THE RELATIONSHIPS OF THE CELLULAR ANTIGEN C OF COLUMBA GUINEA TO ANTIGENIC CHARACTERS IN OTHER SPECIES OF COLUMBIDAE¹

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FOLLOWING the production of species hybrids between *Columba guinea* and *C. livia*, a segregation of cellular antigens which differentiate *guinea* from *livia* has been observed in the offspring resulting from backcrosses to *livia* of the species hybrids and selected backcross hybrids (IRWIN, COLE and GORDON 1936). Each of four such antigenic characters (A, B, C and E) has behaved in inheritance as a single character, in that the offspring from matings to *livia* of backcross birds carrying any one of them either have had the antigenic character *in toto*, or have lacked it entirely, in approximately equal proportions. This evidence indicates that each antigenic substance is the product either of a single gene or of linked genes between which crossing over has not yet been observed.

The transfer to *livia* of the respective chromosomes of *guinea* carrying genes which effect the antigenic characters which differentiate *guinea* from *livia* is only the first step in the study of the evolutionary patterns of these antigenic substances and their causative genes. Such patterns may be deduced in part from the appearance of identical or related substances in other species, as described elsewhere (IRWIN and MILLER 1961). More precise information has resulted from the tests for the relationships of one of the specific antigenic characters, C of *guinea*, among various species of Columbidae. The results of these tests are presented in this paper.

EXPERIMENTAL PROCEDURE

Antisera were produced in rabbits by injections of the cells of backcross birds heterozygous (*C/C'*) for the respective causative genes for the C substance of *guinea* and *C'* of *livia* (MILLER and BRYAN 1953) or derivatives of these backcross hybrids which are homozygotes (*C/C*). Six of 12 antisera against the cells of homozygotes (*C/C*) had antibodies for the C substance ranging in titer from 1:1920 to 1:15,360, and one of these (No. 594F1) was used in performing the

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majority of the tests to be described in this paper. Similar but not completely parallel results were obtained with reagents prepared from two other antisera (709F5 and 710F4) as described by BRYAN (1953). The absorptions of the antisera and the subsequent agglutination tests with the cells of the various species were carried out essentially as previously described (IRWIN and COLE 1936; IRWIN 1939).

The species which have been tested for their content of antigenic specificities related to the C of *guinea* belong to several different genera. Those of the genus *Streptopelia* are: *capicola*, *chinensis*, *humilis*, or *tranquebarica*, *orientalis*, *risoria*, *semitorquata*, *senegalensis*, and *turtur*. Those of the genus *Columba* are: *fasciata*, *guinea*, and *livia*, the domesticated form of the rock pigeon. Those of other genera are: *Chalcophaps indica*, *Ocyphaps lophotes*, *Phaps chalcoptera*, *Phaps elegans*, *Zenaida asiatica* (formerly called *Melopelia leucoptera*), *Zenaidura auriculata*, *Zen. graysoni*, and *Zen. macroura*.

Representatives of these species have been purchased from various dealers in the United States or have been obtained as described elsewhere (IRWIN 1953; IRWIN and MILLER 1961).

EXPERIMENTAL RESULTS

Comparisons of the erythrocytes from heterozygotes and homozygotes

Following matings *inter se* of backcross hybrids carrying the gene or genes for the C character, offspring were obtained which were homozygous for the causative genes. At first, the only criterion for the homozygosity of an individual was that all its offspring resulting from a mating to *livia* would contain the C character. Later, the use of the cells of the homozygotes (C/C) in absorption of anti-*livia* serum provided a reagent which was reactive primarily, and seemingly only, with the contrasting substance C' in *livia* (MILLER and BRYAN 1953). Thus the respective reagents for C and C' became available for use in differentiating the heterozygotes and homozygotes. A further test to differentiate the homozygote from the heterozygote depended upon the reactivity of cells of the heterozygotes (C/C') with a reagent for a hybrid substance (BRYAN and MILLER 1953). The cells of the homozygotes were not reactive with this reagent.

It will be noted from the data in the following insert that the interpretation appears valid that the C and C' substances are respectively produced by single genes, or by two or more genes linked so closely that crossing over has not yet been observed. (For several years DR. W. J. MILLER was responsible for supervising these matings and for testing the offspring.)

Kind of mating	Offspring	
	With C	Lacking C
$C/C' \times C'/C'$	51	33
$C/C' \times C/C'$	49	13
$C/C \times C'/C'$	55	0
$C/C \times C/C'$	20	0
$C/C \times C/C$	12	0

All the birds were tested for the presence or absence of C, but only part of them were tested with the reagent for C' of *livia*. The presence of C' is therefore assumed, on the basis that C and C' are antigens of a closed system (MILLER and BRYAN 1953).

Only rarely have definite differences been noted in the reactivities—agglutination or lysis—of the cells from heterozygotes and homozygotes. WIENER (1943) cites the experimental work which indicates that no differentiation between AA and AO, or BB and BO bloods has been possible, but that slight differences in reactivity have been noted between bloods from AB or MN individuals and those of their respective homozygotes. OLSON (1943) noted a quantitative difference in the reactivities of chicken cells from homozygotes and heterozygotes, and STORMONT (1952) found differences in reactivity and in absorbing capacity between the cells from homozygotes and heterozygotes in two genetic systems—FV and Z—of cellular antigens in cattle.

Definite differences in reactivity of the cells of the heterozygotes (C/C') and those of the homozygotes (C/C and C'/C') have not been observed in tests with the respective reagents from the different antisera (anti-*guinea*, anti-*livia*, anti-CC and anti-CC'). However, one of us (BRYAN) noted that a smaller quantity of cells from the homozygotes (C/C) than from the heterozygotes (C/C') was required to exhaust an anti-CC serum of antibodies. An example of these tests with one (No. 710F4) of the three antisera to cells of homozygotes, with which such comparative tests were made, is given in Table 1.

It may be noted in Table 1 that a smaller quantity of cells of the homozygotes than of the heterozygotes was required to remove antibodies for the different test cells from the antiserum. Results varying only slightly from those of the table were obtained in parallel tests of the absorption of two other antisera against the cells of homozygotes (BRYAN 1953). One discrepancy in results is puzzling; there was a slight but definite reactivity of the cells of both homozygotes and heterozy-

TABLE 1

Differential absorbing capacities of cells of backcross birds homozygous or heterozygous for the gene(s) for the C character

Reactions of anti-CC serum (710F4) following absorptions with varying volumes of cells from birds homozygous (C/C) or heterozygous (C/C') for the causative genes for the C substance						
Time of absorption (minutes)	Volume of anti-CC serum (1:60)	Volume of cells in absorption	Cells tested and titers			
			<i>livia</i> (C'/C')	<i>guinea</i> (C/C)	C/C	C/C'
Unabsorbed	....	....	9*	8	10	8
5	1 ml	1 ml	4/5†	3/4	5/8	4/7
5 + 30	1 ml	2 ml	0/0	0/1	1/6	1/3
5 + 30 + 30	1 ml	3 ml	0/0	0/0	0/4	0/2
5 + 30 + 30 + 30	1 ml	4 ml	0/0	0/0	0/3	0/1

* The digits refer to the titers of the doubled dilutions of the reagents, beginning at 1:60. Thus 1=60, 2=120 . . . 10=30,720.

† The numbers in the numerators refer to titers of the test cells following absorption with the cells of homozygotes (C/C), those in the denominators refer to the titers following absorptions by the cells of heterozygotes (C/C'). Equal volumes of cells and of antiserum (1 ml) were used at each absorption.

gotes following two absorptions of the antiserum with CC cells, but no reaction was noted with the cells of *guinea* (C/C). (Results quite contrary to these have been obtained occasionally in parallel tests with other antisera to C, in that the cells of *guinea* were agglutinated but those of the homozygotes or heterozygotes were not.) A satisfactory explanation for the failure of the cells of *guinea* to be reactive with these fluids is not apparent. However, the conclusion appears valid that the erythrocytes of the heterozygotes possess completely the C of *guinea* and the C' of *livia* (MILLER and BRYAN 1953), but seemingly in lesser amounts than in the respective homozygotes. In addition to C and C', the cells of the heterozygotes carry the "hybrid substance" (BRYAN and MILLER 1953).

The antigenic specificities, or antigenic groupings, of the C antigen of guinea

The agglutination reactions of the cells of the various species which were tested with the reagent for the C character of *guinea* prepared by absorption of an anti-C serum with the cells of *livia*, and with this reagent further absorbed with the cells of each of the species listed, are given in Table 2. The reagent prepared from antiserum 594F1 was reactive in varying degrees with cells of other species of Columbidae than are listed in the table, as *Caloenas nicobarica*, *Ducula spilorrhoa*, *Gallicolumba luzonica*, *Geopelia humeralis*, and *Goura cristata*. Unfortunately, the cells of these five species were not available for tests other than with the reagents for C of *guinea*. (We are indebted to DR. W. J. MILLER for making the cells of three of these five species available for these tests, and to MR. KARL PLATH, Curator of Birds, Chicago Zoological Park, Brookfield, Ill., and to MR. R. M. PERKINS, Director, Lincoln Park Zoological Gardens, Chicago, Ill., who kindly allowed blood to be taken by DR. MILLER from certain of these three species.)

Similar but not parallel reactivities to those given in Table 2 (line 1) were obtained in testing the cells of these 20-odd species with the reagents for the C character prepared from two other antisera, 709F5 and 710F4, following absorption with the cells of *livia*. The cells of *nicobarica*, *spilorrhoa*, *luzonica* and *humeralis* were not used in combination with these reagents. One of them, 709F5, was not reactive with the cells of *indica*, *cristata*, *chalconoptera*, *elegans* or *lophotes*; the other, 710F4, produced no observable reactions with the cells of *auriculata*, *chalconoptera*, *elegans* or *lophotes*, but both reagents were reactive with the cells of all the other species listed in Table 2.

There are a few reactions listed in Table 2 which are at variance either in different reactivities of the cells of a species with a given reagent at different times of testing, or in lack of agreement with the expectancy of the reactivities of reciprocal tests, as outlined by KRUMWIEDE, COOPER and PROVOST (1925). The interlocking relationships of the antigenic specificities (antigenic groupings) of the C substance in these various species are proposed despite these discrepancies.

The differential reactivities of the three reagents for the C character toward the cells of a few species suggested a complexity of the C substance, and a part of this complexity is revealed in the various reactivities summarized in Table 2. Thus, the cells of *indica* removed antibodies only for themselves from the re-

TABLE 2

The presence in various species of Columbidae of antigenic specificities related to the C character of Columba guinea

Absorptions by the cell of:		capicola	chinensis	humilis	orientalis	risoria	semi- torquata	senegalensis	turtur	fasciata	guinea	C/C	livia	asiatica	auriculata	chalcop- tera	elegans	graysoni	indica	lophotes	macroura
livia	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	±	+	C
livia + capicola	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + chinensis	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + humilis	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + orientalis	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + risoria	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + semitorquata	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + senegalensis	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + turtur	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + fasciata	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + guinea	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + asiatica	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + auriculata	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + chalcoptera	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + elegans	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + graysoni	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + indica	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + lophotes	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + macroura	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + orientalis + asiatica	±	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + semitorquata + graysoni	±	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + risoria + turtur	+	+	+	+	+	+	+	+	+	+	C	C	0	+	+	+	+	+	0	+	+
livia + fasciata + asiatica + chinensis + senegal + lophotes + capicola	0	0	0	0	0	0	0	0	0	0	C	C	0	0	0	0	0	0	0	0	0

Symbols: C=complete agglutination; ++=strong agglutination; +=weak but definite agglutination; ?=questionable agglutination; 0=no agglutination, at the first dilution of the reagent, usually 1:60. If two reactions are listed (as 0, ?; 0, ±; ±, ±), these were obtained at different tests.

agent for the C antigen, since the cells of all other species previously reactive with this reagent were still agglutinated following the absorption with the cells of *indica*. The antigenic specificity on the cells of *indica* related to the C of *guinea* is designated as "a." This specificity "a" is present on the cells of all the species listed in Table 2 except *lophotes*, because antibodies against the cells of *indica* were exhausted in each absorption except that by the cells of *lophotes*.

The cells of *humilis* exhausted the reagent of antibodies for themselves and for those of *indica*, and therefore contain at least one specificity "b" in addition to the a assigned to *indica*; *humilis* = ab. Similarly, the corpuscles of *orientalis* removed antibodies for the cells of both *indica* and *humilis*, as well as for themselves, so a third specificity "c" is present in *orientalis* in addition to the a and b of *humilis*; *orientalis* = abc.

The specificity a is present on the cells of *auriculata*, and also an additional specificity "d" which differentiated the reactivities of the cells of this species from those of *humilis* and *orientalis*. Similarly, *chinensis* carries a on its cells and a different specificity "e" from those previously assigned. The reactivities of *risoria* and *semitorquata* were parallel in these tests. Both species removed antibodies for the cells of each other and for those of *auriculata* (ad). The cells of both species were strongly agglutinated by the reagent made by the exhaustion of the antiserum with the cells of *auriculata*, so an additional specificity "f" is assigned to these two species, making them adf.

The cells of *turtur* exhausted the antiserum of antibodies for themselves, and also for the cells of *indica* (a), *orientalis* (abc) and *auriculata* (ad). Absorption by a combination of the cells of *orientalis* and *auriculata* (not given in Table 1) did not remove all the antibodies for the cells of *turtur*, hence an additional specificity "g" is assigned to *turtur* (abcdg). Further, the cells of *chalconoptera* and *elegans* removed antibodies for each other by absorption, and for those of *indica* (a) and *auriculata* (ad), but not for those of any other species. The antigenic specificity of the C character assigned to *chalconoptera* and *elegans*, in addition to a and d, is called "h". The cells of the two species, *graysoni* and *macroura*, were indistinguishable and presumably identical in their reactivities and capacity for absorption with this antiserum (with certain exceptions), exhausting it of antibodies for the cells of *indica* (a), *risoria* and *semitorquata* (adf) and probably of those for the cells of *auriculata* (ad), and *chalconoptera* and *elegans* (adh). Antibodies for the cells of *graysoni* and *macroura* were not removed by the pooled cells of *risoria* (adf) and *turtur* (abcdg), and the specificities adfhi are proposed as the minimum number in *graysoni* and *macroura* to explain the results.

The cells of *asiatica* exhausted the antiserum of antibodies for the cells of *graysoni* and *macroura*, among others, and were agglutinated by the reagents resulting from the use in absorption of the cells of either *graysoni* or *macroura* (adfhi), so an additional specificity "j", to those of the other two species, characterizes the cells of *asiatica* (adfhi). There are two species, *lophotes* and *senegalensis*, whose cells were agglutinated by each of the reagents prepared by absorption of the antiserum by the cells of each of the different species, with questionable exceptions. In absorption, the cells of *senegalensis* removed anti-

bodies for those of *indica*, whereas those of *lophotes* did not. A specificity in addition to the *a* of *indica* is assigned to *senegalensis* (*ak*), a different one to *lophotes* (*al*).

In view of the differing reactivities of the cells of *capicola* and *fasciata*, from each other and from all other species, and the agglutinations of the cells of the various species following the exhaustion of the antibodies from the antiserum by the cells of each of these two species, the specificities *abcdgm* are assigned to *capicola*, and *abcdfgn* to *fasciata*. Finally, since the cells of *guinea* in absorption removed antibodies from the antiserum for the cells of each of the species, but a combination of cells of the species (see last row of Table 2) carrying all the specificities *a . . . n* did not exhaust the antibodies for the cells of *guinea*, at least one additional specificity "o" is invoked for the cells of *guinea* or of backcross hybrids with the C character. Thus, 15 different specificities—*a b c d e f g h i j k l m n o*—are the minimum required to explain the relationships of the C character of *guinea* to related antigens on the cells of 17 species of Columbidae, as revealed by the interactions in absorption and agglutination with the antibodies in one antiserum to the C character.

BRYAN (1953) noted that two other antisera to the C character of *guinea* contained antibodies which by parallel analyses of the relationships of C-like substances in the various species revealed the same kind of pattern described above, but with respective differences obtained by the use of each antiserum. Presumably these differences in specificities of the antibodies and the specificities of the antigens with which they reacted are an index of the response of the individual rabbit to immunization with portions of a single suspension of blood cells. For example, the reagent for C prepared from one antiserum (No. 710F4) reacted with the cells of *indica*, that prepared from the other (709F5) did not. Further, the cells of *risoria* and *semitorquata* were indistinguishable in their reactivities with antibodies to C in antiserum No. 594F1, as described above, but were readily differentiated in tests with the other two antisera. The reactivities of the respective reagents prepared from the three antisera to C of *guinea* indicate strongly that the number of antigenic specificities proposed above for the C character is a minimum.

Unfortunately, other species of the genus *Columba* than are listed in the table were not available to be included in these tests. A previous report (IRWIN and MILLER 1961) has presented evidence that the antisera of seven of eight species of *Columba*, in addition to the three species included in the present report, contained antibodies to the C of *guinea*, and therefore these species undoubtedly share antigenic specificities of the C substance. Only one of these species, *palumbus*, appears to contain all or nearly all the antigenic specificities of the C character of *guinea*.

It seems reasonable to conclude that the antigenic substance or substances related to the C of *guinea* in the various species definitely share antigenic specificities with *guinea*, but that one or more other antigenic specificities may distinguish the C-like character in each species from the C of *guinea* and possibly from that of each other species. Examples of comparable relationships are those

of the d-1 substance of *chinensis* and the s-2 of *senegalensis*, which have been shown to share antigenic specificities as well as to possess those which differentiate each antigenic character from the other (IRWIN 1949; STIMPFLING and IRWIN 1960).

Further complexities of the C character of guinea

The C character has followed the pattern in the backcross populations which would be expected if it were effected either by a single gene or by linked genes between which a crossover has not yet been detected. No individual differences in respect to the C character have been noted among the backcross birds carrying the C of *guinea* since the antibodies for C were always completely removed from the antiserum by the calls of any such individual. This finding is not surprising because all backcross hybrids possessing the C character are the descendants of a single male of *guinea* (D172.1) which was mated to *livia* by the late PROFESSOR L. J. COLE before 1930.

However, observations over a period of years, and summarized in Table 3, have indicated definitely that the C character of *guinea* shares antigenic specificities with two genetically and serologically independent characters peculiar to *chinensis*, in contrast to *risoria*, viz., the d-4 and d-11 antigens (IRWIN 1939), and to a character in *senegalensis* which is distinct from either of these two characters of *chinensis* (BRYAN 1953). Thus, the reagents respectively specific to d-4 and d-11 of *chinensis* were not reciprocally cross-reactive, but each agglutinated (Table 3) the cells of backcross hybrids with C of *guinea*, whether

TABLE 3

Tests of the antigenic relationships of the C of guinea with the d-4 and d-11 of chinensis and the s-8 of senegalensis

Anti-sera	Absorptions by the cells of	Test cells										
		<i>chin-ensis</i>	<i>guinea</i>	<i>livia</i>	<i>risoria</i>	<i>senegal-ensis</i>	d-4	E561G	d-11	s-8	CC'	CC
C of <i>guinea</i>	<i>livia</i>	+	++	0	+	0,+	+	..	+	0	++	++
C	<i>livia</i> and <i>risoria</i>	+	++	0	0	0,+	+	..	+	0	++	++
d-4	<i>risoria</i>	+	+	0	0	++	++	+	0	0	±	+
d-4	<i>risoria</i> and <i>guinea</i>	±	0	0	0	+	++	0,2,±	0	0	0	..
d-4	<i>risoria</i> and C/C'	±	0	0	0	++	++	?,±	0	0	0	0
d-4	<i>risoria</i> and E561D	..	0	0	0	±	±	0	..	..	0	..
d-4	<i>risoria</i> and d-4	0	0	..	0	0	0	0	..	..	..	..
d-4	<i>risoria</i> and <i>chinensis</i>	0	0	0	0	++	++	..	0	0	0	0
d-11	<i>risoria</i>	++	+	0	0	+	0	..	++	+	±	+
s-8	<i>risoria</i>	+	0	0	0	++	0	..	+	++	0	0

Symbols: Same as in Table 2.

homozygous or heterozygous. (Not all the reagents from antisera prepared against the cells containing d-4 or d-11 were reactive with cells carrying C, nor were all reagents from anti-C sera reactive with either the d-4 or d-11 antigens, rarely with both.) In view of the antigenic and presumed genetic relationship between d-11 of *chinensis* and s-8 of *senegalensis* (IRWIN 1949) it is somewhat surprising that cross-reactivity has not been observed between C of *guinea* and s-8 of *senegalensis* (Table 3). Seemingly, the antigenic grouping of d-11 which is shared with C of *guinea* is at least a part of the d-11 antigen which is not shared with s-8. Specificities *a* and *e* of the C antigen were assigned above to *chinensis*. If the specificity of the C of *guinea* called "*e*" is shared with d-11 of *chinensis*, an additional specificity is shared with *chinensis* by virtue of the relationship of C to the d-4 antigen of *chinensis*.

As is given in Table 3, the specific reagent for C was reactive with the cells of some individuals of *senegalensis*, but not with those of others. The cells of 11 birds of this species were reactive with this reagent, those of seven others were not. The presence or absence of this reactivity was independent of the presence or absence of the s-4 of *senegalensis* (previously called s-6, an homologous substance with d-4; IRWIN 1949). Of 11 birds whose cells were reactive with the reagent for C, seven carried s-4 and four did not; of the seven birds whose cells were not reactive with the C reagent, six carried s-4 and one did not (this reagent for C did not agglutinate cells with d-4 or s-4). Thus it appears that the C substance of *guinea* is cross-reactive with two of the independently occurring antigenic components of *chinensis* (d-4 and d-11) and also with two components of *senegalensis* (s-4, which is indistinguishable in backcross birds from d-4, and another component). BRYAN (1953) reported that the C of *guinea* was probably related to s-10 of *senegalensis*. No reagent specific only for s-10 has ever been obtained, so the identity of the antigenic character of *senegalensis* (not s-4) which is responsible for the reactions of some individuals of *senegalensis* with the reagent for C is not known.

The data of Table 3 show further that one backcross hybrid (E561G), the offspring of a mating to *risoria* of a backcross bird carrying only d-4 of *chinensis*, did not possess all the antigenic components of other backcross birds with d-4. That is, the cells of this bird did not by absorption remove all the antibodies from a potent d-4 antiserum for the cells of other birds (including a sib) carrying d-4. Presumably this bird resulted from either a crossover between linked genes effecting d-4, or a mutation. Two other examples of this kind have been noted in less than 250 backcross birds carrying this character, suggesting strongly that crossing over of linked genes is a more rational explanation of the fractionation of this substance than mutation. It may be noted in the data of Table 3 that the cells of this exceptional bird (E561G) removed all, or nearly all, the antibodies from a d-4 antiserum for the C character of *guinea*, whether in backcross birds or in *guinea*, but that a faint reactivity for the cells of E561G usually was observed after absorption of the antiserum by cells with the C antigen. Thus a major part, but not all, of the d-4 antigen of *chinensis* appears to be shared with C of *guinea*, and that part was carried on the cells of the exceptional individual, E561G.

DISCUSSION

The C antigenic character of *guinea* and the C' of *livia* appear to be contrasting characters, according to the genetic behavior of each in the backcross population (IRWIN *et al.* 1936; MILLER and BRYAN 1953; and unpublished data). The progeny of *inter se* matings of backcross hybrids carrying the C antigen also conformed to genetic expectation on the assumption that C and C' are single characters. However, the data presented in this paper show that the C antigen of *guinea* is antigenically related to each of two genetically and immunologically distinct antigens of *chinensis*, d-4 and d-11, and to an antigen of *senegalensis*, possibly s-10, (BRYAN 1953) also distinct from both d-4 and d-11.

If a single gene in *guinea* produces the C antigen, one would explain the cross-reactivities of C with the d-4, d-11 of *chinensis* and (possibly) s-10 of *senegalensis* on the assumption that these cross-reactivities were due to a fortuitous production of related antigenic groupings by the respective causative genes in each of the species. On this basis, one would expect that there should be cross-reactivity between each pair of the three antigens—d-4, d-11 and s-10—as well as between each of them and the C antigen. No cross-reactivity has been noted between the two antigens for which specific reagents are available, d-4 and d-11, according to the summary of these tests given in Table 3. If then, a single gene effects the C character, the cross-reactivities with the three different antigenic characters seemingly must be by virtue of the relationship of each of three different antigenic groupings of C to the respective differing antigens of the two other species (*chinensis* and *senegalensis*). It is possible that the relationship of C to both d-4 and d-11 suggests that d-4 and d-11 may be related, as proposed by MILLER (1956), but these findings may also be explained by assuming that genes at more than one locus in *guinea* are concerned in the production of the C antigen. This latter explanation appears more probable in the light of the available evidence.

If genes at two, possibly three or more, loci in *guinea*, with products related to the different antigens (d-4, d-11 and s-10) are acting together in *guinea* to effect the C antigen, one may question why crossing over has not been demonstrated in the backcross progeny. One possibility could be that the pertinent homologous chromosomes of the two species, *guinea* and *livia*, have accumulated inversions which effectively suppress crossing over between them. (The number and small size of the chromosomes in Columbidae have prevented extensive cytological analysis of the species and species hybrids.) Inversion differences may often exist between closely related species, but not always, according to DOBZHANSKY (1941, 1951), and PATTERSON and STONE (1952).

If the C of *guinea* is produced by a single gene, the relationships of the C-like substances in the other species of Columbidae to the C of *guinea* and to the C' of *livia* could be satisfactorily explained by assuming that the causative genes in the various species constitute an allelic series. Of the species tested, only *palumbus* contains a substance indistinguishable from and presumably identical to C of *guinea*, implying then that the gene for C in *palumbus* is the same as in *guinea*

(IRWIN and MILLER 1961). In the other species, the presumed alleles would effect antigens related to C of *guinea*, as has been noted for a related group of antigens of another system in *chinensis*, *humilis*, *orientalis*, *senegalensis* and *guinea* (STIMPFLING and IRWIN 1960).

On the other hand, if genes at more than one locus in *guinea* effect the C character, it would seem reasonable to propose that at least two multiple allelic series would obtain among the various species to account for the observed relationships. The C of *guinea* is related to the independent characters d-4 and d-11 of *chinensis*, and to one in *senegalensis*, possibly s-10. Both d-4 and d-11 of *chinensis* are related to C of *guinea* and have been shown to be complex characters, by virtue of their relationships to related antigenic characters in various species of Columbidae (BRYAN 1953; PALM 1955). The most probable explanation of the interlocking relationships existing for the antigenic groupings of the C antigen to those of the antigenic characters in the other species appears to be to assume the existence in *guinea* and in the other species of two series of allelic genes. One series would produce antigenic groupings similar to the d-4 antigen, the other to the d-11 antigen. Other species of Columbidae might carry a gene in either or both series of allelic genes. This situation could, but need not, obtain also for the gene responsible for the antigenic grouping of C related to *senegalensis* (possibly s-10).

The experimental results show definitely that there are antigenic characters in species of Columbidae with varying degrees of relationship to the C of *guinea*. The assumption of the existence of two series of multiple alleles in these various species conforms to the genetic expectation that the members of an allelic series produce related effects, rather than essentially different effects of the final gene product.

At present very little information is available concerning the chemical bases of the specificities of the antigens of the red blood cells. The A and B substances of humans have been studied most intensively and these appear to be mucopolysaccharides—i.e., macromolecules containing carbohydrate chains and peptide units bound together (KABAT 1956). MORGAN and WATKINS (1959) indicate, as has been known for many years, that the specificities of the A and B soluble substances reside in the carbohydrate portion of the mucopolysaccharide molecules.

Extensive findings in the field of immunochemistry permit the general conclusion that antibodies to a particular antigenic substance may be, but need not be, reactive with a wide range of related chemical substances. Thus AVERY and GOEBEL (1929) noted that derivations of two simple monosaccharides—glucose and galactose—exhibited completely distinct immunological specificity when combined with proteins from widely separated species. In contrast, the antibodies against the α and β azophenol glucosides of glucose, when combined with distinct proteins, were cross-reactive (AVERY, GOEBEL and BABERS 1932). Many other examples of comparable cross-reactions of relatively simple but related chemical substances, made antigenic by attachment to proteins, are cited by LANDSTEINER (1945). OWEN (1959) has given examples of how the immunological reactions of

chemically related haptens (themselves not antigenic) attached to proteins may simulate the reactions of antisera to blood cells, prior to and following antibody absorption.

The possibility cannot be ignored that slight differences in the chemical makeup of relatively simple compounds may account for the antigenic differences which have been observed among related "antigens" of an antigenic system, such as the "B system" of cattle (STORMONT, OWEN and IRWIN 1951) or of the antigens in the various species of Columbidae which are related to the C antigen of *guinea*, or which belong in other antigenic systems. However, the authors believe that the chemical relationships among naturally occurring antigens may be more appropriately illustrated by invoking other examples. HARRIS and KNIGHT (1955) showed that there is a serological difference in both directions between normal tobacco mosaic virus (TMV) and TMV which had been treated with carboxypeptidase. The carboxypeptidase exclusively splits off threonine from the carboxyl ends of the peptide chains of the virus. Therefore, removal of the threonine groups (about 3000), comprising about 0.7 percent of the molecule, has changed the immunological specificity. Another example of a change in antigenic specificity of a protein molecule by virtue of a substitution, not a loss, of an amino acid has been reported by GOODMAN and CAMPBELL (1953) between normal adult hemoglobin and sickle-cell anemia hemoglobin. These examples suggest that definitive groupings play a major role in immunological specificity of proteins.

The cross-reactivities existing between certain types of the pneumococci may be fully as pertinent, possibly even more so, as examples of the possible chemical bases of cross-reactions of blood cells. Types III and VIII have long been known to be immunologically cross-reactive. The specific capsular substance of Type III is a polycellobiuronic acid, meaning that it is composed of glucose and glucuronic acid in definite linkage (GOEBEL 1935). The capsular substance of Type VIII has the same cellobiuronic acid units as part of its molecule, but it also has additional glucose and galactose. The reason for the reciprocal cross-reactivity of the antibodies to these two types is because of the occurrence of multiple cellobiuronic acid units in both capsular substances, although the linkages of the glucose and glucuronic acid units in the two capsular substances are different. According to HEIDELBERGER (1956) "there are many cross-reactions and it is possible now to show that these cross-reactions are due, in the case of the carbohydrate antigens, to similar groupings in similar linkages".

However, mere possession of the same basic units need not result in cross-reactivity, as exemplified by the presence of *L*-rhamnose and *D*-glucose in both types II and XVIII, but no cross-reactivity has been noted between these two types (for references on this general topic see HEIDELBERGER 1956; see also for a table showing the content of polysaccharides in ten of the pneumococcal types). One may draw the general conclusion that immunological cross-reactivity of antigenic substances indicates a chemical similarity, but chemical similarity can exist without cross-reactivity.

Transformation of the pneumococcal types is recognized as having fundamental implications in biology, particularly in genetics, primarily following the

recognition of the role of desoxyribonucleic acid (DNA) in directing the transformation (AVERY, MACLEOD and McCARTY 1944). The findings of EPHRUSSI-TAYLOR (1951) with variant forms of Type III indicate that six different transforming agents can be detected in the desoxyribonucleate of a single Type III smooth strain. Further studies of mutant types of both Types I and III by AUSTRIAN and BERNHEIMER (1959) and AUSTRIAN, BERNHEIMER, SMITH and MILLS (1959) have elucidated possible biochemical pathways concerned with the formation of uronic acids in the pneumococci. Analogous pathways to these may obtain in the formation of cellular antigens.

It is unfortunate that no information is available concerning the chemical nature of the cellular antigens in Columbidae. As stated above, the chemical basis of the cross-reactions among the pneumococcal types appears to the authors to be the most pertinent models of the possible chemical relationships of the cellular antigens, in the Columbidae and in other species. One can also visualize a possible explanation for the appearance of the hybrid substance, as in the heterozygotes (C/C'). As stated above, the same basic units of polysaccharides may be present in different types of the pneumococci, but without cross-reactivity being demonstrable. If one would then assume that in the respective homozygotes, C/C and C'/C' , there exist one or more common antigenic groupings which do not engender specific antibodies, but in the heterozygote (C/C') these in some way became antigenically active, the appearance of a new antigenic specificity becomes understandable. However, a pertinent argument against this assumption is that an animal does not produce antibodies against the antigens of its own tissues. (The only known exceptions to this rule may occur because of isolation of the tissue from the antibody-forming cells.) Whether the new specificity is the result of an interaction between genes, possibly of alleles, or of an interaction between antigens, is still an open question.

SUMMARY

Backcross birds which carry only the C antigenic character of *guinea*, in contrast to *livia*, have been obtained by mating to *livia* the species hybrids and selected backcross hybrids from the mating of *guinea* $\times$ *livia*. *Inter se* matings of these backcross birds have produced offspring which are homozygous for the gene or genes producing the C antigen. The heterozygotes contain all of the C antigen of *guinea*, but less in amount than either *guinea* or C/C birds.

The reactions or lack of reactions of the cells of 18 species of Columbidae, following absorptions of antisera to the C antigen, showed clearly that the C of *guinea* is a complex character. The available evidence suggests that the C antigen of *guinea* is produced by the joint action of two, probably three, linked genes, based on the cross reactions existing between C and at least three genetically and serologically distinct antigenic characters in two other species.

Each of the species of Columbidae contained an antigenic character related to the C of *guinea* by one, usually two or more, antigenic groupings, or specificities, of the total of 15 antigenic groupings required as a minimum to explain the experimental results. The relationship of the causative genes in these species of

Columbidae producing the antigens related to C can be explained by assuming that the genes in the various species form a multiple series of alleles, belonging to one or the other, or both, of at least two such allelic series presumably existing in *guinea*, and that each member of the allelic series produces multiple antigenic specificities.

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LITERATURE CITED

- AUSTRIAN, R., and HARRIET P. BERNHEIMER, 1959 Simultaneous production of two capsular polysaccharides by pneumococcus. I. Properties of a pneumococcus manifesting binary capsulation. *J. Exptl. Med.* **110**: 571-584.
- AUSTRIAN, R., HARRIET P. BERNHEIMER, EVELYN E. B. SMITH and G. T. MILLS, 1959 Simultaneous production of two capsular polysaccharides by pneumococci. II. The genetic and biochemical bases of binary capsulation. *J. Exptl. Med.* **110**: 585-602.
- AVERY, O. T., and W. F. GOEBEL, 1929 Chemo-immunological studies on conjugated carbohydrate-proteins. II. Immunological specificity of synthetic sugar-protein antigens. *J. Exptl. Med.* **50**: 533-550.
- AVERY, O. T., W. F. GOEBEL, and F. H. BABERS, 1932 Chemo-immunological studies on conjugated carbohydrate-proteins. VII. Immunological specificity of antigens prepared by combining α - and β -glucosides of glucose with proteins. *J. Exptl. Med.* **55**: 769-780.
- AVERY, O. T., C. M. MACLEOD, and M. McCARTY, 1944 Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from *Pneumococcus* Type III. *J. Exptl. Med.* **79**: 137-158.
- BRYAN, C. R., 1953 Genetic studies of cellular antigens in Columbidae. Thesis, Library, The University of Wisconsin.
- BRYAN, C. R., and W. J. MILLER, 1953' Interaction between alleles affecting cellular antigens following a species cross in Columbidae. *Proc. Natl. Acad. Sci. U.S.A.* **39**: 412-416.
- DOBZHANSKY, T., 1941 *Genetics and the Origin of Species*. 2nd ed. Columbia University Press. New York.
- 1951 *Genetics and the Origin of Species*. 3rd ed. Columbia University Press. New York.
- EPHRUSSI-TAYLOR, HARRIET, 1951 Genetic aspects of transformations of pneumococci. Cold Spring Harbor Symposia Quant. Biol. **16**: 445-456.
- GOEBEL, W. F., 1935 Chemo-immunological studies on the soluble specific substance of pneumococcus. II. The chemical basis for the immunological relationship between the capsular polysaccharides of Types III and VIII pneumococcus. *J. Biol. Chem.* **110**: 391.
- GOODMAN, M., and D. H. CAMPBELL, 1953 Differences in antigenic specificities of human normal adult, fetal and sickle-cell anemia hemoglobin. *Blood* **8**: 422-433.
- HARRIS, J. I., and C. A. KNIGHT, 1955 Studies on the action of carboxypeptidase on tobacco mosaic virus. *J. Biol. Chem.* **214**: 215-230.
- HEIDELBERGER, M., 1956 *Lectures in Immunochemistry*. pp. 150. Academic Press. New York.
- IRWIN, M. R., 1939 A genetic analysis of species differences in Columbidae. *Genetics* **27**: 709-721.
- 1949 On immunogenetic relationships between the antigenic characters specific to two species of Columbidae. *Genetics* **34**: 586-606.

- 1953 Evolutionary patterns of antigenic substances of the blood corpuscles in Columbidae. *Evolution* **7**: 31-50.
- IRWIN, M. R., and L. J. COLE, 1936 Immunogenetic studies of species and of species-hybrids in doves, and the separation of species-specific substances in the backcross. *J. Exptl. Zool.* **73**: 85-108.
- IRWIN, M. R., L. J. COLE, and C. D. GORDON, 1936 Immunogenetic studies of species and of hybrids in pigeons, and the separation of species-specific characters in backcross generations. *J. Exptl. Zool.* **73**: 285-308.
- IRWIN, M. R. and W. J. MILLER, 1961 Interrelationships and evolutionary patterns of cellular antigens in Columbidae. *Evolution* (in press).
- KABAT, E. A., 1956 *Blood Group Substances*. Academic Press. New York.
- KRUMWIEDE, C., G. COOPER, and D. J. PROVOST, 1925 Agglutinin absorption. *J. Immunol.* **10**: 55-239.
- LANDSTEINER, K., 1945 *The Specificity of Serological Reactions*. Harvard Univ. Press. Cambridge, Mass.
- MILLER, W. J., 1956 The hybrid-substance of the erythrocytes of the hybrids between *Columba livia* and *Streptopelia risoria*. *Genetics* **41**: 700-714.
- MILLER, W. J., and C. R. BRYAN, 1953 Serological differentiation of the homozygotes and heterozygotes in backcross birds following a species cross in Columbidae. *Proc. Natl. Acad. Sci. U.S.* **39**: 407-412.
- MORGAN, W. T. J., and W. M. WATKINS, 1959 Some aspects of the biochemistry of the human blood-group substances. *Brit. Med. Bull.* **15**: 109-113.
- OLSON, C., 1943 The inheritance of an agglutinin of the chicken erythrocyte. *J. Immunol.* **47**: 149-154.
- OWEN, R. D., 1959 Immunogenetics. *Proc. 10th Intern. Congr. Genet.* **1**: 364-374. (University of Toronto Press, Toronto.)
- PALM, JOY E., 1955 Species relationships in Columbidae. Ph.D. Thesis, Library, University of Wisconsin.
- PATTERSON, J. T., and W. S. STONE, 1952 *Evolution in the Genus Drosophila*. The Macmillan Company. New York.
- STIMPFLING, J. H., and M. R. IRWIN, 1960 Gene homologies in Columbidae. *Genetics* **45**: 233-242.
- STORMONT, CLYDE, 1952 The F-V and Z systems of bovine groups. *Genetics* **37**: 39-48.
- STORMONT, C., R. D. OWEN, and M. R. IRWIN, 1951 The B and C systems of bovine blood groups. *Genetics* **36**: 134-161.
- WIENER, A. S., 1943 *Blood Groups and Transfusion*. 3rd rev. ed. Charles C Thomas. Springfield.