FURTHER STUDIES OF INTERACTION PRODUCTS OF GENES EFFECTING CELLULAR ANTIGENS IN SPECIES HYBRIDS IN COLUMBIDAE¹

JUDITH W. UNDERKOFER AND M. R. IRWIN

Department of Genetics, University of Wisconsin, Madison

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THE "hybrid substance" of erythrocytes may be described as an antigenic product on the cells of an individual which is not demonstrable on the cells of either parent. It was originally noted on the cells of species hybrids of doves (IRWIN 1932) and later on the cells of other species hybrids of birds: between pigeons and doves (IRWIN and COLE 1936b), between species of ducks (MCGIBBON 1944) and pigeons (BRYAN and MILLER, 1953), and between chickens and pheasants (BACHARACH, MCGIBBON and IRWIN 1960). However, a hybrid substance has not been demonstrated on the cells of all species hybrids in birds (IRWIN 1952). The only example to date of a hybrid substance on the cells of individuals *within* a species was reported for the rabbit by COHEN (1956, 1960).

One purpose of this study was to provide additional evidence to that previously obtained by PALM (1955) of the genetic relationship of serologically related and presumably antithetical antigens, seemingly comprising one antigenic system, of each of five species of the genus *Streptopelia*. Also, the production of new combinations of the antithetical characters has made it possible to study the relationships of interaction products (hybrid substances) associated with them.

MATERIALS AND METHODS

Repeated backcrosses to *Streptopelia risoria* of species hybrids and selected backcross hybrids from respective matings of *chinensis*, *humilis* (also called *tranquebarica*), *orientalis* and *senegalensis* with *risoria* have resulted in the establishment of many different backcross populations. The individuals comprising each of these are essentially identical to *risoria* except for their content of a gene from one of the other species for an antigenic character that differentiates this species from *risoria*. Previous findings (IRWIN 1949) have shown that *chinensis* and *senegalensis* share antigenic characters, not shared with *risoria*, in either an indistinguishable and presumably homologous or a related form. Two of the related characters (d-11 of *chinensis*, now called ch-8, and s-8 of *senegalensis*, now called se-8) are serologically related to a cellular antigen of *humilis* (hu-8 and *orientalis* (or-8) (PALM 1955). Because of their serological resemblance, these four related antigens may be considered as members of a single antigenic system, called group 8. Also included in this system is the contrasting character of *risoria*, called ri-8. It is recognized by serological methods paralleling those described for other antigens by MILLER and BRYAN (1953) and STIMFFLING and IRWIN (1960).

The cells of each backcross hybrid of group 8 (ch-8/ri-8, hu-8/ri-8, or-8/ri-8 and se-8/ri-8)

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invariably possess a hybrid substance (IRWIN and CUMLEY 1945; PALM 1955), but with distinguishing antigenic specificities. Each of these is identified by the reactions of these cells with a specific reagent prepared by the absorption of antisera against each kind of heterozygote with cells of the respective parental species.

Homozygotes (ch-8/ch-8, hu-8/hu-8, or-8/or-8 and se-8/se-8) for each of the four characters of group 8, not in *risoria*, were produced by *inter se* matings of the respective heterozygotes. The cells of the respective homozygotes differ from those of the heterozygotes in that they do not carry either the hybrid substance or the ri-8 character of *risoria*. They may be substituted for the cells of a parental species (as ch-8/ch-8 for *chinensis* cells), but not for those of *risoria* in the absorptions of antisera to the heterozygotes to provide a reagent for each hybrid substance.

Matings between homozygotes, or between a homozygote and a heterozygote, carrying different group-8 antigens produced the six kinds of new heterozygotes: ch-8/hu-8, ch-8/or-8, ch-8/se-8, hu-8/or-8, hu-8/se-8, and or-8/se-8. The presence of both antigens on the cells of these new heterozygotes was confirmed by their reaction with the reagents respectively specific for each character. The absence of ri-8 from each of these six types of cells provided additional evidence of the antithetical relationship of the antigenic characters of group 8.

Similarly, four or five antigens possessed by *Columba guinea* in contrast to *livia* have been transferred to *livia* (IRWIN, COLE and GORDON 1936). One of these, C^s formerly called C) has been shown to be serologically related to ch-8 (BRYAN and IRWIN 1961) but not to se-8 nor to hu-8 and or-8 (unpublished data). Thus, C^s and the contrasting character of *livia*, C¹ (formerly called C'), have been tentatively included in group 8. Appropriate matings between *livia*, or backcross birds containing the C^s antigen of *guinea*, and *risoria* or backcross birds carrying a group 8 antigen other than ri-8, have resulted in a few *livia/risoria* hybrids with new antigenic combinations of group 8, as C^s/ch-8, C^s/ri-8, C^s/se-8, and C¹/ch-8, rather than the typical hybrids between *livia* and *risoria* (C¹/ri-8). The cells of hybrids between *livia* and *risoria* (C¹/ri-8) have previously been shown to possess a hybrid substance which is serologically related to that of the cells of ch-8/ri-8 heterozygotes (IRWIN and CUMLEY 1945), and the chromosome bearing the gene or genes for C¹ is undoubtedly involved (MILLER 1956).

Antisera production, antibody absorptions and subsequent agglutination tests were carried out by methods comparable to those previously reported (IRWIN and COLE 1936a, IRWIN 1939). Antisera were produced in rabbits against the cells of the respective kinds of group-8 birds. From these antisera, reagents against the respective characters, or against the hybrid substances, were prepared at the absorbing dilutions indicated in the tables.

RESULTS

Relationships of the antigenic characters of group 8: The antithetical relationship between the ch-8 and ri-8 characters is amply illustrated in the results of various matings as follows: ch-8/ri-8 × ri-8/ri-8 produced 128 offspring with ch-8/ri-8 and 113 with ri-8/ri-8; ch-8/ri-8 × ch-8/ri-8 produced 30 with ch-8/ch-8, 72 with ch-8/ri-8 and 31 with ri-8/ri-8; ch-8/ch-8 × ch-8/ri-8 produced 41 with ch-8/ch-8 and 36 with ch-8/ri-8; ch-8/ch-8 × ch-8/ch-8 produced 21 with ch-8/ch-8. These data include some previously reported by BRYAN (1953) and PALM (1955). Parallel data on the ratios of the offspring from matings involving hu-8, or-8, or se-8 and *risoria* (ri-8) also indicated that these three antigenic characters are respectively antithetical to ri-8. A deviation from the expected ratio was obtained in the offspring of 12 matings of or-8/ri-8 × or-8/ri-8, resulting in 20 or-8/or-8: 34 or-8/ri-8: 7 ri-8/ri-8. Otherwise, the ratios in the various matings agreed with those expected of contrasting characters. The pertinent data will be furnished on request.

It has not been possible to differentiate serologically between hu-8 and or-8, either as heterozygote with ri-8 or as homozygote (hu-8/hu-8 or or-8/or-8). Each of these characters has absorbed completely from an antiserum the antibodies for the other, and the heterozygote between the two (hu-8/or-8) could not be distinguished from either homozygote (hu-8/hu-8 or or-8/or-8). Thus, the present evidence indicates that hu-8 and or-8 are homologous characters in the parental species, *humilis* and *orientalis*, and therefore these will be considered as a single character, and usually only one (as hu-8) will be listed.

If ch-8, hu-8 (or-8) and se-8 are respectively antithetical to ri-8, they must be antithetical to each other. Also, matings between two homozygotes differing in the antigens of group 8 (as ch-8/ch-8 $\times$ hu-8/hu-8) should produce a new heterozygote (ch-8/hu-8) in which a sister chromosome from each of the two parental species (*chinensis* and *humilis*) has been transferred to *risoria*. In four matings of ch-8/ch-8 with hu-8/hu-8, four of ch-8/ch-8 $\times$ se-8/se-8, and six of hu-8/hu-8 $\times$ se-8/se-8, all the offspring were the expected new heterozygotes. If the matings were made between homozygotes and heterozygotes, as ch-8/ch-8 $\times$ hu-8/ri-8, half of the resulting offspring were new heterozygotes (ch-8/hu-8). In the offspring of matings between a new heterozygote and *risoria*, the two antigens involved have segregated in a 1:1 ratio. For example, from matings to *risoria* (ri-8/ri-8) of the presumed heterozygote ch-8/hu-8, 9 progeny were ch-8/ri-8 and 7 were hu-8/ri-8; from ch-8/or-8, 21 were ch-8/ri-8 and 15 were or-8/ri-8; from ch-8/se-8, 25 were ch-8/ri-8 and 31 were se-8/ri-8; from hu-8/se-8, 48 were hu-8/ri-8 and 46 were se-8/ri-8; and from or-8/se-8, 32 were or-8/ri-8 and 34 were se-8/ri-8. Matings to *risoria* of the heterozygotes hu-8/or-8 produced 19 progeny, either hu-8/ri-8 or or-8/ri-8, but these could not be differentiated. These results are those expected if the antigenic characters of group 8 from the respective species are contrasting characters.

The genetic data can best be explained by concluding that the ch-8, hu-8, or-8, and se-8 characters are antithetical to ri-8 and to each other. The causative gene in each species behaves as an allele to those of the other species, and thus the five alleles for the antigens of group 8 form a multiple allelic series in these five species (*chinensis*, *humilis*, *orientalis*, *risoria*, and *senegalensis*). As implied above, the genes for hu-8 and or-8 are seemingly identical in the two species.

Antigenic specificities of the ch-8 character: Antisera to the cells of birds carrying ch-8/ch-8, after absorption with cells of *risoria* (ri-8/ri-8) should agglutinate only cells carrying ch-8 or a related substance. As shown in Table 1, this reagent (prepared from a pool of five antisera) agglutinated ch-8 cells and also was reactive with cells carrying hu-8 (or-8) and se-8, indicating that one or more antigenic factors or specificities are shared among the antigens of group 8, in contrast to ri-8. It was also reactive with C^s/ri-8 cells, but not with C¹/ri-8 cells. The reaction with C^s/ri-8 cells was by virtue of the cross reaction between C^s and ch-8, as previously reported (BRYAN and IRWIN 1961). Absorptions by C^s/ri-8 cells removed antibodies only for the absorbing cells, whereas all other cells reactive with the original reagent absorbed antibodies for cells with C^s/ri-8. The antigenic specificity shared by ch-8 and C^s/ri-8 is called "a". Further,

TABLE 1

Absorption analysis of pooled antisera prepared against the cells of birds homozygous for the ch-8 antigen (ch-8/ch-8) of chinensis

Antiserum	Absorbed by cells of:	Agglutinations of the cells of:					
		<i>risoria</i>	C ^g / ri-8	hu-8* hu-8	se-8† se-8	ch-8‡ ch-8	C ¹ / ri-8
ch-8/ch-8	<i>risoria</i>	0	+	+	+	+	0
ch-8/ch-8	<i>risoria</i> + C ^g /ri-8	0	0	+	+	+	0
ch-8/ch-8	<i>risoria</i> + or-8/or-8*	0	0	0	0	+	0
ch-8/ch-8	<i>risoria</i> + se-8/se-8†	0	0	0	0	+	0
ch-8/ch-8	<i>risoria</i> + ch-8/hu-8‡	0	0	0	0	0	0
Proposed antigenic specificities of cells		—	a	ab	abc§	abcd	—

Symbols: + = strong agglutination; = weak agglutination; ? = questionable agglutination (microscopic); 0 = no agglutination — at the absorbing dilution (1:30).

Parallel reactions to those listed were obtained in testing the cells of, and in tests with, other types, following absorptions with the cells of:

* hu-8/hu-8 or or-8/or-8.

† hu-8/se-8 and or-8/se-8.

‡ Any combination with ch-8, as ch-8/hu-8, ch-8/or-8, ch-8/se-8 and C¹/ch-8.

§ Specificity "c" was reported by PALM (1955); antibodies to the c specificity were not demonstrable in these antisera.

absorptions with cells of birds possessing hu-8 or se-8 in any combination except with ch-8 removed antibodies for each other but not for any cells carrying ch-8. Thus, hu-8 (or-8) and se-8 share a, and an additional specificity (called b) with the ch-8 cellular antigen. PALM (1955) noted that ch-8 and se-8 share a specificity not shared with hu-8, here called c. Since antibodies to ch-8 remained in the antiserum after absorption with cells carrying either hu-8 or se-8, at least one additional specificity d to a, b, and c of the ch-8 character is required to explain the results listed in Table 1. No differences were detected between the reactions of the homozygotes for ch-8/ch-8, hu-8/hu-8 and se-8/se-8 and the respective heterozygotes with ri-8: ch-8/ri-8, hu-8/ri-8 and se-8/ri-8.

Interaction products on ch-8/ri-8 cells: A reagent against the hybrid substance of cells with ch-8/ri-8 was prepared by absorbing an antiserum with the cells of *risoria* (ri-8/ri-8) and *chinensis* (ch-8/ch-8) or ch-8/ch-8 cells. As given in Table 2, such a reagent reacted with cells having the following combinations of antigens: ch-8/hu-8, ch-8/ri-8, ch-8/se-8, hu-8/se-8, se-8/ri-8, se-8/se-8, C^g/ri-8, C¹/ri-8, and C^g/se-8. Of these, cells carrying ch-8/hu-8, or se-8 in any combination, removed antibodies from this reagent for all cells except those with ch-8/ri-8, C^g/ri-8 and C¹/ri-8. The homozygotes and heterozygotes for se-8 gave parallel reactivities with this reagent, indicating that the interaction product of ch-8/ri-8 cells is related to the cellular antigen se-8 that is normally present on the cells of *senegalensis* (all individuals tested of *senegalensis* have carried se-8). This antigenic factor of the hybrid substance of ch-8/ri-8 cells is called e.

Although the cells of the two kinds of hybrids between *livia* and *risoria* (C^g/ri-8 and C¹/ri-8) appeared to be nearly equivalent in their respective reactivities to the reagent analyzed in Table 2, C^g/ri-8 cells did not always absorb antibodies for all the cells carrying specificity e. Definite agglutination with this reagent was noted of cells ch-8/hu-8, ch-8/se-8, se-8/ri-8 and se-8/se-8, with no or question-

TABLE 2

Reactions of the reagents for the hybrid substance prepared from pooled antisera (84S1 and 87S2) against ch-8/ri-8 cells*

Antiserum	Absorbed by the cells of:	Agglutinations of the cells of:					
		<i>risoria</i>	ch-8 ch-8	se-8† se-8	C ^g ri-8	C* ri-8	ch-8 ri-8
ch-8/ri-8	<i>risoria</i> + ch-8/ch-8†	0	0	+	+	+	+
ch-8/ri-8	<i>risoria</i> + ch-8/ch-8 + se-8/se-8‡	0	0	0	+	+	+
ch-8/ri-8	<i>risoria</i> + ch-8/ch-8 + C ^g /ri-8	0	0	0, +	0	0, ?	+
ch-8/ri-8	<i>risoria</i> + ch-8/ch-8 + C ¹ /ri-8	0	0	0	0	0	+
Proposed antigenic specificities of cells		—	—	e	f	ef	efg

Symbols: Same as in Table 1. Absorbing dilution = 1:10.

* These antisera were used in previous tests by PALM (1955).

† Cells of the following phenotypes were not reactive with this reagent: hu-8/hu-8, hu-8/ri-8, C^g/C^g, C¹/C¹ and C¹/ch-8.

‡ Parallel reactions to those listed (with exceptions as noted in the text) were obtained in testing the cells of, and following absorptions with the cells of: ch-8/hu-8, ch-8/se-8, hu-8/se-8, or-8/se-8, se-8/ri-8 and C^g/se-8.

able reactions in tests with hu-8/se-8, or-8/se-8 and C^g/se-8. In contrast the cells of the *livia/risoria* hybrid (C¹/ri-8) consistently exhausted the reagent of antibodies for all cells with the e specificity, except at one test with ch-8/se-8 cells. Tentatively, specificity f is assigned to C^g/ri-8 cells and e f to those with C¹/ri-8. Cells with ch-8/ri-8 were agglutinated by each of the different absorbed fluids given in Table 2, indicating the presence of factors e and f, and also of a third specificity g of the hybrid substance.

On the basis of these results, it appears that the interaction product of the genes for ch-8 of *chinensis* and ri-8 of *risoria* is related to se-8 of *senegalensis* (e) but not to hu-8 and or-8 of *humilis* and *orientalis*. The presence of this specificity (e) on the ch-8/hu-8 cells (also ch-8/or-8) implies an interaction product in this heterozygote. (As will be stated later, the reciprocal reaction was not observed in the antisera to ch-8/hu-8.) Further, this factor (e) normally carried by se-8 cells, is also present on the cells of the F₁ of *livia/risoria* (C¹/ri-8), but seemingly not on C^g/ri-8 cells. Since cells with C¹/ch-8 were not reactive with this reagent for the hybrid substance, it appears that the cells of this heterozygote did not possess a hybrid substance recognized by this reagent.

Other antisera with antibodies to the interaction product of ch-8/ri-8 cells have revealed additional specificities of this hybrid substance. In Table 3 (Part A), the reagent for the hybrid substance was reactive with cells of the heterozygote, se-8/ri-8 (see columns 1, 2 and 3) but not with those of the homozygote se-8/se-8. In fact, absorption of the reagent by se-8/se-8 cells did not noticeably affect the reactivity of the reagent (compare reactions in columns 1 and 2). The antigenic factor shared by ch-8/ri-8 with se-8/ri-8 cells is called h, and is present also on C^g/ri-8 and C¹/ri-8 cells (columns 4 and 5), since these two kinds of cells removed antibodies for se-8/ri-8 cells.

The antibodies to the hybrid substance in an antiserum engendered against the cells of *chinensis/risoria* hybrids were directed against only ch-8/ri-8 cells, since absorption by these cells removed all antibodies from the reagent (Part B, Table

TABLE 3

Further analyses of antibodies to the hybrid substance of ch-8/ri-8 cells

Test cells	A. Absorption analysis of pooled antisera against ch-8/ri-8 cells absorbed by cells of <i>risoria</i> and ch-8/ch-8*, plus those listed:					B. Absorption analysis of an antiserum against cells of the <i>F₁</i> - <i>chinensis/risoria</i> , absorbed with the cells of <i>chinensis</i> and <i>risoria</i> †, plus those listed:								
	se-8/ se-8	se-8/ ri-8	C ^g / ri-8	C ^l / ri-8		ch-8/ ch-8	ch-8/ ri-8	hu-8/ ri-8	or-8/ ri-8	se-8/ ri-8	C ^g / ri-8	C ^l / ri-8	hu-8/ hu-8	
<i>risoria</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>chinensis</i>	.	.	.	.	.	0	0	0	0	0	0	0	0	0
ch-8/ch-8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
se-8/se-8	0	0	0	0	0	0	.	.	.	.	.	.	.	.
ch-8/ri-8	+	+	+	+	+	+	+	0	+	+	+	+	+	+
hu-8/ri-8	0	.	.	.	.	+	+	0	0	0	0	0	0	+
or-8/ri-8	.	.	.	.	.	+	?	0	0	0	0	0	0	?
se-8/ri-8	+	+	0	0	0	?	?	0	0	0	0	0	0	?
C ^g /ri-8	+	+	+	0	0,?	+	+	0	+	+	+	0	0	+
C ^l /ri-8	+	+	+	0,?	0	+	+	0	+	+	+	0	0	+
Column	1	2	3	4	5	6	7	8	9	10	11	12	13	14

Symbols: Same as in Table 1.

* This reagent was not reactive with cells carrying hu-8/hu-8, ch-8/hu-8, ch-8/se-8, C^l/ch-8, C^g/se-8.

† This reagent was not reactive with the cells carrying hu-8/hu-8 or or-8/or-8.

3, column 8). The reactions of this reagent differed from those given in Part A of the table in that this second reagent agglutinated the cells of the heterozygotes hu-8/ri-8 and or-8/ri-8 (column 6) and these by absorption removed the antibodies for each other (columns 9 and 10). Although the cells with se-8/ri-8 were only faintly reactive, if at all, with this reagent (column 6), they removed antibodies for hu-8/ri-8 and or-8/ri-8 cells (column 11) suggesting that the heterozygotes hu-8/ri-8 (or-8/ri-8), and se-8/ri-8 shared a common constituent of the hybrid substance with ch-8/ri-8 cells. This antigenic specificity is termed i, and is shared also with C^g/ri-8 and C^l/ri-8 cells (see reactions in columns 12 and 13).

Interaction products on ch-8/or-8 cells: Although the hu-8 and or-8 characters have been indistinguishable, antisera to ch-8/or-8 cells and two antisera against ch-8/hu-8 cells have exhibited a difference in antibody specificities. However, this difference is more readily explained by assuming individuality in the response of the rabbits than to differences in the antigenic characters. The data of Table 4, Part A, show that the reagent for the hybrid substance of ch-8/or-8 contained antibodies with at least two specificities. One of these, called j, was directed against ch-8/se-8 and hu-8/se-8 cells as well as those with ch-8/or-8. Neither of these cells removed completely the antibodies from the antiserum for the homologous cells, ch-8/hu-8, requiring the assumption of another specificity (k) for the interaction product on ch-8/hu-8 cells, both j and k being present.

A mixture of two antisera against ch-8/or-8 cells, when absorbed to provide a reagent for the hybrid substance, was reactive with ch-8/se-8 cells as well as with ch-8/or-8 cells, but not with hu-8/se-8 cells (Part B, Table 4). At least one specificity (l) in addition to the j and k previously assigned to ch-8/hu-8 cells is required to explain the reactions of Part B of Table 4. Comparable results were

TABLE 4

Absorption analysis of an antiserum against ch-8/or-8 cells (A) and of a mixture of two antisera against ch-8/hu-8 cells (B)

Test cells	Absorbed by cells of <i>risoria</i> , ch-8/ch-8 and hu-8/hu-8, plus those listed (1:10)							Proposed antigenic specificities
	Part A				Part B			
	—*	ch-8/hu-8	ch-8/se-8	hu-8/se-8	—*	ch-8/hu-8	ch-8/se-8	
<i>risoria</i>	0	0	0	0	0	0	0	..
ch-8/ch-8	0	0	0	0	0	0	0	..
ch-8/hu-8†	+	0	+	+	+	0	?	jkl
ch-8/ri-8	0	0	0	0	0	0	0	..
ch-8/se-8	+	0	0	0	+	0	0	j l
hu-8/hu-8	0	0	0	0	0	0	0	..
hu-8/se-8	+	0	0	0	0	.	.	j

Symbols: Same as in Table 1.

* These reagents were not reactive with the following cells: hu-8/ri-8, hu-8/or-8, or-8/ri-8, se-8/ri-8, C^κ/ri-8, C^κ/se-8, C^λ/ch-8 and C^λ/ri-8.

† The cells of a hybrid between *chinensis* and *risoria* (ch-8/or-8) were indistinguishable from those of ch-8/hu-8 and ch-8/or-8 heterozygotes in agglutinations and in absorbing capacities of the antibodies to the hybrid substance.

obtained by the use of an antiserum to the cells of the F₁-*chinensis/orientalis* (therefore ch-8/or-8), in that strong antibodies of this antiserum for the hybrid substance were completely absorbed by ch-8/hu-8 and ch-8/or-8 cells. None of these antisera to ch-8/hu-8 or ch-8/or-8 cells contained antibodies to the factor "e" of ch-8/ri-8 cells as described above, and thus did not conform to expectation.

Interaction products of ch-8/se-8 cells: Two antisera have been obtained against ch-8/se-8 cells which contained weakly expressed antibodies for a hybrid substance of ch-8/se-8 cells. Neither reagent was reactive with ch-8/ri-8 cells. These antibodies were not stable, and disappeared before further tests were made. It is not known whether these antibodies were direct against specificity l, or were peculiar to the heterozygote ch-8/se-8.

The various antigenic factors that have been postulated by virtue of the experimental results indicate the relationship of the factors of ch-8, as a homozygote and in heterozygous combinations, to those of various combinations of other members of group 8. These are listed as follows:

Cells	Antigenic factors	Cells	Antigenic factors
ch-8/ch-8	abcd	hu-8/hu-8	ab
ch-8/hu-8	abcd e jkl	hu-8/ri-8	ab i
ch-8/or-8	abcd e jkl	hu-8/se-8	abc e j
		or-8/or-8	ab
ch-8/ri-8	abcd efghi	or-8/ri-8	ab i
		or-8/se-8	abc e j
ch-8/se-8	abcd e j l	se-8/ri-8	abc e hi
ch-8/C ^κ	abcd	se-8/se-8	abc e
ch-8/C ^λ	abcd	ri-8/ri-8	
C ^κ /ri-8	a f hi		
C ^λ /ri-8	ef hi		

In future papers the relationship of the interaction products associated with heterozygotes other than those considered here will be presented.

DISCUSSION

In discussing these experimental results, the assumption is made that the respective hybrid substances are the result of the interaction of genes. Granted that the interaction of the antigens *per se* cannot be excluded as a possible explanation, the fact that no interaction products have been observed in heterozygotes of some other antigenic systems makes such an explanation less plausible for the hybrid substances of the group-8 characters. The term "interaction of genes" does not imply that there has been a change in the genes themselves, only in their final product. Presumably the interaction occurs at some place in the chain of reactions between the causative genes and their end products, the cellular antigens.

The data presented indicate strongly that the interaction products associated with various combinations of the ch-8 cellular antigen of *chinensis* (ch-8/ri-8, ch-8/hu-8 or ch-8/or-8, ch-8/se-8) are limited to the heterozygotes. In contrast, the cells of the homozygotes (ch-8/ch-8, hu-8/hu-8, or-8/or-8, ri-8/ri-8, se-8/se-8, and C^s/C^s), derived from *inter se* matings of the respective backcross birds, do not carry a hybrid substance (BRYAN and MILLER 1953; PALM 1955).

The ability of two alleles as heterozygotes to produce the same character that each effects as a homozygote, plus an additional product, may well constitute a basis for an explanation of the phenomenon of heterosis.

At present, the only difference that seemingly exists between the different group-8 antigens in the respective heterozygotes and homozygotes (as ch-8 and ri-8 in the heterozygote ch-8/ri-8, or in the homozygotes ch-8/ch-8 and ri-8/ri-8) is that which might be explained by gene dosage. That is, the homozygotes, as ch-8/ch-8 and ri-8/ri-8, seemingly possess more of the ch-8 or ri-8 characters than do the heterozygotes, as ch-8/ri-8 (unpublished data). The difference then is quantitative, not qualitative. One might visualize the homozygotes as possessing more antigenic sites for the ch-8 and ri-8 antigens on the surface of the erythrocytes than are present on the cells of the heterozygotes. Alternatively, there might be a greater concentration of these antigens at the sites on the cell surfaces in the respective homozygotes. In addition, however, the heterozygotes carry the hybrid substance, so that each causative gene involved in the production of the cellular antigen of group 8, peculiar to the original species, continues to effect this antigen and by interaction with its allele, from another species, produces the hybrid substance. This statement is based on the assumption that the genes which interact to effect the hybrid substances are those that effect the group-8 antigens. It is possible that genes linked to those producing the antigens of group 8 may be interacting. The principal evidence indicating that the genes for the group-8 characters are those that by interaction effect the hybrid substance is that no separation of the group-8 character and the hybrid substance of the different heterozygotes has ever been observed. The possibility of linked genes cannot be excluded, but seems less likely.

If the contrasting antigens in the respective heterozygotes are contained in a single antigenic molecule, the addition of the antigenic specificities of the hybrid substance is presumably an addition to the same molecule. In contrast, if the contrasting antigens are located in separate molecules, an interesting question presents itself. Are the antigenic specificities of the hybrid substance also in a separate molecule, possibly forming a bridge between the two independent molecules, or are they combined with one or the other of the other two molecules? Unfortunately, no method is presently available for testing these possibilities. The only information available on this question known to the authors is the finding by MORGAN and WATKINS (1956) that the A and B characters of humans are members of a single molecule in saliva of AB secretors and ovarian cyst material from AB heterozygotes. Whether the AB substances on the erythrocytes of the heterozygotes would belong to a single molecule is an open question.

Two general points emerge from an examination of the data. First, the antigenic specificities of the hybrid substance of the ch-8/ri-8 cells are shared more widely with other heterozygotes with ri-8 in the group-8 system than are those of ch-8/hu-8 (therefore ch-8/or-8) and ch-8/se-8 (compare specificities of Tables 2, 3 and 4). Secondly, there appears to be an inverse relationship between the number of antigenic specificities shared by ch-8 with other members of group-8 and the appearance of a well defined hybrid substance. Thus, ch-8 is more closely related to se-8 than to hu-8 and or-8 (see data of Table 1), and only weakly expressed antibodies to the interaction product of ch-8/se-8 cells have been obtained to date, in contrast to potent antibodies against the hybrid substance of ch-8/hu-8 and ch-8/or-8 cells.

The analyses of the interaction products of C^s/ch-8 and C¹/ch-8 cells will be published elsewhere. However, definite antibodies have been obtained against the hybrid substance of these two presumed heterozygotes. The C^s and ch-8 antigens are reciprocally cross reactive, but those of C¹ and ch-8 are not. Furthermore, in these tests the interaction product of C¹/ri-8 cells appears to be more similar to the hybrid substance of ch-8/ri-8 cells than is the interaction product of C^s/ri-8 cells, whereas C¹/ch-8 cells were not reactive with the reagents available for the hybrid substance of ch-8/ri-8 cells. These results raise the question of what will be the relationships of the hybrid substances that may be demonstrable among new combinations of C^s and C¹ in *livia*/*risoria* hybrids: as C^s/ri-8, C^s/ch-8, C^s/se-8, C¹/ch-8, C¹/ri-8 and C¹/se-8 cells.

SUMMARY

One of the antigenic characters of the erythrocytes that differentiates each of four species of *Streptopelia*—*chinensis*, *humilis*, *orientalis* and *senegalensis*—from *risoria* has been isolated in the backcross populations following the production of the respective species hybrids in matings with *risoria*. These form a genetic system of related and antithetical antigens called group 8: ch-8 of *chinensis*, hu-8 of *humilis*, or-8 of *orientalis*, ri-8 of *risoria*, se-8 of *senegalensis*, and possibly C^s of *Columba guinea* and C¹ of *livia*. Ch-8 of *chinensis* shares one or more of four antigenic specificities (abcd), not present in ri-8, with the other three characters

in the *Streptopelia*, one with C^s of *guinea*, but none with *livia* (C¹). Also, the backcross birds with ch-8/ri-8 antigens carry a minimum of five antigenic specificities (efghi) of a hybrid substance not present in either parental species. Of these, from one to four are shared with other heterozygotes, either of group 8 or related to it.

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