GENETIC STUDIES OF CELLULAR ANTIGENS IN THE CHICKEN¹

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Received June 26, 1956

LINKAGE involving a gene for a cellular antigen was first reported by SAWIN, GRIFFITH and STUART (1944) for brachydactyly and the A agglutinin in rabbits. In chickens a recombination frequency of 39.8% was reported for the crest gene and the gene for the cellular antigen X3 (BRILES 1951). The latter report followed closely the investigations of BRILES, MCGIBBON and IRWIN (1950) which clearly demonstrated the existence of two loci with multiple alleles for cellular antigens in the chicken. The evidence for two linkage groups of genes for erythrocyte antigens in the chicken will be presented in this paper as well as data for blood group genes whose linkage relations are not known.

METHODS AND MATERIALS

In general, the procedures used in this study are similar to those of BRILES, MCGIBBON and IRWIN (1950). Reagents were obtained by differential absorption of chicken isoantisera and antisera obtained from rabbits. The titers of the reagents used in this study ranged from 1:4 to 1:64. Agglutination tests were used to detect the presence of antigen or antibody specificities following pertinent absorptions. In these tests mixtures of red blood cells and antiserum were incubated at 31°–32°C for two hours, at which time the tubes were examined for evidence of agglutination. The tubes were then kept in the refrigerator at 4°C for 18 to 24 hours and again examined. The effect of the additional period of incubation at 4°C is to increase slightly the amount of agglutination, possibly to the next or second higher score (defined below). Incubation for four hours at 31°–32°C is comparable to the additional incubation at 4°C in that the change is toward increased agglutination. Very rarely (3 out of 499 mixtures of cells and reagent) was agglutination present at four hours of incubation when it was not observed at two hours. Incubation for four hours has been used in some agglutination tests reported in this paper and is now standard practice.

Tubes in which agglutination could not be observed or in which weak agglutination occurred were rechecked microscopically. The method of scoring agglutination is similar to the one used by BRILES *et al.* (1950). The absence of a reaction is scored 0 and a score of 4 is recorded for maximum agglutination, i.e. a solid clump of all cells in the tube. Scores between zero and four are recorded for degrees of reaction less than maximum. A score of $\pm$ is used to indicate a questionable reaction which was checked microscopically. Reagents were designated by the prefix, "anti", e.g.

¹ Paper No. 623 from the Department of Genetics. This project was supported in part by grants from the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. Part of the project was carried out while the author was a post-doctorate fellow of the National Institutes of Health.

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anti-M. Antigenic substances and their causative genes were designated by the same letter with subscript numbers used to depict each allele and its antigenic product. Reagents which have not been used in progeny tests or which are in the process of being tested have been designated solely by number in the sequence of discovery. The rationale involved in the analysis of anti-sera from which reagents were obtained is similar to that used by BRILES, MCGIBBON and IRWIN (1950), the latter reference providing a detailed account of the analysis of two antisera. The details of absorptions carried out by the author will be furnished on request.

The reagents obtained were assumed to contain a single antibody specificity. Progeny tests indicated whether the antigenic substance was inherited monofactorially and therefore indirectly the unity of the antigenic substance and the serological purity of the reagent. Only progeny from dihybrid or higher order testcross matings were used since such progeny furnished a maximum amount of information with a minimum expenditure of reagent. The heterozygosity of a parent was assumed if the cells of some of its progeny were non-reactive with the reagents employed. Each reagent was tested with at least 20 progeny in two or more sire families, therefore, sampling errors involving small family size are believed to be at a minimum.

RESULTS

Specificity of reagents

Table 1 provides a summary of agglutination tests of progeny from testcrosses in which results with each reagent have been considered separately, i.e. a monohybrid testcross. The expectation from such matings is a 1:1 ratio of reactive to non-reactive progeny. The ($\pm$) class indicates doubtful cases which for the most part could not be retested. The probable causes for such weak reactions are numerous, including errors in technique, a functional disorder in the bird tested, etc. These

TABLE 1
Reactions of the cells of progeny in monohybrid testcrosses Hy:1:1

Reagents for	No. of sires	No. of dams	Phenotypic classes		
			+	0	$\pm$
M_2	3	4	63	48	
M_5	9	20	128	103	
M'_5	5	17	75	69	
O_z	10	37	133	127	
Q_4	7	25	120	114	2
Q'_4	7	23	21	26	
S_2	7	34	104	109	
S'_2	6	14	74	72	
S_3	11	30	90	106	
2	4	18	69	67	1
5	9	25	71	102*	7
7	8	24	51	57	2
8	4	10	38	38	1
9	13	35	87	93	

* $\chi^2_1 = 5.56$, 1 df., $P = .01-.02$.

doubtful reactions do not in any way alter the conclusion that all antigenic substances but #5 appear to be inherited in a simple mendelian fashion. Each of the reagents except anti-#5 presumably detected the antigenic product of a single locus. In that sense they can be regarded as unitary for the population sampled. Generalizations to other populations cannot be made until they are tested. Reagents which are designated by primes (M_5' , etc.) are those which were found to be duplicates of reagents bearing the same letter and numeral.

In the antiserum analyses, each antiserum fraction was designated numerically, 1, 2 or 3 in sequence. Following the progeny tests the designations were altered to conform to the relations among the genes responsible for the antigenic substances; the latter designations were used throughout this paper. Antiserum fractions which could not be obtained in concentrations sufficiently high to be useful as reagents were not isolated, but still retained their initial designations.

Additional evidence for the serological purity of the reagents lay in the demonstration of their independence when comparing the results of agglutination of these reagents with the same array of bird cells. A portion of these results is presented in table 2 and a more complete table is available on request. These results confirm the identity of M_5 with M_5' , Q_4 with Q_4' and S_2 with S_2' . Reagents anti- S_3 and anti-#9 may possess a common specificity but anti- M_2 , M_5 , O_5 , Q_4 , S_2 , #2, #5, #7 and #8 appear entirely distinct one from the other and from anti- S_2 or anti-#9.

Reagents for A_7 , A_9 , B_2 and B_6 were kindly provided by W. E. BRILES of Texas Agricultural and Mechanical College. Agglutination tests with anti- Q_4 paralleled

TABLE 2
Typing tests of the flock of the Department of Poultry Husbandry

Reagents for	Part A Agglutination with cells of birds listed														Part B % of birds whose cells reacted with reagents				
	3204	3212	E295	Q791	Q832	Q928	S570	3191	A2282	C98	C2685	C4253	C4254	E137	All breeds	Barred Plymouth Rocks	New Hampshire Reds	White Plymouth Rocks	Rhode Island Reds
M_2	0	0	0	0	0	+	0	+	+	+	+	+	0	0	38.4 (112) ¹	16.0 (25)	70.9 (55)	0.0 (14)	0.0 (18)
M_5	0	+	+	0	0	0	0	0	0	0	+	0	0	0	11.6 (112)	0.0 (25)	12.1 (58)	0.0 (15)	42.9 (14)
M_5'	0	+	+	0	0	0	0	0	0	0	+	0	0	0	10.6 (113)	0.0 (25)	14.5 (62)	0.0 (13)	23.1 (13)
O_5	+	+	-	0	+	0	+	+	0	0	+	+	+	+	61.6 (112)	29.6 (27)	66.6 (54)	60.0 (15)	100.0 (16)
Q_4	+	0	0	+	+	0	0	0	0	0	0	0	+	+	57.1 (98)	87.5 (24)	51.0 (47)	45.5 (11)	37.5 (16)
Q_4'	+	0	0	+	+	0	0	0	0	0	0	+	+	+	57.5 (106)	87.5 (24)	51.7 (56)	45.5 (11)	40.0 (15)
S_2	+	+	-	+	+	0	+	0	0	0	+	0	0	+	57.9 (107)	21.4 (28)	59.2 (54)	88.8 (9)	100.0 (16)
S_2'	-	+	0	+	+	0	+	0	0	0	+	0	0	+	58.4 (101)	21.4 (28)	61.2 (49)	88.8 (9)	100.0 (12)
S_3	0	+	0	0	0	0	0	+	0	0	+	+	0	+	8.7 (104)	0.0 (24)	12.3 (49)	0.0 (14)	17.6 (17)
2	0	+	+	0	0	±	+	+	0	+	0	0	0	+	44.4 (108)	4.3 (23)	35.0 (57)	92.3 (13)	100.0 (15)
5	+	+	0	+	+	0	+	+	0	+	+	+	+	+	78.4 (102)	87.5 (24)	72.0 (50)	60.0 (10)	94.4 (18)
7	-	-	-	-	-	-	-	-	+	+	0	0	0	0	-	-	6.5 (31)	-	-
8	0	+	+	0	0	+	0	0	0	0	0	0	+	+	-	-	62.5 (8)	-	-
9	-	-	-	-	-	-	-	+	-	-	0	0	0	+	28.2 (79)	4.5 (22)	35.5 (45)	25.0 (4)	25.0 (8)
A_7	0	+	0	0	0	+	+	+	-	-	-	-	-	-	-	-	-	-	-
A_9	+	+	0	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
B_2	+	+	0	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
B_6	+	+	0	+	+	-	0	-	-	-	-	-	-	-	-	-	-	-	-

All agglutinations indicated by (+) regardless of degree of reaction. No reaction is designated by (0). Where test was not performed a dash is used.

¹ Number tested in parentheses.

those with anti- B_6 (table 2) with four exceptions in which the cells reacted with anti- B_6 but not with anti- Q_4 ; one of the latter cases is shown in table 2. These reagents cannot, therefore, be considered identical but may possess an antibody specificity in common. Additional tests will be necessary to determine the relationships between anti- A_7 , anti- A_9 , anti- B_2 and those prepared by the author. The results of typing tests with the parents used in the progeny tests are summarized in part B of table 2. The latter section shows the number tested and the frequency of reactors of the various breeds used. The cells of all of these birds were used in comparisons typified in part A of table 2.

TESTS FOR GENE INDEPENDENCE

The antigenic substances appeared to be inherited in a simple monofactorial fashion. It was, therefore, of interest to determine whether the causative genes were independent or whether any of them were linked or allelic to one another. The data presented in table 3 were obtained from dihybrid testcrosses in which the expectation for the progeny was 1:1:1:1 with an hypothesis of independence. The recombination frequency observed between M_2 and Q_4 was $5.4 \pm 4.2\%$ and between O_5 and S_2 , $2.6 \pm 2\%$, indicative of close linkage. S_2 and S_3 segregated as alleles and O_5 and S_3 segregated as if completely linked in the coupling phase. In an analogous fashion M_2 and M_5 segregated as if they were alleles. Independent segregation was noted in matings with M_3 or M_5 on the one hand and either O_5 , S_2 or S_3 , members of the second linkage group on the other hand. There appear to be a minimum of four

TABLE 3
Reactions of progeny in dihybrid testcrosses Hy:1:1:1:1

Reagents for	No. of sires	No. of dams	Phenotypic classes				$\chi^2 P$
			++	+0	0+	00	
$M_2 M_5$	1	3	0	14	15	0	< .01
$M_2 O_5$	2	3	3	3	6	7	.3-.5
$M_2 Q_4$	3	14	4	59	46	2	< .01
$M_2 S_2$	2	7	8	12	8	11	.7-.8
$M_2 S_3$	3	9	24	26	18	23	.5-.7
$M_5 O_5$	2	2	10	12	6	6	.3-.5
$M_5 S_2$	3	6	24	27	18	18	.3-.5
$M_5 S_3$	2	4	13	12	13	13	> .99
$O_5 S_2$	8	34	113	3	3	109	< .01
$O_5 S_3$	3	4	33	0	0	55	< .01
$O_5 2$	1	2	4	6	4	3	.7-.8
$O_5 7$	3	14	22	31	31	23	.3-.5
$Q_4 S_3$	1	1	14	6	21	19	.02-.05
$S_2 S_3$	1	5	0	41	39	0	< .01
$S_3 2$	2	6	15	15	10	12	.5-.7
$S_3 5$	2	3	5	8	6	5	.8-.9

++—Cells possessing both substances

+0—Cells possessing the first but not the second

0+—Cells possessing the second but not the first

00—Cells lacking both substances

loci with M_2 and Q_4 forming one linkage group and a second group comprised of O_5 and S_2 . Further studies are necessary in order to elucidate the chromosomal relations of the genes for substances #2, #5, #7, #8 and #9.

In all progeny tests made thus far, genes O_5 and S_2 have been closely associated in apparently the coupling phase of linkage with the exception of the observed crossover types. This close association is not evident from more recent progeny tests. One sire, #3212, is apparently homozygous for the O_5 locus since thirteen of his progeny possess the O_5 substance. However, there is segregation of the S_2 and S_3 substances among his progeny. Among 33 progeny in another testcross 14 possessed S_3 and 19 did not. In yet a third testcross with another group of hens, #3212 sired nine progeny five of which possessed S_2 and four did not. Whereas #3212 possessed O_5 , S_2 and S_3 the hens to which he was mated lacked one or two but not three of these substances so that a simultaneous trihybrid testcross was not possible. The grandson, #A8406 ($O_5^+S_2^+S_3^+$) of #3212, sired progeny with a similar segregation pattern. In a testcross, 61 progeny were obtained all of which possessed O_5 but segregated with respect to the S_2 and S_3 substances as follows: 28 with S_2 and 31 with S_3 . Thus there is further evidence that the O_5 and S_2 substances are genetically distinct.

In table 3 it was indicated that the linkage between the O_5 and S_2 loci was in the coupling phase and that between M_2 and Q_4 in the repulsion phase. Recently progeny tests were made of the linkage in a phase reciprocal to that indicated in table 3. A number of progeny were obtained from matings of a crossover containing O_5 but lacking S_2 with an unrelated female containing only S_2 . From this mating two males were obtained which contained both O_5 and S_2 and they in turn were mated to females lacking both substances. This constituted a testcross in which the linkage of the O_5 and S_2 genes was in the repulsion phase. The results obtained to date are shown in part A, of table 4. As expected with this small sample the progeny fall in the two noncrossover classes.

In the M_2Q_4 system two hens of the crossover type containing both M_2 and Q_4 were available for mating but only one laid fertile eggs. The results of mating the latter individual (#R999) to two males lacking M_2 and Q_4 are shown in part B, of table 4. It was expected that most of the progeny would segregate into two classes. Instead an apparent independent segregation was obtained.

Considering the closeness of linkage observed in the repulsion phase these results are surprising. It is possible that R999 contains a reciprocal translocation which would explain the observed findings. In such an event the M_2 gene would be found on a chromosome non-homologous to Q_4 and an apparently independent segregation would be obtained. Of 98 eggs obtained from R999 only 24 hatched of which only the 14 shown in table 4 were available for bleeding, the balance having died within a week after hatching, a datum which is of interest with respect to the possibility of translocation.

SEROLOGICAL PURITY, CROSSREACTION AND POSITION EFFECT

In addition to the tests of serological purity already considered the reagents which detected the crossover individuals were subjected to further analyses to determine

TABLE 4

Testcrosses of repulsion phase heterozygotes containing O_5 and S_2 and coupling phase heterozygotes containing M_2 and Q_4

A. Sires with O_5 and S_2	Dams without O_5 and S_2	Phenotypic classes			
		++	+0	+0	00
A9618	M580	0	3	3	0
A9618	R674	0	0	1	0
A9618	H1000	0	4	5	0
A9754	M578	0	1	1	0
A9754	R669	0	2	1	0
Total		0	10	11	0
B. Sires without M_2 and Q_4	Dams with M_2 and Q_4				
E1814	R999	2	2	3	4
3212	R999	0	1	2	0
Total		2	3	5	4

whether the postulated crossover progeny were merely serological artifacts, reacting with the reagent because of an antibody specificity not previously detected. The anti- M_2 reagent, for example, could be represented as containing anti- M_2 and an unknown type of antibody. If the reagents contained primarily one antibody specificity it should be possible to absorb the reagents completely with cells of birds possessing only M_2 , or in the case of anti- Q_4 with cells of birds possessing only Q_4 . Similar absorptions were carried out with reagents anti- O_5 and anti- S_2 . The results of these absorptions are summarized in table 5; the detailed tables are available on request. Following absorption of the anti- M_2 reagent with the cells of an $M_2^+Q_4^0$ bird (a bird which contained M_2 but lacked Q_4) the reagent did not react with the cells of M_2Q_4 birds. Comparable results were obtained from absorptions with anti- Q_4 , anti- O_5 and anti- S_2 . Following absorption with the cells of a bird lacking both M_2 and Q_4 or O_5 and S_2 the respective reagents were still strongly reactive with the cells of birds possessing these substances. It may be concluded that the reagents anti- M_2 , Q_4 , O_5 and S_2 did not contain additional specificities which distinguished the crossover and non-crossover classes.

Since the genes M_2 and Q_4 , and O_5 and S_2 respectively appeared to be closely linked it was of interest to determine whether crossreactivity existed between the antigenic products of the linked genes. Two criteria, cross absorption and reciprocal immunization, were employed to test for cross reactivity. With the criterion of absorption, the antigenic products of the linked genes would be considered crossreactive if each could absorb antibodies for the other antigen.

The cells of birds with the phenotype $M_2^0Q_4^+$ were used to absorb the non-homologous anti- M_2 reagent and reciprocally $M_2^+Q_4^0$ cells were used to absorb the anti- Q_4 reagent also non-homologous. Following absorption, the sera were titrated and the

TABLE 5
Summary of absorption results

Re- agents for	Absorbed with cells of	Tested with cells of							
		$M_2^+Q_4^+$ (3)*	$M_2^+Q_4^0$ (13)	$M_2^0Q_4^+$ (6)	$M_2^0Q_4^0$ (1)	$M_2^+Q_4^+$ (3)	$M_2^+Q_4^0$ (13)	$M_2^0Q_4^+$ (11)	$M_2^0Q_4^0$ (3)
Part A anti- M_2 and anti- Q_4 reagents									
M_2	$M_2^+ Q_4^0$ (2)	0	0	0	0				
	$M_2^+ Q_4^+$ (4)	+	+	0	0				
Q_4	$M_2^0 Q_4^+$ (1)	+	+	0	0				
	$M_2^+ Q_4^0$ (4)					+	0	+	0
	$M_2^0 Q_4^+$ (4)					0	0	0	0
	$M_2^+ Q_4^+$ (4)					+	0	+	0
Part B anti- Q_5 and S_2 reagents									
Re- agents for	Absorbed with cells of	Tested with cells of							
		$O_5^+S_2^+$ (17)	$O_5^+S_2^0$ (9)	$O_5^0S_2^+$ (3)	$O_5^0S_2^0$ (2)	$O_5^+S_2^+$ (14)	$O_5^+S_2^0$ (2)	$O_5^0S_2^+$ (2)	$O_5^0S_2^0$ (1)
O_5	$O_5^+ S_2^0$ (3)	0	0	0	0				
	$O_5^0 S_2^+$ (3)	+	+	0	0				
	$O_5^0 S_2^0$ (2)	+	+	0	0				
	$O_5^+ S_2^+$ (2)					+	0	+	0
S_2	$O_5^0 S_2^+$ (1)					0	0	0	0
	$O_5^0 S_2^0$ (1)					+	0	+	0

* Number of birds tested and the number of absorbed reagents used are shown in parentheses.

tested cells reacted with these sera to a degree comparable to reagents absorbed with the cells of birds lacking both M_2 and Q_4 substances. These results are summarized in table 5 and the details will be furnished on request. Absorptions of this kind were also carried out with reagents anti- O_5 and S_2 . The results obtained paralleled those from absorptions of anti- M_2 and Q_4 in that cross reactivity or antigenic similarity between O_5 and S_2 was not detected.

A further test of the specificity of the substances produced by the linked genes was afforded by reciprocal immunization between birds possessing only one of the two substances, e.g. $O_5^+S_2^0 \rightleftharpoons O_5^0S_2^+$ and $M_2^+Q_4^0 \rightleftharpoons M_2^0Q_4^+$. All birds which were immunized produced antisera which contained a number of different antibody specificities. In order to obtain a reagent specific for each of these antigenic substances differential absorptions were necessary. Duplicate reagents for each of the substances, M_2 , Q_4 , O_5 and S_2 agglutination tests gave results which paralleled those with anti- O_5 , anti- O_6 , anti- M_2 or anti- Q_4 . Since specific sera were obtained following immunization this provided evidence against the similarity or crossreactivity of antigens associated with the linked loci.

Subsequent to the matings considered in table 3 birds were obtained which were heterozygous for O_5 and S_2 . In some of these birds the genes were linked in the coupling phase and in others in the repulsion phase, thus permitting a test for the presence

of the cis-trans form of position effect. If contrasting but unknown alleles are depicted with the subscript "x", the cis form could be represented as $\frac{O_5 S_2}{O_x S_x}$ and the trans form as $\frac{O_5 S_x}{O_x S_2}$. The criterion for position effect was the amount of O_5 and S_2 substance possessed by each genotypic class. The difference in amounts were estimated by titration and absorption of reagents against O_5 and S_2 with the cells of the respective heterozygotes. No significant differences could be detected in titrations with anti- S_2 and anti- $O_5 S_2$, the latter reagent containing both specificities. A typical titration with one of the 11 repulsion phase heterozygotes with reagent anti- $O_5 S_2$ is as follows: $\frac{1}{3}$ -3; $\frac{1}{6}$ -3; $\frac{1}{12}$ -3; $\frac{1}{24}$ -2 (the fraction shows the proportion of reagent in the total volume of reagent and diluent with the integer the agglutination score at the indicated dilution). Of the three coupling phase heterozygotes, a typical reaction is the following: $\frac{1}{3}$ -4, $\frac{1}{6}$ -3, $\frac{1}{12}$ -3, $\frac{1}{24}$ -3. With the reagent for S_2 the repulsion phase heterozygote, $\frac{1}{2}$ -2, $\frac{1}{4}$ -2, $\frac{1}{8}$ -1, $\frac{1}{16}$ -0 and the coupling phase heterozygote: $\frac{1}{2}$ -3, $\frac{1}{4}$ -2, $\frac{1}{8}$ -+, $\frac{1}{16}$ -+. Nor were significant differences detected in agglutination when absorptions of anti- $O_5 S_2$ were carried out with 4 repulsion phase and 3 coupling phase heterozygotes. The details of these tests will be furnished on request. These tests indicate that detectable differences were not present in the amounts of either or both antigens (O_5 and S_2) possessed by the two classes of heterozygotes although variations in the amounts of antigen may be present within each group. In order to detect altered antigenicity or change in cross reaction of the O_5 and S_2 antigenic specificities it would be necessary to obtain birds which differ genetically only with respect to the positions of the O_5 and S_2 genes; such stocks are not now available.

DISCUSSION

Studies of linkage in man involving genes for cellular antigens have been reviewed by RACE and SANGER (1954). Among the 42 studies was the proposal of linkage between the Lutheran and Lewis loci first reported by MOHR in 1951. The data reported by MOHR included one family containing a crossover, the suspected crossing over having occurred in the father. RACE, LAWLER and PENROSE, quoted in MOHR (1954) also tested a family in which a crossover type was observed.

A number of families have been studied for the relation of the *Rh* locus with ovalocytosis, with indications that the two loci are linked (LAWLER 1954; CHALMERS and LAWLER 1953; GOODALL, *et al.* 1953; and MARSHALL, *et al.* 1954). One family was reported (LAWLER 1954) in which the segregation indicated independence of the two loci. As an explanation for this exceptional family it was suggested either that two loci were responsible for the ovalocytosis condition or some chromosomal aberration such as translocation had taken place. In a study involving four Japanese families, FUJII, MOLONEY and MORTON (1955) did not find significant evidence of linkage between ovalocytosis and the *Rh* system. Since the sample was quite small the authors make a plea for continued study of the relation between *Rh* and ovalocytosis. RENWICK and LAWLER (1955) reported on a study of families in which the nail-patella syndrome occurred. They found significant linkage between the locus for this syndrome and the *ABO* locus. As additional markers are discovered and newer statistical techniques are employed such as sequential analyses (WALD 1947; MORTON

1955) further studies will be conducted which may lead to new discoveries of linkage in man.

Attempts to find additional linkages in chickens involve the difficulty that the chicken possesses a large number of chromosomes, on the order of 39 pairs. There is the possibility, however, that in this species the genes are present exclusively in the 7 large chromosomes, the dot-like chromosomes are dispensable (NEWCOMER 1953), a condition favorable to the discovery of linkage. There are two approaches which might be more productive in the search for antigenic products of linked loci. The first of these is to look for reagents which among testcross progeny detect an excess of offspring whose cells react with the reagent. The excess of reactors would be less than the three to one ratio expected in the case of independent genes and slightly more than the one to one ratio expected for a single gene. For small samples of the order of 20 to 30 the deviations from 1:1 would probably not be significant statistically.

All linkage studies of blood group genes with genes for other characteristics followed the discovery of the cellular antigen. Immunological procedures permit the acquisition of linkage information without the necessity of first finding the cellular antigen. This would have great value in human genetics where it is desired to determine an antigenic marker linked to a gene for a physiological trait. An example with chickens will illustrate this method. The blood of a bird known to be heterozygous for rose comb for example would be injected into a single comb bird. The immune sera obtained from the latter chicken would be absorbed with pooled cells of all single comb progeny from the mating of rose comb with single comb. This absorption is made to eliminate all antibody specificities except those for antigens whose genes are closely linked to the rose comb locus. Remaining specificities should be reactive only with the antigenic product of the linked gene. In a similar way all of the chromosomes in the fowl could be examined for the presence of blood group genes as they are linked to genes marking the chromosomes.

This type of experiment is similar in principle to the one carried out by LEVIT, *et al.* (1936) in which serological differences were reported between XX and XXY females in *Drosophila*. An experiment was carried out by the author to determine if blood group genes are located in the "X" chromosome and the postulated "Y" chromosome of chickens. The assumptions involved were (1) that blood group genes are randomly distributed and that some would be found in the X chromosome and the Y chromosome, if the latter is present in the chicken, and (2) that blood group loci in the X chromosome would contain as many different alleles as has been found for other loci studied (BRILES, *et al.* 1950). With the latter assumption in mind, breed crosses were made. The matings and the number of progeny available for testing follow: Buff Orpington $\times$ Single Comb White Leghorn (39), S. C. Barred Plymouth Rock $\times$ S.C. White Leghorn (68), Silver Oklabar $\times$ S.C. White Leghorn (31), New Hampshire Red $\times$ White Plymouth Rock (57). Such matings provided a wide array of genotypes for sampling and permitted the detection of locus effects if more than one allele were present. The mating of a New Hampshire Red male to a White Plymouth Rock female involved only the two individuals but in all other matings a single male was mated to five to seven S.C. White Leghorn females all from the same

or related inbred line. The immunizations were made by injecting the cells of a female into the sire and obtaining an immune serum. Absorptions were carried out with the pooled cells of the progeny of each sex as well as by a series of absorptions with the cells of individuals from each sex. In all cases when absorption removed antibodies for all the members of one sex the absorbed sera did not react with the cells of members from the opposite sex. It may be concluded that if blood group genes are located in either the X or Y chromosomes of chickens their products are so weakly antigenic that detectable antibodies were not produced.

A program of gene mapping would be highly desirable for the solution of many problems confronting immunogeneticists. Correlations between blood groups and physiological traits have been reported by BRILES *et al.* (1953) and SHULTZ and BRILES (1953). These correlations might be due to linkage of genes responsible for the characters, but even if they were not, linkage information would be of value in attempting to understand the nature of the correlation.

As close linkages are found the possibilities become greater for testing for the presence of cross reaction, which it is hoped will lead to the solution of a fundamental immunogenetic question, "What is the nature of antigenic specificity imparted by the gene?"

SUMMARY

Two independent linkage groups involving blood group genes have been found in the chicken. A recombination frequency of $5.4 \pm 4.2\%$ has been found between M_2 and Q_4 , and of $2.6 \pm 2.0\%$ between O_5 and S_2 . S_3 has been found allelic to S_2 and M_5 allelic to M_2 . Reagents for four other antigenic substances have been obtained but the linkage relations of the responsible genes have not been determined.

ACKNOWLEDGMENT

The author is grateful to DR. M. R. IRWIN for his many helpful criticisms and suggestions in the preparation of this paper and to DR. W. H. MCGIBBON for the use of poultry stock and equipment.

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