

*INTERACTION BETWEEN ALLELES AFFECTING CELLULAR
ANTIGENS FOLLOWING A SPECIES CROSS IN COLUMBIDAE**

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The majority of the cellular antigens now recognized in various animal species appear to be the more or less direct products of their causative genes. However, a few exceptions to this general rule have been noted in species hybrids in pigeons and doves^{1, 2} and in ducks,³ in which the blood cells of the species hybrids and some back-cross hybrids⁴ have possessed new antigenic specificities which were not detectable in the respective parental species. These new specificities have been called "hybrid substances."

This paper deals with the detection of a hybrid substance in the cells of certain back-cross hybrids from the mating of *Columba livia* and *C. guinea*, and presents evidence that this hybrid substance is probably due to the interactions of allelic genes, peculiar to *livia* and to *guinea*, respectively.

Materials and Methods.—The techniques employed in this experiment with respect to the production of antibodies, and the absorptions to make specific reagents are essentially the same as those previously described.^{2, 4, 5} The presence of agglutination of the various kinds of cells with specific reagents is the index of the presence of an antigenic substance, as contrasted to the absence of the antigenic substance if no agglutination is observed.

Following back-crosses to *livia* of the F₁ hybrids and selected back-cross hybrids from the mating of *guinea* and *livia*, a cellular antigen called C specific to *guinea*, has been obtained in unit form.⁶ By matings *inter se* of these back-cross birds, heterozygous for the C substance, offspring were obtained which were homozygous for the C antigen. The homozygotes could be differentiated from the heterozygotes serologically and by progeny

tests as described.⁵ Both homozygotes and heterozygotes were available for the tests. The antigenic substance in livia, antithetical to the C of guinea, has been called C'.⁵ The C and C' substances apparently form a closed antigenic system; that is, all the back-cross hybrids from matings to livia or *inter se* have been homozygous CC, homozygous C'C', or heterozygous CC'.

Experimental Results.—Twelve antisera against the cells of the homozygotes (CC) were produced in rabbits. These antisera, when absorbed with an excess of the cells of livia (C'C'), provided reagents specific for the C substance. Consequently, they reacted with the cells of guinea, the homo

TABLE 1

THE AGGLUTINATIONS OF THE ERYTHROCYTES OF LIVIA, GUINEA, AND OF BACK-CROSS BIRDS HOMOZYGOUS (CC) AND HETEROZYGOUS (CC') FOR THE C ANTIGENS OF GUINEA, WITH REAGENTS PREPARED FROM ANTISERA TO THE C SUBSTANCE FROM HOMOZYGOTES AND HETEROZYGOTES

TEST CELLS	THE RANGE OF REACTIONS OF 6 ANTI-CC SERA AFTER ABSORPTION WITH CELLS AS LISTED				THE RANGE OF REACTIONS OF 5 ANTI-CC' SERA (LACKING ANTIHYBRID ANTIBODIES) AFTER ABSORPTION WITH CELLS AS LISTED				
	UNABSORBED	LIVIA (C'C')	CC	CC'	UNABSORBED	LIVIA (C'C')	CC	CC'	CC + LIVIA
Livia	8-13 ^a	0	0	0	9-13	0	8	0	0
CC	9-12	6-9	0	0	8-13	6-11	0	0	0
CC'	8-13	6-8	0	0	9-13	6-11	9	0	0
Guinea	8-13	6-9	0	0	8-12	6-9	0	0	0

TEST CELLS	THE RANGE OF REACTIONS OF 3 ANTI-CC' SERA AFTER ABSORPTION WITH CELLS AS LISTED					
	UNABSORBED	LIVIA (C'C')	CC	CC'	CC + LIVIA	GUINEA + LIVIA
Livia	8	0	4	0	0	0
CC	8	5	0	0	0	0
CC'	8	5	4	0	1-3	1-3
Guinea	7	4	0	0	0	0

^a The numbers refer to titers based on doubling dilutions of the reagents, starting at 1:60; e.g. 1 = 1:60, 2 = 1:120, 3 = 1:240 . . . 13 = 1:245,760; 0 indicates no agglutination at the first dilution of the reagent.

zygotes (CC), and the heterozygotes (CC'), but not at all with the cells of livia (C'C'). However, when absorbed with an excess of the cells of either the homozygotes (CC) or the heterozygotes (CC') they were completely depleted of antibodies. All these anti-C reagents reacted at dilutions from 1:1920 to 1:15,360, as given in table 1.

Thirteen antisera were produced against the cells of the heterozygotes (CC'). These, after absorptions with the cells of livia, also reacted strongly with the cells of guinea, the homozygotes (CC) and the heterozygotes (CC'). They were completely depleted of antibodies following absorptions with the cells of the heterozygotes (CC'). However, unlike the anti-CC sera, following absorptions with an excess of the pooled cells of two or more ho-

mozygotes (CC), they became anti- C' reagents which reacted strongly (1:7680 to 1:30,720) with the cells of livia and of the heterozygotes (CC') but not at all with the cells of guinea or of the homozygotes (CC), as is given in table 1. This latter test clearly differentiated between the cells of the homozygotes (CC) and those of the heterozygotes (CC'). This differentiation is in complete accord with the differentiations based on progeny and pedigree tests as reported elsewhere.⁵

Of these 13 antisera produced against the cells of the heterozygotes (CC'), ten were completely depleted of antibodies when absorbed (*a*) with pooled cells of both parental species (livia and guinea) or (*b*) with cells of the homozygotes (CC) pooled with cells of livia. However, two antisera, similarly absorbed, would react weakly at the absorbing dilution (1:60) only with cells of the heterozygotes (CC') and one antiserum (300F9), thus absorbed, reacted strongly (titer of 1:240) only with the cells of the heterozygotes (CC').

This reaction was undoubtedly due to a new specificity peculiar to the cells of the heterozygotes (CC'). To minimize the possibility of individual differences among the birds giving rise to this phenomenon, the cells of 90 representatives of livia, of 55 homozygotes (CC), of 93 heterozygotes (CC') and of 7 birds of guinea were tested with the reagent for this hybrid substance. The cells of all the heterozygotes (CC') were strongly agglutinated by this reagent but none of the cells in the other three groups reacted with it. Furthermore, this reagent agglutinated strongly the cells of 35 offspring (CC') of matings to livia of birds homozygous for C , even when the pooled cells of their respective parents were employed in absorptions for the preparation of the reagent.

As previously reported,⁶ there are four other distinct antigenic substances, A , B , E , and F , particular to guinea as contrasted to livia. Following the same procedures as described above for the detection of the C substance, antithetical substances designated as A' , B' , E' , and F' have been demonstrated in the cells of livia⁵ and corresponding back-cross hybrids. Both homozygotes and heterozygotes for the gene or genes producing the respective substances have been recognized. It was therefore pertinent to determine whether the erythrocytes of the respective heterozygotes (AA' , BB' , EE' , and FF') also possessed a hybrid substance. Repeated immunizations of rabbits were made with the cells of each of these heterozygote types, but to date no antibody specific to a hybrid substance has been demonstrated for the four kinds of heterozygotes.

The erythrocytes of Ring dove (*Streptopelia risoria*) are agglutinated by the reagent for the C of guinea, and therefore possess one or more "reactive groups" of the C substance, a C -like substance (unpublished data). The hybrids from matings of livia ($C'C'$) and Ring dove (presumably homozygous for the C -like substance) will be heterozygous for the genes pro-

ducing C' and the C-like substance. Such hybrids possess a hybrid substance,² which may be, but need not be, related serologically to the interaction products of the heterozygotes CC'. The reagent for the CC' hybrid substance was tested at the absorbing dilution (1:60) with the cells of six hybrids from the mating of livia and Ring dove, and no agglutination was observed. These results indicate that, since the antibodies in the specific reagent are not reactive with the cells tested of hybrids between livia and Ring dove, the gene or genes in guinea, which interact with those of livia to effect a new antigenic specificity, are not found in Ring dove, at least not as homologs. That is, the gene or genes of guinea, associated with the C substance, which interact with one or more genes of livia to effect the new or changed antigenic specificity of the heterozygous CC' cells, are not present in Ring dove, although an antigenic substance, and therefore a causative gene, related to the C of guinea exists in Ring dove. The specificities of other antisera need to be examined for possible interrelationships of these two hybrid substances before concluding that an interrelationship does not exist.

The present evidence indicates that a hybrid substance is associated only with the CC' heterozygotes, not with the four other heterozygotes (AA', BB', EE', or FF'). It might be argued that the new or changed antigenic specificity of the cells of the CC' birds was simply a change in specificity conferred upon the heterozygotes by some sort of rearrangement of the antigenic substances C and C' when together. This might be called an "interaction product" of the antigens C and C'. Such an explanation appears much less tenable than if each of the five kinds of heterozygotes were characterized by a new antigenic specificity. Therefore a somewhat more plausible explanation appears to be that this hybrid substance of the CC' heterozygote is the result of genic interaction, but whether of alleles producing C and C', respectively, or of other genes on the same chromosomes, is not yet determined.

Summary.—Following backcrosses to *C. livia* of the hybrids and selected back-cross hybrids from the mating of *C. guinea* and *C. livia*, a cellular antigen called C specific to guinea has been obtained in unit form. A contrasting substance called C' has been demonstrated in livia. The C' of livia and C of guinea are presumably produced by one or more pairs of allelic genes. Antisera were produced against the cells of the heterozygotes (CC') which, when absorbed (a) with pooled cells of both parental species or (b) with the cells of the homozygotes (CC) pooled with cells of livia, provided reagents which reacted only with cells of the heterozygotes (CC'). These antisera therefore contained antibodies specific to an antigenic factor different from those found in either parental species or the homozygotes (CC).

Presumably this hybrid substance is the result of genic interaction, but

whether of alleles producing C and C', respectively, or of other genes on the same chromosomes, is an open question. ♀

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¹ Irwin, M. R., and Cole, L. J., *J. Exptl. Zool.*, **73**, 85 (1936).

² Irwin, M. R., and Cole, L. J., *Ibid.*, **73**, 309 (1936).

³ McGibbon, W. H., *Genetics*, **29**, 407 (1944).

⁴ Irwin, M. R., *Ibid.*, **24**, 709 (1939).

⁵ Miller, W. J., and Bryan, C. R., these PROCEEDINGS, **39**, 407-412 (1953).

⁶ Irwin, M. R., Cole, L. J., and Gordon, C. D., *J. Exptl. Zool.*, **73**, 285 (1936).
