the above series of alleles are thus designated respectively as A_{123}, A_{1236}, A_{23}, A_{237}, A_{2346}, A_{23456}, A_{3456} and A_{8}.

A second series, given the locus designation *B*, consists of five alleles—B^{15}, B^{25}, B^{3}, B^{4} and *b*. The antigens produced by the *B* series of alleles are identified by means of the separate reagents B1, B15, B2, B3 and B4.

In general, an antigen is usually defined simply as any substance which, when introduced parenterally into an animal, stimulates the production of antibodies with which it will react. In discussing the results obtained in this study the term *antigen* will be used only in referring to the *total antigenic product* of an allele, while the term *antigenic factor* will be used in referring to the "serological components" of which the antigen appears to be composed. For example, the total antigenic product of the allele B^{15} is referred to as antigen B_{15}, which is composed of antigenic factors B_1 and B_5. These designations are made for the sake of convenience in discussion and should not be viewed necessarily as representing natural units.

PREPARATION OF REAGENTS

The various reagents resulted from the serological fractionation by selective absorption of the agglutinins present in iso-immune sera. As all of the reagents

were obtained in essentially the same manner, the preparation of reagents from only two antisera will be discussed as illustrative of the general method; however, the authors will supply on request the detailed procedure followed in obtaining any of the other reagents. The antisera to be used as examples do not necessarily correspond to the order of the discovery of the various antigenic factors.

The serological analysis of antiserum E962(E965), resulting from the injection of the blood of bird E965 into E962, is presented in table 1. The general procedure in carrying out an analysis of this kind was, first, to absorb the antiserum individually with the cells of birds selected at random or because of

TABLE 1

*Differential agglutination of test cells by test-fluids resulting from the absorption of antiserum E962(E965) with selected cells.**

TEST CELLS	PROPOSED ANTIGENIC FACTORS IN CELLS	ABSORBING CELLS: I989 (OR UNABSORBED)	I993	P143	P144	W29	W30	W33	W70	W29 AND I993
		AGGLUTININS REMAINING IN RESPECTIVE TEST-FLUIDS: A2, A4	A2	None	None	A4	A4	A4	A4	None
2712	A_2, A_4	++	+	0	0	++	++	++	++	0
I977	None	0	0	0	0	0	0	0	0	0
I988	None	0	0	0	0	0	0	0	0	0
I989	None	0	0	0	0	0	0	0	0	0
I992	A_4	++	0	0	0	++	++	++	++	0
I993	A_4	++	0	0	0	++	++	++	++	0
P72	None	0	0	0	0	0	0	0	0	0
P107	None	0	0	0	0	0	0	0	0	0
P142	None	0	0	0	0	0	0	0	0	0
P143	A_2, A_4	++	+	0	0	++	++	++	++	0
P144	A_2, A_4	++	+	0	0	++	++	++	++	0
P178	None	0	0	0	0	0	0	0	0	0
P179	None	0	0	0	0	0	0	0	0	0
W28	A_2	++	++	0	0	0	0	0	0	0
W29	A_2	++	++	0	0	0	0	0	0	0
W30	A_2	++	++	0	0	0	0	0	0	0
W31	A_2	++	++	0	0	0	0	0	0	0
W32	A_2	++	++	0	0	0	0	0	0	0
W33	A_2	++	++	0	0	0	0	0	0	0
W61	A_2, A_4	++	++	0	0	++	++	++	++	0
W62	A_2	++	++	0	0	0	0	0	0	0
W70	A_2	++	++	0	0	0	0	0	0	0

* The serum was diluted 1:2 for the absorptions; the test-fluids were diluted again 1:2 for the agglutination tests, except for the absorption controls, where no dilution was made.

Symbols: ++=strong agglutination, +=definite agglutination observable macroscopically, 0=no agglutination.

their antigenic constitution and, second, to test the absorbed fluids with the cells of selected individuals. After the agglutination results were obtained following the exploratory single absorptions, it was frequently necessary to make absorptions with the pooled cells from two or more individuals in order to complete the analysis.

It may be seen in table 1 that there were four kinds of cells among those tested for agglutination. The first type of cell (I977, I988, I989 and others as listed in the first column) failed to react with the unabsorbed antiserum or with any of the absorbed fluids prepared from this antiserum.

When cells from this non-reactive group (that is, I989) were used in absorbing this antiserum, agglutinins remained for the cells of various individuals, which subsequently could be divided into three groups. The cells of the second group (W29, W30, W33, W70 and others) reacted with the test-fluid prepared by absorbing this antiserum with the cells of I993. To account for the reaction of these cells with this test-fluid it was necessary to postulate at least one antigenic factor for the cells and a corresponding agglutinin for the fluid, which were given the symbols A_2 and A2, respectively.

When the cells possessing the A_2 factor (W29, W30, W33 and W70) were used in absorbing the anti-serum, agglutinins were left for the third and fourth classes of reactive cells. The reaction of one of these classes of cells (I992 and I993) requires an antigenic factor different from A_2, and a corresponding agglutinin for the test-fluids; this second antigenic factor and its homologous antibody were given the symbols A_4 and A4 respectively. The fourth class of cells (2712, P143, P144 and W61) reacted with both the A2 and A4 test-fluids. Further, when the cells of two of these, P143 and P144, were used separately in absorbing the antiserum, the agglutinins were removed for all of the test cells (table 1). Therefore, it is reasonable to conclude that the cells of these two birds possessed the two antigenic factors A_2 and A_4 *in toto*. Because of the similarity of the reactions of the cells of these two individuals and those of 2712 and W61, it is reasonable to assume that the cells of the latter two birds also possessed the A_2 and A_4 factors. Evidence that the antiserum did not contain more agglutinins reactive with the test cells than those represented by the A2 and A4 antibody fractions is afforded by the ability of pooled A_2 (W29) and A_4 (I993) cells to exhaust this antiserum of antibodies (table 1).

The A2 reagent used in detecting the A_2 antigenic factor referred to in subsequent sections of this paper was prepared by absorbing antiserum E962 (E965) with the cells of bird I993. Because of its special value as a source of A2 reagent, this antiserum was not utilized subsequently as a source of A4 reagent.

Additional immunizations were made to obtain a second source of A4 reagent using as donors birds whose cells possessed the A_4 antigenic factor and as recipients, birds whose cells lacked it. One antiserum—resulting from the injection of the blood of male R439, with factors A_1, A_2, A_3 and A_4, into male R428, with factors A_2 and A_3—contained A4 antibodies in relatively high concentration. The antigenic factors A_1 and A_3, involved in this immunization, were detected by reagents prepared in essentially the same manner as the A2

reagent just considered. When this antiserum, R428(R439), was absorbed with selected cells, a fluid was produced which reacted only with the cells of those individuals previously shown to possess the A_4 antigenic factor.

Since this absorbed fluid appeared to contain only antibodies reactive with the A_4 antigenic factor, it was designated as an A4 reagent. However, as a result of employing this reagent in testing the cells of the progeny from appropriate matings, it was later shown that this A4 reagent contained at least three fractions of antibody—A4, A5 and A6. The procedure used in detecting serologically these antibody fractions and in identifying simultaneously the genetic relationships of the homologous antigenic factors follows. Birds possessing both the A_1 and A_4 factors were mated to individuals possessing neither of them. The birds involved in these matings were Barred Plymouth Rocks, except for one New Hampshire male, 2738, whose cells were reactive with both the A1 and A4 reagents. The progeny of the Barred Plymouth Rocks showed a 1:1 segregation of the genes determining the A_1 and A_4 antigens, suggesting that the two antigens were determined by allelic genes. However, the cells of all the progeny resulting from the New Hampshire male reacted with the A4 reagent, while the cells of only half of his progeny reacted with the A1 reagent. This difference in segregation indicated that the genes or gene combinations effecting the A_1 and A_4 antigenic factors in this male (2738) and in the Barred Plymouth Rocks were different. It was noticed that the cells of the A_1 progeny of male 2738 reacted more weakly with the A4 reagent than did the cells of the non-A_1 segregates. This suggested that this male possessed two allelic genes each of which produced similar but not identical antigens; that is, while the two antigens produced by the *A* alleles contributed by this male were agglutinable to a different degree by the A4 reagent, only the antigen least reactive with A4 possessed the ability to react with the A1 reagent. To test this possibility, A_1 and A_4 progeny from the Barred Plymouth Rock matings and the two kinds of segregates from the New Hampshire male were used individually in absorbing antiserum R428(R439). As can be seen from examining table 2 it was necessary to postulate two additional antigenic factors, A_5 and A_6, to explain the reactions obtained in the analysis of this antiserum.

In interpreting the data obtained from the differential absorption of a complex antiserum, as shown in table 2, it is advisable to group together cells showing the same reaction pattern and to consider the cell-groups in the order of their reactivity; that is, the group or cell-type showing agglutination with the smallest number of absorbed fluids (or with none of them) is considered first, followed by the consideration of the next most reactive cell-type, etc. The cell-type to be considered first in table 2 is R2094, representing the A_1 segregates from the Barred Plymouth Rock matings; this type of cell did not react with any of the absorbed fluids. The second cell-type in order of reactivity is represented by those of chicks R2100 and R2102—the A_1 segregates from New Hampshire male 2738. Cells of this type were agglutinated only by the absorbed fluid indicated by code number 6. Since the cells of bird R2094 used in absorbing fluid 6 contained A_1, any antibodies remaining must have reacted with an additional antigenic factor, arbitrarily assigned the symbol A_6.

The third cell-type in order of reactivity is represented by R2071 and R2076—the non-A_1 segregates from the New Hampshire male. To explain the reactions of this type of cell with fluids 7 and 8 it was necessary to hypothesize an additional antigenic factor; since this factor segregated with respect to A_1 in the progeny of male 2738, it was given the symbol A_4 in accordance with the behavior of the A_1 and A_4 factors present in the Barred Plymouth Rocks. The most reactive cell-type is represented by birds R2090, R2092, R2093 and R2117—the non-A_1 segregates from the Barred Plymouth Rock matings. To

TABLE 2

*Differential agglutination of test cells by test-fluids resulting from the absorption of antiserum R428(R439) with selected cells.**

TEST CELLS	PROPOSED ANTIGENIC FACTORS IN TEST CELLS	ABSORBING CELLS: AGGLUTININS REMAINING IN RESPECTIVE TEST-FLUIDS								
		R2071†	R2076†	R2090	R2092	R2093	R2094	R2100†	R2102†	R2117
		A5	A5	NONE	NONE	NONE	A4,A5,A6	A4,A5	A4,A5	NONE
R2071†	A_4, A_6	0	0	0	0	0	++	+	+	0
R2076†	A_4, A_6	0	0	0	0	0	++	+	+	0
R2090	A_4, A_5, A_6	++	++	0	0	0	++	++	++	0
R2092	A_4, A_5, A_6	++	++	0	0	0	++	++	++	0
R2093	A_4, A_5, A_6	++	++	0	0	0	++	++	++	0
R2094	A_1	0	0	0	0	0	0	0	0	0
R2100†	A_1, A_6	0	0	0	0	0	++	0	0	0
R2102†	A_1, A_6	0	0	0	0	0	++	0	0	0
R2117	A_4, A_5, A_6	++	++	0	0	0	++	++	++	0
Code number of test-fluids		1	2	3	4	5	6	7	8	9

* The serum was diluted 1:8 for the absorptions; test-fluids were used undiluted.

† These birds resulted from the mating of New Hampshire male 2738 with Barred Plymouth Rock hens; the remainder of the birds were Barred Plymouth Rocks.

For explanation of symbols, see table 1.

account for the reactions of this type of cell with fluids 1 and 2 a third antigenic factor, A_5, was hypothesized. Thus, a minimum of three antigenic factors was necessary to explain adequately the agglutination reactions obtained after this antiserum was individually absorbed with four serologically distinct cell-types.

To determine the combinations of antigenic factors comprising the different cell-types, it was necessary to ascertain what antibody fractions remained in the antiserum after absorption by each of the cell-types. At this point it is preferable to consider first the simplest or least reactive test-fluid just as above the least reactive cell-type was considered first. Test-fluids designated in the table as code numbers 3, 4, 5 and 9 contained no antibodies; this means that the cells used to absorb the antiserum in producing these fluids contained all three of the antigenic factors—A_4, A_5 and A_6—for which the antiserum contained specific antibody fractions. The simplest type of test-fluid having antibodies remaining after absorption is represented by code numbers 1 and 2. The agglutination reactions produced by these fluids were previously accounted for

by antigenic factor A_5; therefore, the antibody fraction remaining in this fluid was designated A5. In order for the A5 antibody fraction to have remained after absorption, the cells of R2071 and R2076 must have removed antibody fractions A4 and A6; therefore, the cells of these two birds were assigned antigenic factors A_4 and A_6. The third type of test-fluid in order of reactivity is represented by code numbers 7 and 8. These fluids contained antibody fractions A4 and A5 since the cells used in absorbing the antiserum to prepare these fluids had been previously shown to possess only the antigenic factor A_6. Finally, the absorbed fluid producing agglutination with the greatest number of cell-types is that designated by code number 6. Since the cells of bird R2094, used in absorbing this fluid did not react with any of the test-fluids, it would not be expected to remove any one of the three antibody fractions—A4, A5 or A6—from the antiserum. This result was actually obtained when the cells of this bird were used in absorption; that is, when the resulting fluid was subjected to further individual absorptions with the three reactive cell-types, the presence of the three antibody fractions could be demonstrated (in the same manner as for the original antiserum in table 2).

Apart from their association with other antigenic factors, A_4, A_5 and A_6 have been found to exist only in certain combinations; namely, A_6, A_4A_6 and $A_4A_5A_6$ (table 2), henceforth designated as antigens A_6, A_{46} and A_{456}. By absorbing antiserum (R428(R439) with the cell-types possessing known antigenic combinations it was possible to prepare only three reagents—A5, A45 and A456. Reagents specific for the A_4 or A_6 antigenic factors could not be prepared from antiserum R428(R439) because the cell-types or combinations of types, A_5A_6 and A_4A_5, with which absorption would be necessary for the preparation of such reagents, have not been found.

The remainder of the reagents used in testing the cells of the progeny from the matings to be presented subsequently were all prepared in a manner essentially the same as that discussed above, and the presentation of the details of their preparation does not seem warranted in this report.

GENETIC RESULTS

The Genetic Relations of the Genes Effecting the A Group of Antigens

Initially, when the work involved primarily serological techniques, each reagent was designated by a different letter of the alphabet, on the assumption that the various reagents were reacting with independently inherited antigens. However, as soon as data on genetic segregation were obtained, it was found that the antigens with which the reagents reacted were frequently associated in complexes; that is, the ability of the cells of the progeny from certain matings to react with one reagent was perfectly correlated with their ability to react with a second reagent. For example, when male 2758, whose cells reacted with both the A2 and A3 reagents, was mated with hens W30, W31, W32, W65 and W68, whose cells likewise reacted with the A2 and A3 reagents, two kinds of progeny resulted—35 individuals whose cells reacted with both of the reagents and 13 individuals whose cells failed to react with either of the reagents.

Each dam produced at least one chick whose cells were non-reactive. The mating of these hens with this male constitutes mating 1 of table 3. A single pair of allelic characters and therefore, a single pair of genes is sufficient to account for this simple 3:1 ratio. These proposed alleles were given the locus designation *A*, and the two reagents were thereafter designated A with numbers following the letter to distinguish them—A2 and A3 being used as symbols to represent these two serologically distinct reagents. The allele having the

TABLE 3

The distribution of the progeny resulting from parents selected on the basis of the reaction of their cells with the A reagents.

MATING	PROPOSED GENOTYPE OF PARENTS	ANTIGENS POSSESSED BY THE PROGENY							
		a	A_{23}	A_{123}	A_{1236}	A_{2346}	A_{23456}	A_{237}	A_8
1	$A^{23}/a \times A^{23}/a$	13	35	—	—	—	—	—	—
2	$A^{234(56)*}/a \times a/a$	4	—	—	—	—	6	—	—
3	$A^{23456}/a \times a/a$	11	—	—	—	—	9	—	—
4	$A^{(23)456}/a \times A^{(23)}/A^{(23)}$ or $A^{(23)}/a$	87	—	—	—	—	84	—	—
5	$A^{1(2)3}/A^{(2)3456} \times A^{(2)3}/A^{(2)3}$ or $A^{(2)3}/a$	—	—	26	—	—	25	—	—
6	$A^{123}/A^{23} \times a/a$	—	2	4	—	—	—	—	—
7	$A^{1(2)36}/A^{(2)346} \times A^{(2)3}/a$ or $A^{(2)3}/A^{(2)3}$	—	—	—	37	48	—	—	—
8	$A^{(23)456}/A^{(23)7} \times A^{(23)}/A^{(23)}$	—	—	—	—	—	43	46	—
9	$A^{(23)4.6}/A^{8} \times A^{(237)}/A^{(23)}$	—	—	—	—	—	11	—	10

* When a digit of the superscript is enclosed in parenthesis, it indicates that the reagent detecting that particular antigenic component in the parent was not used in testing the cells of the progeny.

recognizable antigenic effect was given a numerical superscript indicating the reagents with which its antigenic product reacted; for example, since the cells of male 2758 and each of the hens W30, W31, W32, W65, and W68, reacted with the reagents A2 and A3, they were assigned the genotype A^{23}/a, *a* being the designation for any gene or genes at the *A* locus for which no antigenic effect had been recognized.

During this same early stage of the work, evidence was obtained that the ability of cells to react simultaneously with the A2, A3 and A4 reagents was probably also determined by a single gene, or at least by a chromosome unit failing to fractionate at segregation. This evidence consisted of the agglutination reactions obtained with ten progeny resulting from the mating of male 2712 (whose cells reacted with reagents A2, A3 and A4) with females P178 and P179 (the cells of neither of which were reactive with any of the three reagents). Mating 2 of table 3 represents the data obtained from these two matings. Among the ten progeny only two types were obtained; six whose cells reacted with all three of the A reagents (A2, A3 and A4) and four whose cells were not agglutinated by any of them. Thus, tentatively at least, it seemed plausible to assign all three reactions to the product of a single gene; this gene was designated A^{234}. Further work disclosed the ability of the pre-

sumed antigenic product of this gene, A^{234}, to react with the two other fractions of antibody, A5 and A6. From the same type of mating as that just considered, male R426 (A^{23456}/a) mated to female P827(a/a) (mating 3 of table 6), there resulted 20 progeny, which, when tested with reagents A2, A3, A5, A45, and A456, fell into two distinct classes—nine individuals whose cells reacted with all five reagents and 11 whose cells did not react with any of them. The 11 negative individuals are of special importance since they represent gametes resulting from simultaneous segregation toward one pole during the reduction division of genic material having to do with the production of the five antigenic components. If any breakdown had occurred in the apparently constant association of the five antigenic components, individuals would be expected whose cells were capable of agglutination with only a portion of the five reagents employed. The nine individuals of the "positive" class resulting from this mating showed definitely that the A_2, A_3 and A_5 factors segregated together, but whether or not the factors A_4 and A_6 were also present could not be determined, due to the fact that the reagents A45 and A456, available for the detection of these factors, also contained antibody for the A_5 factor. For example, if the antigenic complex A_{235} had appeared in the cells of certain progeny, it would have reacted with reagents A45 and A456 by virtue of the A_5 antigenic component. However, any of the progeny whose cells contained factors A_4, A_6 or A_{46} in the absence of A_5 would have been readily identified as antigenic variants by the reagents employed in testing the progeny.

Additional progeny from the mating of heterozygotes (A^{23456}/a) with homozygotes (a/a) were not obtained, seemingly due to the low fecundity of the homozygous type. However, numerous individuals carrying the A^{23} allele, usually in the heterozygous condition, were available for use in matings with individuals of the A^{23456}/a genotype. Mating 4 of table 3 gives the data obtained from testing the progeny resulting from the mating of male R426 (A^{23456}/a) to hens whose cells reacted only with the A2 and A3 reagents. Three of these hens carried the A^{23} allele in heterozygous condition (A^{23}/a) while two appeared to be homozygous for A^{23}. The cells of all of the progeny were tested with reagents A5, A45 and A456, but they were not tested with reagents A2 or A3 (in table 3 any antigenic factor possessed by a parent and not tested for in the progeny is enclosed by parenthesis in the proposed genotype of the parent). As a result of testing their red cells for agglutination, the progeny fell into two classes—87 non-reactors and 84 whose cells reacted with all three reagents. As pointed out above, in direct agglutination tests employing the available reagents the antigenic factors A_4 and A_6 could not be detected in the presence of the A_5 factor; therefore, in order to determine the extent to which the "positive" segregates of mating 4 received all three of the components (A_4, A_5 and A_6), the cells of 26 of these segregates, chosen at random, were used individually to absorb the A456 reagent. In every instance the cells of each of the 26 positively reacting segregates removed the three antibody fractions, as evidenced by the failure of the absorbed fluids to agglutinate the cells of male R439, against which these antibody fractions were originally produced in immunization. Therefore, the cells of at least 26 of the 84 positively reacting

segregates contained *in toto* the antigenic factors A_4, A_5 and A_6.

In mating 5 ($A^{123}/A^{23456} \times A^{23}/A^{23}$ or A^{23}/a) of table 3 the allele A^{123} is introduced. The cells of the 51 progeny resulting from this mating were tested with reagents A1, A3, A5, A45 and A456. (The reagent A2 was not available in sufficient quantity to allow its use in testing these birds.) Here again, although tested with five serologically different reagents, the progeny fell into only two groups; 26 of them reacting with reagents A1 and A3 and 25 reacting with reagents A3, A5, A45 and A456. Of the 25 individuals belonging to the latter class, 17 were used in absorbing the A456 reagent in the same manner as was done with a portion of the positively reacting progeny from mating 4. Here, also, no antibodies remained after absorption, showing that the blood of each individual used in absorption possessed the entire A_{456} complex. The data from matings 4 and 5 indicate strongly that the genic material responsible for the appearance of the A_{456} antigenic complex is unitary—presumably a single gene.

Further serological characterization of the antigenic substance produced by the allele responsible for the A_1 factor is afforded by the agglutination reactions obtained with the cells of the progeny resulting from mating 6 ($A^{123}/A^{23} \times a/a$). The ability of the four chicks receiving the A_1 factor, presumably of the genotype A^{123}/a, to react also with the A2 and A3 reagents indicates that the antigen produced by this allele also possessed the capacity to react with the A2 and A3 reagents. Additional evidence for the multiple serological properties of the substance produced by the A^{123} allele is afforded by hen P720, whose cells were agglutinable by reagents A1, A2 and A3 and who was proven to have the genotype A^{123}/a. When mated to male 2776 (A^{23456}/A^{23}), this hen produced among her progeny three females R407 (A^{23}/a), R408 (A^{23456}/a) and R411 (A^{23456}/a). Each of these three birds was shown to carry the a allele by virtue of the a/a progeny which resulted when they were mated to male R426(A^{23456}/a). In view of these findings it seems reasonably certain that the allele A^{123} produces an antigen capable of reacting with the reagents A1, A2 and A3.

Two additional antigenic complexes probably effected by genes at the A locus were derived from New Hampshire male 2738. As given in mating 7 of table 3, the probable genotype of this male was A^{1236}/A^{2346}. This male was placed in a mating pen with ten females, five of which were known as a result of a previous progeny test to possess the genotype A^{23}/a; the remaining five hens could have had either genotype A^{23}/a or A^{23}/A^{23}, although none of them were proven to be of the latter type. The ten dams were either sibs or half sibs, and since all those adequately progeny-tested had been proven to be heterozygous, it is likely that most, if not all, of those with indefinite genotypes were also of the A^{23}/a type. The progeny from this mating were tested with reagents A1, A3, A45 and A456. Since at least half of the dams were transmitting the a allele, it is not likely that the universal presence of the A_3 factor in the cells of the 85 progeny could be accounted for by the A^{23} allele derived only from the dams; it is highly probable that both alleles transmitted by male 2738 were capable of producing the A_3 factor. Rather conclusive evidence that

both of the alleles carried by the male 2738 produced antigens capable of reacting with the A2 and A3 reagents consists of data collected on four chicks before the A_5 and A_6 factors had been recognized. These chicks resulted from the mating of male 2738 (A^{1236}/A^{2346}) with a hen of the genotype a/a. All four of the chicks reacted with both the A2 and A3 reagents; the cells of two of the chicks were positive with the A4 reagent while those of the other two were positive with the A1 reagent. These results can most satisfactorily be accounted for by assuming that the genotypes of the chicks were either $A^{123(6)}/a$ or $A^{234(6)}/a$; as shown above these appear to constitute the two segregating genotypes found among the progeny of male 2738.

Due to the serological similarity of the antigens produced by the alleles A^{1236} and A^{2346} with the antigens produced by the A alleles, it is highly probable that they also occupy the A locus, but because of the lack of matings between birds of appropriate genotypes genetic proof of the allelic relationship has not been obtained.

The most recently identified alleles of the A series are A^{237} and A^8. From mating 8 ($A^{23456}/A^{237} \times A^{23}/A^{23}$) there resulted 43 progeny whose cells were reactive with the A456 reagent and 46 progeny whose cells were reactive with the A7 reagent, indicating that the genes responsible for the presence of these two serologically distinct substances were segregating in a manner characteristic of alleles. Likewise, in observing the antigenic constitution of the 21 progeny from mating 9 ($A^{23456}/A^8 \times A^{237}/A^{23}$) it is evident that the 11 progeny positive with the A456 reagent and the 10 progeny positive with the A8 reagent indicate an allelic relationship of the causative genes. That the antigenic substance reactive with the A7 reagent is also capable of reacting with the A2 and A3 reagents is indicated by the positive reactions of these reagents with the cells of three individuals of the genotype A^{237}/A^8 resulting from mating 9. Since the reagent detecting the presence of antigen A_8 was not available until recently, the gene responsible for the production of this antigen previously constituted one of the alleles designated by the symbol a, given to any A allele for which no antigenic effect has been detected.

The antigenic combination A_{3456} has been found in only two sisters, I992 and I993, neither or which produced progeny; genetic proof, therefore, that this antigenic combination is due to a gene of the A locus is lacking. However, in view of the particular absorptive capacity and cross reactivity of this antigen with the A reagents, it is clear that these two birds possessed an A allele different from those present in the cells of the other birds utilized in these studies.

Genetic proof that the antigenic complex A_{23} is produced by an A allele is also lacking at the present time, largely due to the difficulty of setting up appropriate matings for testing allelism when the antigenic factors A_2 and A_3 occur in most of the antigens produced by this series of alleles. With the recent discovery of the allele A^8 it is likely that the desired segregation data can be obtained. However, the serological similarity of the A_{23} antigenic substance with those produced by the other members of the A locus indicates strongly that its causative gene is a member of the A series of alleles.

The Genetic Relations of the Genes Effecting the B Antigens

A second series of antigens, effected by genes occupying a locus different from that of the *A* alleles, has been tentatively designated by the symbols B_{15}, B_{25}, B_3 and B_4. Evidence indicating the allelic nature of the genes effecting these antigens is presented in table 4. In each mating listed in the table the cells of one of the parents gave positive reactions with each of two B reagents, that is, one parent carried genes effecting two B antigens; while the other parent of each mating, represented by the genotype *b/b*, was negative with the

TABLE 4

The distribution of the progeny resulting from parents selected on the basis of the reaction of their cells with the B reagents.

MATING	PROPOSED GENOTYPE OF PARENTS	ANTIGENS POSSESSED BY THE PROGENY				
		b	B_{15}	B_{25}	B_3	B_4
1	$B^{1(5)*}/B^{2(5)}\times b/b$	—	41	40	—	—
2	$B^{1(5)}/B^{3}\times b/b$	—	118	—	111	—
3	$B^{2(5)}/B^{3}\times b/b$	—	—	73	97	—
4	$B^{2(5)}/B^{4}\times b/b$	1(?)	—	16	—	24
5	$B^{3}/B^{4}\times b/b$	—	—	—	26	24
6	$B^{25}/B^{3}\times b/b$	—	—	14	10	—

* When a digit of the superscript is enclosed in parenthesis, it indicates that the reagent detecting that particular antigenic component was not used in testing the cells of the progeny.

(?) The parentage of this chick was doubtful; for explanation, see text.

same two reagents. Neglecting for the present the doubtful exception to be considered later, it may be seen that from each mating listed in table 4 there resulted only progeny possessing one or the other of the two antigens characterizing the first parent. For example, when the cells of the progeny of mating 1 ($B^{15}/B^{25}\times b/b$) were tested with reagents B1 and B2, 41 offspring were reactive with the B1 reagent and 40 were reactive with the B2 reagent. The appearance of the two contrasting classes among the progeny indicates that the genes controlling the ability of the cells to react with the reagents were segregating in a manner characteristic of alleles. Likewise the progeny resulting from mating 2 ($B^{15}/B^{3}\times b/b$) fell into two classes when tested with the B1 and B3 reagents. Of the 229 offspring from this mating, 118 possessed the antigen B_{15} and 111 possessed the antigen B_3—again indicating an allelic relationship of the causative genes. From these allelic relationships of B^{15} with both B^{25} and B^{3}, it can be concluded that B^{25} and B^{3} are allelic. The classes of offspring resulting from mating 3 ($B^{25}/B^{3}\times b/b$)—73 progeny reactive with the B2 reagent and 97 reactive with the B3 reagent—support the above proposal that the genes effecting antigens B_{25} and B_3 belong to the same allelic series. In the same manner the classes of progeny resulting from matings 4 ($B^{25}/B^{4}\times b/b$) and 5 ($B^{3}/B^{4}\times b/b$) indicate that the gene producing the B_4 antigen also belongs to the *B* series of alleles.

Among the progeny resulting from mating 4 of table 4, there was one bird which failed to react, even after repeated testing, with either the B2 or B4 reagents. If it could be proved that this individual actually resulted from the mating to which it was assigned, the validity of the hypothesis regarding the allelic relationship of the genes producing the B antigens would be doubtful. Hen R409, the supposed dam of this chick, was the only hen of genotype B^{25}/B^4 in a pen mating of seven birds. By assigning an egg of any other hen to R409 the discrepant chick could be accounted for. Furthermore, if the chick had hatched from an egg laid by R409, it would have had a probability of 0.5 of receiving the A^{123} allele from the dam since she was heterozygous for this allele; however, when tested with the A1 reagent, the cells of this chick were not reactive. Had this chick possessed the antigen A_{123}, thus confirming that R409 was the dam, it would have constituted serious evidence against the proposed allelic relationship of the genes producing the antigens B_{25} and B_4.

Until recently, largely due to the manner in which the B reagents were prepared, each allele of the B series of antigens appeared to produce only one antigenic factor. This apparent simplicity probably resulted from the particular combinations of birds used in producing and absorbing the antisera. For example, in preparing the B4 reagent, cells containing antigen B_4 were injected into an individual possessing antigen B_3; this meant that no antibodies could be produced against any antigenic factor common to both the B_3 and B_4 antigens, since a recipient can not produce antibodies reactive with antigens present in its own cells. Further, in the preparation of the B4 reagent from the above antiserum, any antibodies engendered against B_4 which were also cross-reactive with the B_1 and B_2 antigenic factors were removed by absorption with the cells of a bird of the genotype B^1/B^2. As a result, any antibodies present in the B4 reagent were highly specific for the antigen determined by the B^4 allele.

The only component thus far shared between antigens produced by alleles of the *B* locus is the B_5 factor which is common to B_{15} and B_{25}. This common component or factor was detected with antibodies produced as a result of the injection of cells containing the antigen originally termed B_1 into a bird possessing the antigen B_3. When this antiserum was absorbed with the cells of certain individuals, antibodies remained which were reactive with cells containing both antigens B_1 and B_2, as they were then designated. When the cells from an individual possessing the homologous antigen B_1 were used in absorbing this fluid, no antibodies remained. However, when this fluid, reactive with both the B_1 and B_2 antigens, was absorbed with cells possessing the B_2 antigen, there remained only antibodies for the B_1 antigen. Thus, the reagent B15 contained an antibody fraction, designated as B5, which was reactive with both antigens B_1 and B_2 as well as a fraction specific for the antigen B_1. To indicate the common antigenic component, the symbols for the antigens B_1 and B_2 were changed to B_{15} and B_{25} respectively.

Birds of the same genotype as proposed for mating 3 were used in mating 6 ($B^{25}/B^3 \times b/b$), but they are listed separately in table 4 because the progeny were tested with the B15 reagent in addition to the B2 and B3 reagents. When tested with the three reagents, the progeny from mating 6 were found to con-

sist of 14 birds whose cells were reactive with both the B15 and B2 reagents and 10 birds whose cells were reactive with only the B3 reagent. This is tentative evidence that the B_2 and B_5 antigenic factors are produced by a single gene. Further investigation may reveal other antigenic factors common to the antigens determined by the various *B* alleles.

The Genetic Relationships of the A and B Loci

The chromosomal relationship between the *A* and *B* loci is indicated by the data presented in table 5. This table shows the distribution of the classes of progeny resulting from the mating of male R426 of the genotype A^{23456}/a B^{15}/B^3 with females which can be represented by the genotype a/a b/b, since the dams were negative with the reagents A5, A45, A456, B1 and B3 with which the progeny were tested. That is, even though the dams carried the allele A^{23}, they can be represented as a/a for the sake of convenience since the

TABLE 5

*The progeny resulting from the mating of male R426 of genotype A^{23456}/a B^{15}/B^3 with females of genotype a/a b/b.**

AGGLUTINATION OF CELLS OF PROGENY WITH REAGENTS:					NUMBER IN EACH CLASS	PROPOSED GENOTYPES
A5	A45	A456	B1	B3		
+	+	+	+	0	45	A^{23456}/a B^{15}/b
+	+	+	0	+	43	A^{23456}/a B^3/b
0	0	0	+	0	49	a/a B^{15}/b
0	0	0	0	+	48	a/a B^3/b

* The genotype a/a b/b indicates that the cells of the dams did not react with any of the reagents (A5, A45, A456, B1 and B3) used in testing the progeny. The actual dams and their genotypes were R407 and R408 (A^{23}/a B^4/b), R443 and R450 (A^{23}/A^{23} b/b), and W65 (A^{23}/a b/b). The symbols (+) and (0) indicate the presence and absence of agglutination, respectively.

progeny were not tested with the reagents A2 or A3. Also, two of the hens actually carried a third *B* allele, B^4, for which the progeny were not tested.

The 185 progeny from the above mating fell into four equally frequent classes, presumably of the following genotypes: A^{23456}/a B^{15}/b, A^{23456}/a B^3/b, a/a B^{15}/b, and a/a B^3/b (table 5). These data indicate that the genes at the *A* and *B* loci segregated independently. This means that the loci are on different chromosomes or that the two loci are separated on the same chromosome by 50 or more cross-over units. Because of the large number of chromosomes (40 pairs, reported by MILLER, 1938) and the small size of most of them, the possibility of loose linkage is relatively remote, and until both of these loci can be shown to be linked to a third locus, it may be assumed that they are located on different chromosomes.

Table 6 gives the reactions obtained with the cells of individuals possessing representative genotypes, which in each instance were determined by progeny test. However, by considering the pedigree in conjunction with the agglutina-

tion tests on the cells of the progeny, it was frequently possible to establish the presumed genotype of the offspring without resorting to the progeny test. For example, the genotypes of the progeny from the mating of male 2776 ($A^{23456}/A^{23}\ B^3/B^4$) with female P827 ($a/a\ B^{15}/B^{25}$) could be determined by simply testing their cells with the appropriate reagents (A2, A3, A456, B1, B2, B3 and B4). At the *A* locus the offspring would be either A^{23456}/a or A^{23}/a, and at the *B* locus they would be heterozygous for alleles effecting independently recognizable antigens—B^{15}/B^3, B^{15}/B^4, B^{25}/B^3, or B^{25}/B^4. Thus, by making appropriate matings it was possible to determine directly the genotype of the progeny by testing their red cells with suitable serological reagents.

The *A* and *B* loci are autosomal; this was shown by the ability of either

TABLE 6

The reactions obtained when the cells from birds of various genotypes were tested with the A and B reagents.

BIRDS	PROPOSED GENOTYPE	AGGLUTINATION OF CELLS WITH REAGENTS:										
		A1	A2	A3	A5	A45	A456	B1	B2	B3	B4	B15
2712*	$A^{23456}/a\ B^{15}/B^{25}$	0	+	+	+	+	+	+	+	0	0	+
2738*	$A^{1236}/A^{2346}\ b/b$	+	+	+	0	+	+	0	0	0	0	0
2758*	$A^{23}/a\ B^{15}/b$	0	+	+	0	0	0	+	0	0	0	+
2776*	$A^{23456}/A^{23}\ B^3/B^4$	0	+	+	+	+	+	0	0	+	+	0
P178	$a/a\ B^{25}/b$	0	0	0	0	0	0	0	+	0	0	+
P827	$a/a\ B^{15}/B^{25}$	0	0	0	0	0	0	+	+	0	0	+
R404	$A^{123}/A^{23456}\ B^3/b$	+	+	+	+	+	+	0	0	+	0	0
R405	$A^{23}/a\ B^{15}/B^4$	0	+	+	0	0	0	+	0	0	+	+
R407	$A^{23}/a\ B^4/b$	0	+	+	0	0	0	0	0	0	+	0
R409	$A^{123}/A^{23456}\ B^{25}/B^4$	+	+	+	+	+	+	0	+	0	+	+
R426*	$A^{23456}/a\ B^{15}/B^3$	0	+	+	+	+	+	+	0	+	0	+
R427*	$A^{23}/A^{23}\ B^{25}/B^3$	0	+	+	0	0	0	0	+	+	0	+
R439*	$A^{123}/A^{23456}\ B^4/b$	+	+	+	+	+	+	0	0	0	+	0

* Indicates that the bird is a male.
The symbols (+) and (0) indicate the presence and absence of agglutination, respectively.

parent to transmit with equal frequency the genes at either locus to both sexes of the progeny, as indicated by the genotypes (determined by progeny test) of the males and females of table 6.

Possible linkage relationships between the *A* or *B* loci and the loci of other known autosomal marker genes have been investigated only with regard to dominant white (*I*). From the mating of a white crossbred female of the genotype $A^{23456}/A^{23}\ B^3/b\ I/i$ to a Barred Plymouth Rock male of the genotype $A^{23}/A^8\ B^{15}/b\ i/i$ there resulted 36 progeny in which dominant white (*I*) segregated with respect to the *A* and *B* loci in a manner not significantly different from that expected as a result of independent assortment. Thus, even though the number of progeny was small, it can be tentatively concluded that the locus for dominant white is on a third independent chromosome, although the present data do not exclude the possibility of loose linkage.

DISCUSSION

The apparent serological complexity of the "A" series of agglutinogens (A_{23}, A_{123}, A_{23456}, etc.) may be accounted for, according to one explanation, by

assuming that the antigenic factors (A_1, A_2, A_3, etc.) are each due to individual genes, very closely linked so that the various antigenic factors appear to be inherited in certain constant combinations. For example, the "antigenic complex" A_{123} may be inherited as a unit because of very close linkage between the genes producing each of the antigenic factors A_1, A_2 and A_3. This hypothesis presupposes that crossing over does occur between the genes responsible for these antigenic factors and should eventually be observed. Cases of closely linked genes having similar effects have been reported in Drosophila (LEWIS 1945, 1948; and GREEN and GREEN 1949), maize (LAUGHNAN 1949), cotton (STEPHENS 1948, YU and CHANG 1948) and mice (DUNN and CASPARI 1945). On the other hand, the data obtained in these studies may be explained rather simply by hypothesizing that each "antigenic complex" is produced by one of a series of alleles. For example, the single gene, A^{123}, produces an antigenic substance or substances capable of reacting with each of the serological reagents—A1, A2 and A3.

The multiple allele hypothesis could account for the antigenic complexity in either of two ways, depending on the type of gene action presumed to be predominant. Pleiotropic activity on the part of the individual alleles of the series may be assumed; that is, each allele is capable of producing several particulate antigenic substances. For example, allele A^{123} could be viewed as producing three separate antigens—A_1, A_2 and A_3—each of which is reactive with a different reagent. The other type of gene action by which a series of alleles could account for the A series of agglutinogens is based upon the assumption that each allele produces a particulate antigenic substance which is very similar to, yet chemically distinct from, the antigenic substances produced by the other members of the series. This means that a reagent prepared against the antigenic substance produced by one member of the allelic series will frequently cross-react with the antigens produced by one or more of the remaining alleles.

Fundamental work on the specificity of antigens and antibodies (reviewed by LANDSTEINER 1945) shows that in response to the injection of a relatively simple conjugated antigen there results a multiplicity of antibodies of varying specificity, all of which are reactive with the homologous antigen, but show graded affinity for chemically related substances. That an array of antibodies of varying specificity may be produced against a natural antigen is clearly demonstrated by the results obtained by PERLMAN and GOEBEL (1946) with highly purified somatic antigens of the Flexner group of dysentery bacilli. Considering these findings along with the similar effects characteristically produced by allelic genes, it is reasonable to expect that certain portions of the array of antibodies formed against an antigen produced by one allele would frequently cross-react with antigens determined by other alleles of the same series. For example, as a result of the injection of red cells containing antigen A_{23456} into an individual whose cells possessed A_{23}, there were formed antibodies which, in addition to agglutinating cells possessing the immunizing antigen A_{23456}, reacted with cells possessing the antigen A_{1236} or A_{2346}. Thus, the apparent antigenic complexity of many red cell antigens may simply reflect the serological phenomena associated with the production of antibodies and their ensuing cross-reactivity with similar, yet chemically distinct, antigens.

Groups of antigens resembling in serological complexity those reported here for the chicken are known to exist in other species of mammals and birds. In man the linked gene hypothesis has been advanced by FISHER (1944) and FISHER and RACE (1946) to account for the serological complexity of the Rh blood group factors. On the other hand, the complexity of the Rh group has been accounted for on the basis of multiple allelic genes by WIENER and LANDSTEINER (1943) and WIENER (1943, 1944, 1949). The recent studies of WIENER and GORDON (1949) and WIENER, UNDER and MAZZARINO (1949) on the rh^w factor, a new variant of the Rh group, strongly indicate that the type of serological and genetic diversity displayed by the Rh factors is very similar to, if not identical with, the antigenic characters reported in this paper. The discovery of a third antigenic factor (S) belonging to the MN group of agglutinogens of man (WALSH and MONTGOMERY 1947, SANGER and RACE 1947) has prompted RACE, SANGER, LAWLER, and BERTINSHAW (1949) to prefer the linked gene hypothesis over the multiple allele explanation in accounting for the four constantly inherited antigenic combinations M, MS, N and NS, although the latter hypothesis appears to be simpler and adequate to explain the results obtained.

In cattle there exist at least two series of serologically related antigens for which either the linkage or multiple allele hypothesis could be advanced; however, in view of the consistency with which the various combinations of antigenic factors are inherited as units the multiple allele hypothesis has been given preference by STORMONT, IRWIN and OWEN (1945) and STORMONT, OWEN and IRWIN (in press). McGIBBON (1944, 1945) has reported that a group of serologically related antigens also exists in Muscovy ducks. Here, as with the other species mentioned, the antigenic factors appeared to be inherited only in certain combinations, indicating that unitary inheritance, regardless of species, of antigenic factor combinations seems to be a universal rule applicable to all of the extensively studied groups of serologically related red cell antigens.

In view of this common finding, together with the known facts relating to the diversity of antibody production and the cross-reactivity of antibodies with substances similar to the homologous antigen, it is felt that the multiple allele interpretation offers the most acceptable working hypothesis on which to base future work on the cellular antigens of the chicken.

SUMMARY

These studies show that two independently inherited groups of agglutinogens are present in the erythrocytes of the chicken. In view of the serological and genetic data presented, it has been hypothesized that the antigens so far detected are determined by genes belonging to one or the other of two autosomal series of multiple alleles. One of these consists of 9 and the other of 5 members. The type of action displayed by the alleles of each series indicates a complete lack of dominance, in that each allele produces its particular antigenic substance, regardless of the other alleles that may be present.

The antigenic products of many of the alleles have multiple serological properties. To facilitate discussion of this complex situation the total antigenic

product of each allele has been uniformly referred to as an antigen and the serological components which appear to constitute the antigens are designated as "antigenic factors." It is thought that these factors as they appear in the antigens produced by various alleles are probably indicative only of serological similarity existing between respective gene products and do not in themselves represent separate and discrete antigenic units.

LITERATURE CITED

BOYD, W. C., and O. E. ALLEY, 1940 Individual blood differences in chickens. J. Hered. **31**: 135–136.

DUNN, L. C., and E. CASPARI, 1945 A case of neighboring loci with similar effects. Genetics **30**: 543–568.

EHRLICH, P., and J. MORGENROTH, 1900 Ueber Haemolysine (III. Mitteilung). Berl. klin. Wschr. pp. 453–458.

FISCHER, R. A., 1944 Cited by R. R. Race: An "incomplete" antibody in human serum. Nature **153**: 771–772.

FISHER, R. A., and R. R. RACE, 1946 *Rh* gene frequencies in Britain. Nature **157**: 48–49.

GREEN, M. M., and K. C. GREEN, 1949 Crossing-over between alleles at the lozenge locus in *Drosophila melanogaster*. Proc. nat. Acad. Sci. **35**: 586–591.

KOZELKA, A. W., 1933 Individuality of the red blood cells of inbred strains of fowls. J. Immunol. **24**: 519–530.

LANDSTEINER, K., 1900 Zur Kenntniss der antifermentativen, lytischen und agglutinierenden Wirkungen des Blutserums und der Lymphe. Zbl. Bakt. **27**: 357–362.

1901 Ueber Agglutinationserscheinungen normalen menschlichen Blutes. Wien. klin. Wschr. **14**: 1132–1134.

1945 The Specificity of Serological Reactions. xiv+310 pp. Cambridge: Harvard Univ. Press.

LANDSTEINER, K., and P. LEVINE, 1932 On individual differences in chicken blood. Proc. Soc. exp. Biol., N. Y. **30**: 209–212.

LANDSTEINER, K., and C. P. MILLER, 1924 On individual differences in chicken blood. Proc. Soc. exp. Biol. Med. **22**: 100–102.

LAUGHNAN, J. R., 1949 The action of allelic forms of the gene *A* in maize. II. The relation of crossing over to mutation of A^b. Proc. nat. Acad. Sci. **35**: 167–178.

LEWIS, E. B., 1945 The relation of repeats to position effect in *Drosophila melanogaster*. Genetics **30**: 137–166.

1948 Pseudo-allelism in *Drosophila melanogaster*. Genetics **33**: 113.

MCGIBBON, W. H., 1944 Cellular antigens in species and species hybrids in ducks. Genetics **29**: 407–419.

1945 Further division of contrasting antigens in species hybrids in ducks. Genetics **30**: 252–265.

MILLER, R. A., 1938 Spermatogenesis in a sex reversed female and in normal males of the domestic fowl, *Gallus domesticus*. Anat. Rec. **70**: 155–189.

PERLMAN, E., and W. F. GOEBEL, 1946 Studies on the Flexner group of dysentery bacilli. V. A quantitative study of the serological cross-reactions. J. exp. Med. **84**: 235–245.

RACE, R. R., RUTH SANGER, SYLVIA D. LAWLER and DOREEN BERTINSHAW, 1949 The inheritance of the MNS blood groups: a second series of families. Heredity **3**: 205–213.

SANGER, RUTH, and R. R. RACE, 1947 Subdivisions of the MN blood groups in man. Nature **160**: 505.

STEPHENS, S. G., 1948 A biochemical basis for the pseudo-allelic anthocyanin series in Gossypium. Genetics **33**: 191–214.

STORMONT, C., M. R. IRWIN, and R. D. OWEN, 1945 A probable allelic series of genes affecting cellular antigens in cattle. Genetics **30**: 25–26.

STORMONT, C., R. D. OWEN, and M. R. IRWIN, 1951 The B and C systems of bovine blood groups. Genetics **36**: (in press).

THOMSEN, O., 1934 Untersuchungen über erbliche Blutgruppenantigene bei Hühnern. Hereditas **19:** 243–258.

1936 Untersuchungen über erbliche Blutgruppenantigene bei Hühnern. II. Hereditas **22:** 129–144.

TODD, C., 1930 Cellular individuality in the higher animals, with special reference to the individuality of the red blood corpuscle. Proc. Roy. Soc. **106:** 20–44.

1931 Cellular individuality in the higher animals, with special reference to the individuality of the red blood corpuscle. II. Proc. Roy. Soc. **107:** 197–205.

1935 Cellular individuality in the higher animals, with special reference to the individuality of the red blood corpuscle. III. Proc. Roy. Soc. **117:** 358–366.

TODD, C., and R. G. WHITE, 1910 On the haemolytic immune isolysins of the ox and their relation to the question of individuality and blood-relationship. J. Hyg. Camb. **10:** 185–195.

WALSH, R. J., and CARMEL MONTGOMERY, 1947 A new human iso-agglutinin subdividing the MN blood groups. Nature **160:** 504.

WIENER, A. S., 1934 Individuality of the blood in higher animals. II. Agglutinogens in red blood cells of fowls. J. Genet. **29:** 1–8.

1943 Genetic theory of the Rh blood types. Proc. Soc. exp. Biol., N. Y. **54:** 316–319.

1944 The Rh series of allelic genes. Science **100:** 595–597.

1949 Heredity of the Rh blood types. VII. Additional family studies with special reference to the genes R^z and r^y. Proc. 8th Int. Congr. Genet. 500–519.

WIENER, A. S., and E. B. GORDON, 1949 Studies on the blood factor rh^w. Amer. J. clin. Path. **19:** 621–629.

WIENER, A. S., and K. LANDSTEINER, 1943 Heredity of variants of the Rh type. Proc. Soc. exp. Biol., N. Y. **53:** 167–170.

WIENER, A. S., L. J. UNGER, and C. A. MAZZARINO, 1949 Further studies on the rh^w factor. Amer. J. clin. Path. **19:** 779–781.

YU, C. P., and T. S. CHANG, 1948 Further studies on the inheritance of anthocyanin pigmentation in Asiatic cotton. J. Genet. **49:** 46–56.