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Genetic Control of Immunoglobulin Allotypes in the Fowl

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Eight immunoglobulin allotypic specificities have been identified in the fowl by isoimmunization. Aa1 and Aa2 are controlled as codominants at the a *locus, Ab1 and Ab2 at the* b *locus, and Ac1, Ac2, Ac3, and Ac4 at the* c *locus. Column chromatography and ultracentrifugation indicate that the specificities at the* a *locus are located on molecules corresponding to IgG with sedimentation coefficients 7 S. Immunoelectrophoresis results also indicate that we are dealing with an immunoglobulin G molecule. Further tests are underway to resolve this beyond doubt.*

INTRODUCTION

Oudin (1956) showed that serum proteins of certain individuals induce formation of precipitating antibodies in others of the same species. The isoantibodies formed characterize serum protein specificities which may be genetically controlled. The term "allotype" defines a particular serum protein specificity (Dray and Young, 1958).

The Mendelian basis for immunoglobulin allotypes has been established for several species. In rabbits, three alleles at each of two loci (*a* and *b*) have been described (Dray *et al.*, 1962). In mice, allotypes of at least three multiple allelic systems have been demonstrated (Lieberman and Dray, 1964; Warner and Herzenberg, 1967; Warner *et al.*, 1966). Allotypic specificities have also been reported in chickens, pigs, and cattle (Skalba, 1964; Rasmusen, 1965; Rapacz *et al.*, 1968). In this paper information on eight allotypic specificities in chickens is presented. Subjected to breeding tests, these demonstrate genetic control by codominant genes at three genetic loci.

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MATERIALS AND METHODS

Chickens used in the experiment for identification of isoprecipitins came from three White Leghorn lines (Leg-1, Leg-19, and Leg-GH), an inbred Barred Rock line, and several ornamental or exotic breeds. The three Leghorn lines have inbreeding coefficients between 50 and 80%. Samples of the exotic breeds were obtained from a commercial hatchery. Since these were maintained in rather small flocks, they would also be somewhat inbred. Genetic information on allotypes was based on specific matings among these lines and breeds.

Donor cockerels from different lines were immunized with killed *Brucella abortus* cells. Each was given three intravenous injections of 1 ml of bacterial suspension containing approximately 10^8 to 10^9 microorganisms per week for 3 weeks. The chickens were bled from the heart 1 week after the last injection. The harvested serum containing the *Brucella* antibody was stored at −20 C and served as the antigen for the second immunization.

Injection of anti-*Brucella* globulin from an individual donor stimulated the production of isoprecipitins. Five drops of bacterial suspension containing approximately 20×10^9 cells were mixed with 1 ml of homologous serum and incubated for 60 min at 37 C in a water bath. The agglutinate (anti-*Brucella* antibody–*Brucella* antigen complex) was washed three times in saline, mixed with Freund's incomplete adjuvant,[4] and injected subcutaneously into chickens at monthly intervals. Recipient chickens were bled periodically; the sera were tested for precipitating antibodies to chicken globulin using Ouchterlony's agar gel double diffusion procedure on microslides. The diffusion medium consisted of 0.075 M phosphate (Na_2HPO_4–KH_2PO_4) buffer solution containing 8% NaCl, 1% Noble agar,[4] and 1:10,000 Merthiolate. Preimmunization sera from birds injected with *Brucella abortus* cells were placed in the center well, and sera from the birds that received the anti-*Brucella* antibody–*Brucella* cell complex were placed in the surrounding wells of the gel diffusion plate. A serum that produced a precipitin line was further tested for monospecificity and cross reaction against a panel of sera from different strains. A typical pattern is shown in Fig. 1.

Although in a few cases antisera with a single specificity were successfully obtained, usually the antisera contained more than one specificity. Absorptions were then necessary to reduce the sera to a single specificity. This was accomplished directly on the Ouchterlony slide by treating the center or "antibody" well with the absorbing antigen and allowing it to diffuse into the medium prior to adding the antisera. The particular absorbing antigen was also placed in one of the peripheral wells to detect incomplete absorptions.

Figure 2 shows the reaction pattern before and after absorption in a test. Allotypes of individuals can also be determined by analyzing the reaction patterns of multispecific antisera, just as in the case of blood-group reagents with multiple specificities.

A typical arrangement for testing normal sera is illustrated in Fig. 3. A specific allotype antiserum was pipetted into the center well; the normal sera were pipetted

[4] Difco Laboratories, Detroit, Michigan.

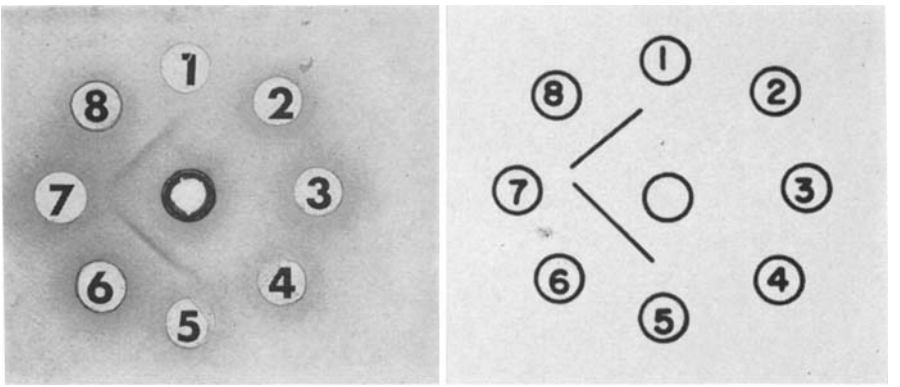

Fig. 1. A typical pattern in testing sera for isoprecipitins. Sera from the donor bird is in the center well and the sera from the recipients in the surrounding wells. The test sera in wells 6 and 8 identify an allotypic specificity.

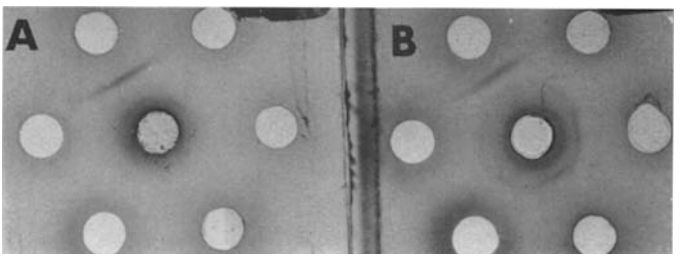

Fig. 2. Reactivity of anti-Aal (GH 745) after absorption with Ac3 (A) and before absorption (B).

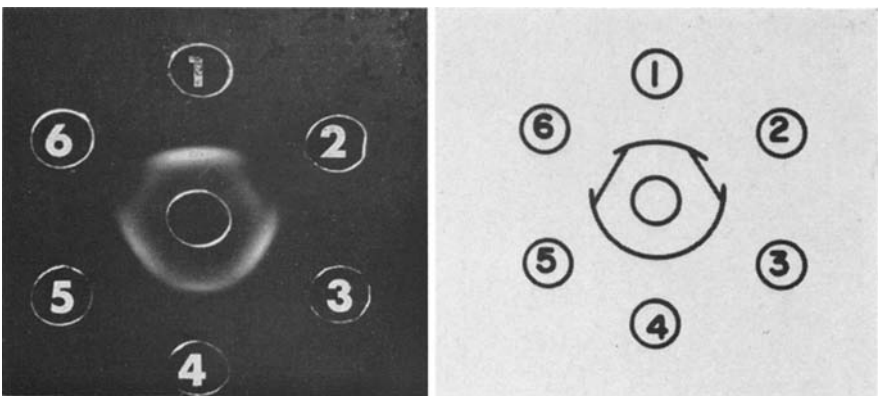

Fig. 3. Typing normal sera for allotypes. Wells 1, 3, and 5 contain the reference serum and wells 2, 4, and 6 the test sera. The anti-allotype serum is in the center well. The test serum in well 4 contains the specific allotype. In wells 2 and 6 cross reactions are indicated by noncoalescence (spurs) with the reference serum.

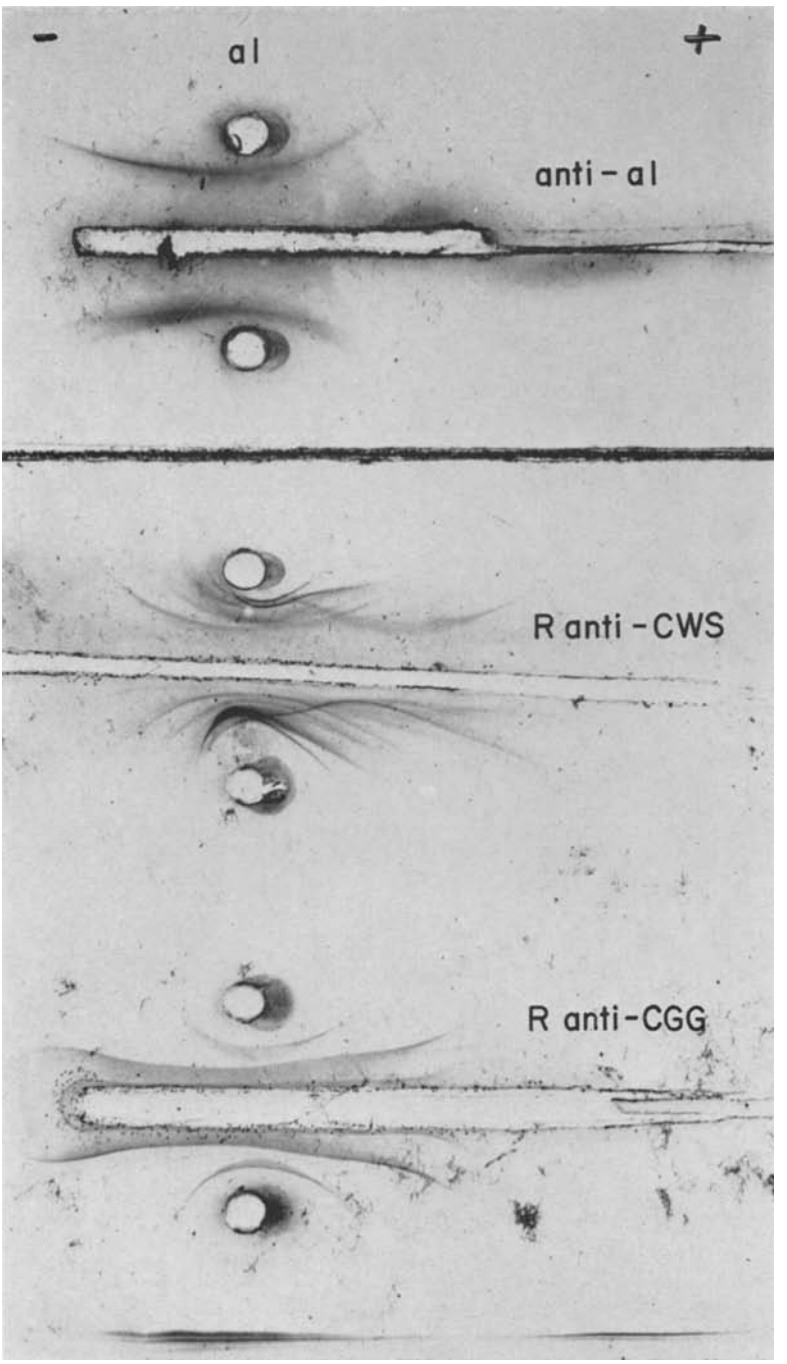

Fig. 4. Immunoelectrophoretic plates showing precipitin reactions of anti-Aa1 allotype antibodies with a1 in normal sera of chickens. The precipitation lines are formed in the region of immunoglobulin G as seen by comparison with lines formed by rabbit anti-chicken whole sera (R/CWS) and rabbit anti-chicken gamma globulin (R/CGG).

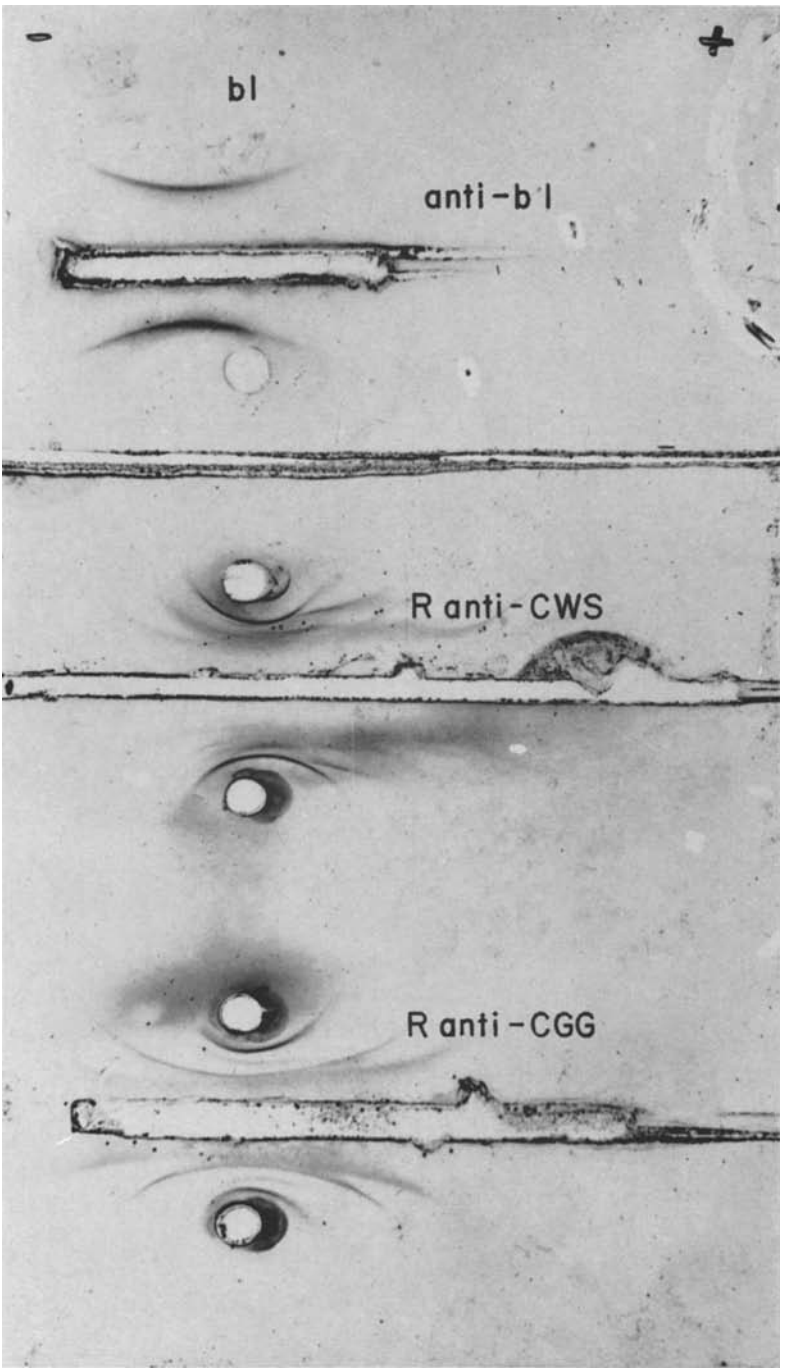

Fig. 5. Same as for Fig. 4, except showing anti-Ab1 allotype antibodies with b1.

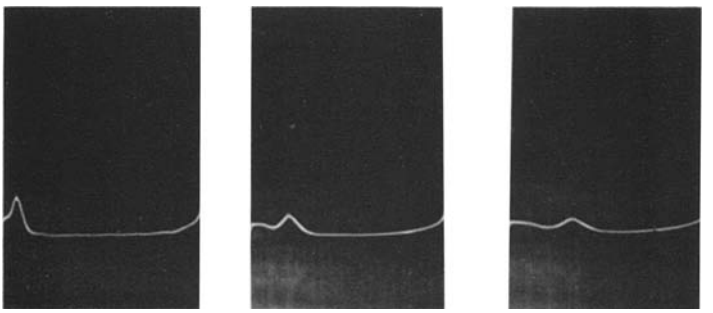

Fig. 6. Ultracentrifuge pattern of immunoglobulin fraction containing allotypic specificity Aa1. Photographs were taken 16 min apart giving a sedimentation value of 6.6 S.

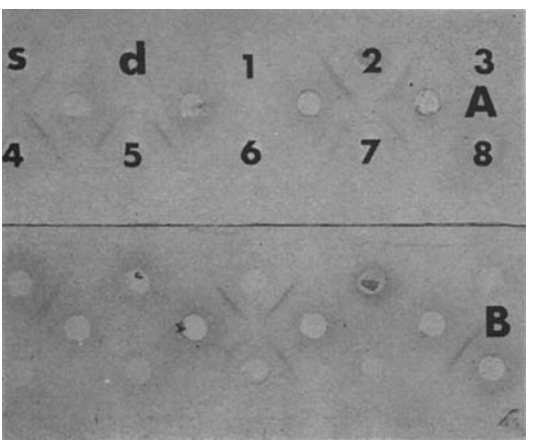

Fig. 7. Family data for segregation at *a* locus. The sire (s) is a^1/a^2 and the dam (d) is $-/-$; the progeny, 1–8, segregate at the *a* locus. The two slides are identical except that the center well in slide A contains anti-Aa1 sera and in slide B anti-Aa2. The sire serum reacts positively in both slides while the dam is negative in both. The progeny show positive reactions in one slide or the other. Progeny numbers 2, 3, 4, 5, and 7 are positive to anti-Aa1 sera and negative to anti-Aa2 sera. Progeny numbers 1, 6, and 8 are negative to anti-Aa1 and positive to anti-Aa2.

Table I. Identification of Allotypes

System	Allotype	Donor		Recipient		Antisera also contain antibodies for	Cross reactions[a]
		Line or breed	Individual no.	Line or breed	Individual no.		
a	Aa1	Barred Rock	7682	Leg-GH	745	Ac3	—
	Aa2	Araucana	5854	Hamburg	5912		?
b	Ab1	Leg-1	R239	Leg-GH	1540		?
	Ab2	Spanish	385	Houdan	5968	Ax2[b]	—
c	Ac1	Red Cap	5938	Leg-19	1415		?
	Ac2	Araucana	5879	Leg-19	2126		?
	Ac3	Barred Rock	7688	Leg-GH	743		Ax2[b]
	Ac4	Buttercup	6012	Leg-19	1570		

[a] The question marks indicate cross reactions with unknown antigens.
[b] The letter x designates an unknown genetic system.

into perimeter wells, alternatively, with known reference sera. This arrangement permitted the detection of cross reactions of closely related but not identical allotypic antigens.

The allotypic globulins were also subjected to immunoelectrophoresis on $3\frac{1}{2} \times 4$ inch plates. The medium consisted of 0.5% ionagar in barbital–lactate buffer (0.1 M, *p*H 8.2). Normal sera of the specific allotypes were placed in the circular wells and electrophoresed. After initial electrophoresis, specific isoprecipitins were added to the troughs. The plates were incubated overnight and blotter paper soaked in saturated salt solution was placed on top of the gel for 30 min. The plates were further incubated for 24–48 hr, washed, dried, and stained. The precipitin lines were compared with the electrophoresed allotype sera tested against rabbit anti-chicken sera and rabbit anti-chicken gamma globulin (Figs. 4 and 5).

The allotypic sera (a1 and a2) were fractionated in a Sephadex[5] G200 column according to the Sephadex instruction manual, and the fraction containing the allotypic globulin in a pure form was ultracentrifuged at 59,780 rpm. Sedimentation coefficients were then calculated (Fig. 6).

The notation used in designating the allotype is in conformity with that of Dray *et al.* (1962). For example, in the case of Ab1, A means "allotype," "*b*" designates the genetic system (locus), and "1" is the specificity. When the number corresponding to the specificity is used as a superscript, this designates the allele of a genetic system. For example (as in Table II), a^1/a^2 is the genotype of an individual at locus *a* with alleles 1 and 2.

RESULTS

The eight allotypic specificities identified by immunizing chickens with the *Brucella*-chicken antibody conjugates are listed in Table I according to the lines and breeds

[5] Pharmacia, Uppsala, Sweden.

Table II. Progeny Test for Segregation of Allotypes

Parent genotype		Number of progeny	Phenotype of progeny[a]	Probability of larger χ^2 value
Sire	Dam			
a^1/a^2	−/−	31	$18a^1$, $13a^2$	0.3–0.4
b^1/b^2	−/−	60	$33b^1$, $27b^2$	0.4–0.5
c^2/c^4	−/−	32	$16c^2$, $16c^4$	
c^1/c^2	−/−	42	$22c^1$, $20c^2$	0.7–0.8
c^1/c^3	−/−	38	$18c^1$, $20c^3$	0.7–0.8
c^3/c^4	−/−	34	$19c^3$, $15c^4$	0.4–0.5

[a] The number of progeny with the allele, e.g., 18 progeny tested positive for a^1 and 13 for a^2.

by which they were originally identified. From breeding tests we have deduced three genetic systems, designated a, b, and c, with 2, 2, and 4 alleles, respectively.

Some allotypic reagents showed cross reactions (Table I). Typically, cross reactions were detected by a spur formation between the normal sera and reference sera. In other cases, spurs were not clearly visible. Usually, in the process of washing the gel before staining, most of the nonspecific reacting material seemed to be removed indicating rather loosely bound antigen–antibody complexes.

The results of the breeding tests are shown in Table II. On the whole, the progeny genotypes segregated as expected according to a 1:1 ratio when tested statistically. Figure 7 shows the results from a typical family segregating at the *a* locus. The sire

Table III. A Typical Set of Matings and Progeny Showing that Allotypic Systems Designated a, b, and c Are Determined by Alleles on Separate Loci

Mating			Progeny			
Sire	Dam					
a^1/b^1	−/−	Phenotype	a^1	b^1	a^1/b	−/−
		Observed frequencies	9	12	8	7
		Expected if a^1 and b^1 are:				
		Nonallelic	9	9	9	9
		Allelic	18	18	0	0
a^2/c^3	−/−	Phenotype	a^2	c^3	a^2/c^3	−/−
		Observed frequencies	13	15	13	14
		Expected if a^2 and c^3 are:				
		Nonallelic	13.75	13.75	13.75	13.75
		Allelic	27.5	27.5	0	0
b^3/c^3	−/−	Phenotype	b^2	c^3	b^2/c^3	−/−
		Observed frequencies	27	20	12	22
		Expected if b^2 and c^3 are:				
		Nonallelic	20.25	20.25	20.25	20.25
		Allelic	40.5	40.5	0	0

genotype was a^1/a^2 and the dam lacked both of these alleles. Of the eight progeny, five received the a^1 allele and three received the a^2 allele from the sire.

Table III presents a typical set of matings and progeny, which demonstrates that the allotypic systems designated a, b, and c are determined by alleles at separate loci. The three loci so far identified are open systems; that is, individuals have been found which lack the known alleles. Hence, each system probably contains many alleles.

Some of the alleles identified were relatively rare while others were in high frequency in most of the test populations. For example, Aa2 and Ac1 were detected in three breeds, while Ac2 was detected only in the Araucana, a South American breed.

Since the original material injected was antibodies (anti-*Brucella*), it is assumed that this would be mostly immunoglobulin. We believe the concentration of possible contaminating proteins during injection of an immune agglutinate was too low to induce antibody formation. Nevertheless, as a precautionary measure, the agglutinates were washed several times in saline before injection.

The results of column chromatography and ultracentrifugation indicated that the allotypic specificities at the *a* locus are located on the IgG molecule. The allotypic globulin fraction, obtained by gel filtration in Sephadex G200, contained a single electrophoretic component with a sedimentation coefficient of 6.6 S.

The results of immunoelectrophoresis indicate that we are dealing with an immunoglobulin G molecule. We are at present performing experiments to resolve this fact beyond doubt for all the specificities, and the results will be published soon. We also hope to relate the allotypes to the immunoglobulin classes, which would make the genetic data more meaningful.

DISCUSSION

The immunoelectrophoretic technique applied to chicken antibody systems created some problems. The salt concentration of the gel required by this system for optimum precipitation in diffusion tests was too high for satisfactory electrophoresis. At low salt concentrations, precipitation failed. After experimenting with several methods, we obtained the best results when blotter paper soaked in saturated salt solution was placed on top of the gel for 30 min after the plate had been incubated overnight with the antiserum. If the antiserum had a low titer, it failed to precipitate even under these circumstances. If the salt is diffused into the gels prior to incubation, considerable protein was lost resulting in a very faint precipitation line.

In our initial studies in 1964 on chicken allotypes, we tested several White Leghorn inbred lines and one Barred Rock inbred. From these, three allotypic specificities were identified. In 1965, a heavy-breed cross population and six White Leghorn inbred lines from a commercial breeder[6] were tested as recipients or donors, or both. From these, two more specificities were identified. Repeated immunizations within or between these populations failed to uncover other specificities. In 1966, 14 ornamental or exotic breeds of chickens were tested from which 14 additional specificities were uncovered.

[6] Hy-Line Poultry Farms, Johnston, Iowa.

Altogether, we have so far identified 19 allotypic specificities. Of these, only eight have given us allelic information in test crosses and are reported in this paper. Perhaps it is surprising that we have identified so many allotypes in chickens. During the several thousands of years that chickens have been domesticated, they have been subjected to diverse forms of selective breeding. Breeders and fanciers have tended to keep the different breeds apart. Therefore, genetic variation in chickens may be relatively greater than among those populations of mice and rabbits which have been studied for allotypic differences. Perhaps sampling of wild populations of rabbits and mice from different geographic regions might result in identification of additional allotypic specificities. Identification of several antigens in the human Gm system indicates the heterogeneity of gamma globulin in human populations.

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