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Relationships among interaction products in hybrids between pigeons and doves, following transfer of genes between species*

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1. INTRODUCTION

Hybrids between the common pigeon, *Columba livia*, and the ringneck dove, *Streptopelia risoria*, possess a complex of antigenic specificities of the red blood cells, the 'hybrid substance', that distinguishes them from either parent (Irwin & Cole, 1936; Miller, 1956; Underkofler & Irwin, 1965). Cohen (1962) used the term 'interaction antigen' for this phenomenon in rabbits.

Following matings to *livia* of the hybrids between *C. guinea* and *livia*, and of selected backcross hybrids in successive backcrosses, four antigens (A^g, B^g, C^g and E^g) peculiar to *guinea* have been isolated as units in *livia* (Irwin, Cole & Gordon 1936; unpublished data). These backcross birds are indistinguishable from *livia* except for the presence on their erythrocytes of the antigen transferred from *guinea*. The respective contrasting characters in *livia* (A^l, B^l, C^l and E^l) were recognized by serological tests after backcross birds homozygous for each of the four antigens from *guinea* had been produced (Miller & Bryan, 1953). A hybrid substance not found in either parental species has been demonstrated (Bryan & Miller, 1953) on the cells of all backcross birds carrying the C^g antigen of *guinea* (i.e. C^g/C^l), but not on the cells of homozygotes (C^g/C^g). The cells of the heterozygotes (C^g/C^l) possess completely the C^g and C^l antigens, seemingly in slightly lesser amounts than in the respective homozygotes (Bryan & Irwin, 1961). In addition, they also carry the hybrid substance. Present evidence indicates that the gene for the C^l antigen of *livia* is involved also in the production of the hybrid substance on the erythrocytes of the hybrids between *livia* and *risoria* (Miller, 1956).

Similarly, antigenic characters peculiar to each of four species of *Streptopelia*—*chinensis*, *humilis* (or *tranquebarica*), *orientalis* and *senegalensis*—in contrast to

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risoria have been transferred to and isolated as units in *risoria*, following matings to *risoria* of the four kinds of species hybrids and selected backcross hybrids (Irwin, 1939; Irwin & Cumley, 1947; unpublished data). Among these there are four antigens which are interrelated and antithetical to each other and to an antigen of *risoria* (Underkofler & Irwin, 1965). These five antigens form an antigenic system, called group-8, and consist of ch-8 from *chinensis*, hu-8 from *humilis*, or-8 from *orientalis*, ri-8 from *risoria* and se-8 from *senegalensis*. The heterozygotes with ri-8—as ch-8/ri-8, hu-8/ri-8, or-8/ri-8 or se-8/ri-8—respectively possess a hybrid substance, but the homozygotes do not.

Cross reactivity has been noted between the C^g antigen of *guinea* and ch-8 of *chinensis* (Bryan & Irwin, 1961), and also between the hybrid substances of the F_1-*livia*/*risoria* hybrids (C^l/ri-8) and the ch-8/ri-8 cells in backcross birds (Irwin & Cumley, 1945). Therefore it seemed pertinent to test in the *livia*/*risoria* hybrids whether a substitution of C^g for C^l in the *livia* parent, and of ch-8 or se-8 for ri-8 in the *risoria* parent, would alter the antigenic specificities of the expected hybrid substances. In essence, for the production of the hybrid substance, the progeny from such matings would simulate the following matings: *livia* × *chinensis*, *livia* × *senegalensis*, *guinea* × *risoria* and *guinea* × *senegalensis*. (The probability of obtaining offspring from matings of these species is extremely low.)

The results of the tests utilizing antisera for and erythrocytes from the six kinds of species hybrids are given in this paper.

2. MATERIALS AND METHODS

Matings between pigeons carrying C^l/C^l, C^g/C^l or C^g/C^g (C^g from *guinea*, C^l of *livia*) and doves (*risoria*) with ch-8 from *chinensis*, ri-8 from *risoria* or se-8 from *senegalensis* have resulted in hybrids with the following new combinations of antigens of the C- and group-8 systems, as compared with the standard F_1-*livia*/*risoria* pattern (C^l/ri-8): C^g/ch-8 (one hybrid), C^g/ri-8 (four hybrids), C^g/se-8 (one hybrid), C^l/ch-8 (one hybrid) and C^l/se-8 (two hybrids). This symbolism is used for simplicity and does not necessarily imply that the genes for the antigens of the C system and of group-8 are allelic.

Antisera were prepared against the F_1-pigeon/dove hybrids of the various types by injecting rabbits with the washed erythrocytes of each type. Such antisera were absorbed by the cells of a pool of parental type birds, including the actual parents when possible, until all agglutinating activity for the parental type cells was removed. Any individual differences that may have existed in the antigens of the parental types were largely, if not completely, erased by pooling the cells of individuals for the absorptions. The fluids remaining after such treatment constituted the *reagents* specific for the respective hybrid substances. Antibody production, absorption, and agglutination testing were done as described in previous papers (for example, Irwin, 1939) except that (*a*) absorptions were usually done at lower dilutions of antiserum (see the tables for the dilutions used), and (*b*) there was no attempt to study agglutination reactions that appeared only after

additional incubation at low temperatures. All reactions which appeared negative to the naked eye were checked microscopically.

Erythrocytes of the following types were tested (with exceptions as noted in the tables) with the reagents for the respective hybrid substances of each of the six kinds of hybrids between *livia* and *risoria*: *livia* (C^l/C^l) *C. rufina*, *risoria* (ri-8/ri-8), F_1-*capicola/senegalensis*, *risoria* birds to which had been transferred an antigen of group-8 from one of the four species and which carried one of the following combinations produced by matings among the backcross birds (ch-8/ch-8, ch-8/hu-8, ch-8/or-8, ch-8/ri-8, ch-8/se-8, hu-8/ri-8, hu-8/se-8, or-8/ri-8, or-8/se-8, ri-8/se-8, se-8/se-8), *livia* to which had been transferred the C^g from *guinea* (C^g/C^g or C^g/C^l), and each of the six kinds of F_1-hybrids between *livia* and *risoria* that carried different combinations of antigens from the C and group-8 systems (C^g/ch-8, C^g/ri-8, C^g/se-8, C^l/ch-8, C^l/ri-8 and C^l/se-8).

3. RESULTS

The results to be presented are based on the analysis of antisera prepared against the erythrocytes of F_1-*livia/risoria*, carrying one of the six different combinations of antigens from the C system of *Columba* and the group-8 system of *Streptopelia*. The terms antigenic specificity and antigenic factor are used interchangeably to describe differences and similarities of the erythrocytes under test.

(i) *Analysis of an anti-C^l/se-8 serum (1J6)*

Five of the six antisera that were produced against the erythrocytes of an F_1-pigeon/dove (C^l/se-8) contained antibodies to the C^l/se-8 interaction antigen. One of these, 1J6, reacted with the widest range of cell types. When this antiserum was absorbed completely by the erythrocytes of livia (C^l/C^l) and se-8/se-8 birds (Table 1), antibodies for a hybrid substance remained which agglutinated only the

Table 1. *Absorption analysis of an antiserum (1J6) to the erythrocyes of an F_1-*livia/risoria *carrying the se-8 antigen of* senegalensis *(C^l/se-8)*

Test cells*	Reactions with reagents prepared by absorption with parental type cells (*livia* and se-8/se-8) only, or additionally in combinations as shown					Proposed antigenic specificities
		C^g/ri-8	C^g/se-8	C^l/ch-8	C^l/ri-8	
C^l/se-8	+	+	+	0	+	**ab**
C^g/ri-8	+	0	0	0	0	**a**
C^g/se-8	+	0	0	0	0	**a**
C^l/ch-8	+	+	+	0	+	**ab**
C^l/ri-8	+, 0	0	0	0	0	**a**

Symbols: + = definite agglutination; 0 = no agglutination; +, 0 = differing results at different tests—at the absorbing dilution (1:10).

* Cells with C^g/ch-8 and ch-8/ch-8 were not tested. No agglutination was observed with any other cell type listed in Materials and Methods.

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cells of hybrids of types C^g/ri-8, C^g/se-8, C^l/ch-8, and C^l/ri-8, as well as those of the homologous (immunizing) cell type (C^l/se-8).

It may be noted in Table 1 that the respective absorptions of the reagent with the cells of types C^g/ri-8, C^g/se-8 and C^l/ri-8 removed antibodies for themselves and the other two types, but not for the cells of the hybrids with types C^l/ch-8 or C^l/se-8. An antigenic specificity, called **a**, distinguishes these three types of cells from the parental types of cells, *livia* (C^l/C^l) and *risoria* with se-8. Since cells of types C^l/ch-8 and C^l/se-8 removed antibodies for each other and also for the other three types of reactive cells, these two types carry factor **a** and at least one in addition, **b**.

The analysis of this antiserum shows that the substitution of se-8 for ri-8 in *risoria* resulted in a partial change in the hybrid substance of the resulting F_1-*livia*/*risoria* hybrids. The existence of a common antigenic specificity **a** among the five types of hybrids is evidence that the hybrid substances of these new types of *livia*/*risoria* hybrids are antigenically related. Further, since the cells of type C^l/ch-8 were indistinguishable by these tests from those of C^l/se-8, it appears that the substitution of ch-8 for ri-8 was equivalent in these *livia*/*risoria* hybrids (with C^l) to the substitution of se-8 for ri-8. However, other antisera might reveal additional antigenic specificities by which the two hybrids (C^l/ch-8 and C^l/se-8) may differ.

Table 2. *Absorption analysis of an antiserum (A7) to the erythrocytes of an F_1*-livia/risoria *carrying the C^gantigen of* guinea *and the ch*-8 *antigen of* chinensis (*C^g/ch*-8)

Test cells*		Reactions with reagents prepared by absorption with parental type cells (C^g/C^g and ch-8/ch-8) only, or additionally in combinations as shown					Proposed antigenic specificity
		C^g/ri-8	C^g/se-8	C^l/ch-8	C^l/ri-8	C^l/se-8	
C^g/ch-8	+			0	0		c
C^g/ri-8	+	0	0	0	0	0	c
C^g/se-8	+	0	0	0	0	0	c
C^l/ch-8	+	0	0	0	0	0	c
C^l/ri-8	+	0	0	0	0	0	c
C^l/se-8	+	0	0	0	0	0	c

Symbols: See Table 1. Absorbing dilution = 1:10.

* Cells with se-8/se-8 were not tested. No agglutination was observed with any other cell type listed in Materials and Methods.

(ii) *Analysis of an antiserum against cells with C^g/ch*-8

One of the six rabbits injected with the cells of a single hybrid with the combination of C^g/ch-8 produced reasonably potent antibodies for a hybrid substance. The reagent prepared from this antiserum (A7), following absorption with C^g/C^g and ch-8/ch-8 cells, reacted only with the cells of each of the six kinds of pigeon/dove hybrids, as is given in Table 2. Seemingly, this antibody reacted with but one antigenic specificity, since absorption by each of five of the reactive types of cells

removed reactivity for themselves and for the others. Unfortunately, the early death of this hybrid (C^g/ch-8) prevented the testing of its cells with three of the reagents of the table, as well as with many other reagents. Furthermore, the cells of C^g/ch-8 and C^l/ch-8 appeared to be indistinguishable, or nearly so, as revealed by their ability reciprocally to absorb antibodies for each other from other antisera (cf. Tables 2 and 5). Therefore the tentative assignment of specificity **c** to each of the six kinds of cells in Table 2 may duplicate the assignment of specificity **a** of Table 1.

(iii) *Analysis of an antiserum to C^g/ri-8 cells*

A wider spectrum of antibody activity was noted in two antisera against cells from hybrids with C^g/ri-8, as is depicted in Table 3, than in those against the cells of hybrids with C^l/se-8 and C^g/ch-8 (Tables 1 and 2). Two antisera of twelve that were produced against C^g/ri-8 cells contained antibodies reactive at low dilutions following absorption with C^g/C^g and *risoria* cells, but with different specificities. Thus, the reagent for the hybrid substance of each antiserum was reactive with four of the five types of pigeon/dove hybrid cells tested, not with the cells of C^l/ch-8, with those

Table 3. *Absorption analysis of antisera (ML35, ML37) to the erythrocytes of F_1-*livia/risoria *hybrids carrying the C^g antigen of* guinea (*C^g*/ri-*8*)

	Reactions with reagents prepared by absorption with C^g/C^g and *risoria* cells only, or additionally in combinations as given below							
	Antiserum ML35				Antiserum ML37			
Test cells*		C^g/se-8†	ch-8/ri-8	F_1-*cap./sen.*		C^l/ri-8	C^l/se-8	Proposed antigenic specificities
C^g/ri-8	+	0	+	+, 0	+	+	+	**defgh**
C^g/se-8	+	0	+	0	+	0	0	**de(f)‡**
C^l/ch-8	0				0			none
C^l/ri-8	+	0	+	0	+	0	0	**de(f)**
C^l/se-8	+	0	+	0	+	+	0	**de(f)g**
ch-8/ri-8	+, 0		0	0	+, 0	0	0	**d**
C. rufina	+	0	+		+	0	0	**de(f)**
F_1-*capicola/senegalensis*	+		+	0	+	0	0	**de**
Antibody specificities in reagent		—	**e, f**	**f**		**g, h**	**h**	

Symbols: See Table 1. Absorbing dilution = 1 : 4.

* Cells with C^g/ch-8 and se-8/se-8 were not tested. No agglutination of any other cell type listed in Materials and Methods was observed.

† Parallel results were obtained following absorptions with each of the following types of cells: C^l/ri-8, C^l/se-8 or *rufina*.

‡ The specificity '**f**', if enclosed in parentheses, was assigned because of the absorptive capacities of the cells, not because of their agglutination.

of ch-8/ri-8, *C. rufina* and some but not all F_1-*capicola/senegalensis* (Table 3). If one assumes from the results that antiserum ML35 contained antibodies reactive with three specificities of the hybrid substances, **d**, **e**, and **f**, the cells with ch-8/ri-8 carry **d**, those of the F_1-*capicola/senegalensis* carry **de**, and those with C^g/ri-8, C^g/se-8, C^l/ri-8, C^l/se-8, and *rufina* carry **def**.

There is a discrepancy between the agglutinability and absorptive capacity of the cells of types C^g/se-8, C^l/ri-8, C^l/se-8 (**def**) in that antibodies to each of these (in antiserum ML35) were absorbed by the cells of F_1-*capicola/senegalensis* (**de**) whereas the cells of each of these three types and those of *rufina* exhausted the antibodies for the immunizing cells, C^g/ri-8. Specificity **f** is assigned to these four kinds of cells because of their absorptive capacity. Differences in agglutination and absorbing capacity of cells have been often encountered, particularly if the antibodies are weakly expressed, as obtains for these two antisera.

It seems unlikely that the antigenic factors **de** assigned to the cells of some of the F_1-*capicola/senegalensis* represent an interaction antigen in these hybrids. The cells of some *senegalensis*, not of all, have been reactive with the reagent for the hybrid substance of F_1-*livia/risoria* (C^l/ri-8), and the reactions here recorded for cells of F_1-*capicola/senegalensis* could have been due to an antigen of *senegalensis*. The cells of backcross birds with se-8 (ri-8/se-8) were weakly reactive with the reagent made from antiserum ML37, but the results following absorption were not satisfactory and are not recorded in the table.

The results obtained with tests of antiserum ML37, as given in Table 3, may be explained by assuming a minimum of three antigenic specificities of the hybrid substance on the reactive cells, **d** as was recognized by antibodies in ML35, and **g** and **h**. The cells of the F_1-*livia/risoria* birds (C^l/ri-8) removed antibodies for **d**, leaving reactivity for cells with C^l/se-8 by virtue of factor **g**, and **h** representing a factor found only on C^g/ri-8 cells.

(iv) *Analysis of an antiserum (ML18) to cells with C^g/se-8*

Antibodies for a hybrid substance were produced in only one (ML18) of twelve rabbits immunized with C^g/se-8 cells. The reagent for the hybrid substance was reactive with four of five kinds of cells from pigeon/dove hybrids, being non-reactive with cells carrying C^l/ch-8 (Table 4). It was weakly reactive with ch-8/ch-8 cells, and these removed reactivity only for themselves. The specificity **i** is assigned to these cells. The appearance of this specificity (**i**) on ch-8/ch-8 cells and not on ch-8/ri-8 cells is an anomaly for which no rational explanation is offered at present.

Both ch-8/se-8 and C^g/ri-8 type cells removed antibodies for specificity **i** (of ch-8/ch-8 cells), but weakly reacting antibodies for the other kinds of cells usually remained in the reagent following the respective absorptions (Table 4). To explain these results, specificities **ij** are assigned to ch-8/se-8 cells, and **ik** to those carrying C^g/ri-8. The erythrocytes with C^l/se-8 removed antibodies for cells with specificities **i**, **ij** and **ik**, and also for the cells of the standard F_1-*livia/risoria* hybrid, C^l/ri-8, to which only specificity **l** is assigned. Thus C^l/se-8 cells contain factors **ijkl**.

Table 4. *Absorption analysis of an antiserum (ML18) to the erythrocytes of an* F_1-livia/risoria *with the* C^g *of* guinea *and the se-8 of* senegalensis (C^g/*se-8*)

Test cells*	Reactions with reagents prepared by absorptions with parental type cells (C^g and se-8) only, or additionally in combinations as given below						Proposed antigenic specificities
		C^g/ri-8	C^l/ri-8	C^l/se-8	ch-8/ch-8	ch-8/se-8	
C^g/se-8	+	+	+	+	+	+	**ijklm**
C^g/ri-8	+	0	+, 0	0	+, 0	+, 0	**ik**
C^l/ch-8	0						none
C^l/ri-8	+	+, 0	0	0	+	+	**l**
C^l/se-8	+			0			**ijkl**
ch-8/ch-8	+	0	+	0	0	0	**i**
ch-8/se-8	+	+, 0	+, 0	0	+, 0	+, 0	**ij**

Symbols: See Table 1. Absorbing dilution = 1 : 7.

* Cells with C^g/ch-8 were not tested. No agglutination was observed with any other cell type listed in Materials and Methods.

Further, since C^l/se-8 cells did not absorb antibodies for the homologous cells, C^g/se-8, an additional specificity **m** is assigned to cells with C^g/se-8. It is interesting that a reciprocal comparison of the reagents prepared from the respective antisera to C^l/se-8 (Table 1) and C^g/se-8, in which the major difference is that C^g has been substituted for C^l in the hybrid, indicates that there is a pronounced antigenic difference between these two kinds of cells.

(v) *Analysis of an antiserum to cells* C^l/*ch-8*

One antiserum of six produced by immunization with the cells of type C^l/ch-8 carried antibodies at a relatively low dilution against four types of cells, after absorption with *livia* and ch-8/ch-8 cells (Table 5). Only three of the six types of *livia/risoria* hybrid cells (C^l/ch-8, C^l/se-8, and C^g/ch-8) were reactive with this reagent, and possibly only one heterozygote (ch-8/se-8) of the doves. The two types of cells (C^g/ch-8 and C^l/se-8) other than the immunizing type (C^l/ch-8) that were reactive with this reagent absorbed antibodies for the homologous cells and for themselves. Hence this reagent seemingly recognized only one antigenic specificity, **n**, of the hybrid substance. As was stated earlier, the reciprocal analyses of antisera to C^g/ch-8 and C^l/ch-8 cells have revealed a close relationship, if not identity, of these two types, indicating that the C^g or C^l antigens from *Columba*, in combination with ch-8 from *Streptopelia*, exhibit the same, or nearly the same, interaction product.

The agglutination of ch-8/se-8 cells by this reagent was only sporadically observed, and a definite assignment of specificity **n** to these cells is not proposed.

(vi) *Analyses of antisera against cells of type* C^l/*ri-8*

None of ten antisera recently produced by immunization with cells of the standard F_1-*livia/risoria* hybrids (C^l/ri-8) contained as potent antibodies specific

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Table 5. *Absorption analysis of an antiserum (AC1) to the erythrocytes of an F_1-livia/risoria carrying the ch-8 of* chinensis *(C^l/ch-8)*

Test cells*	Reactions with reagents prepared by absorption with parental type cells (*livia* and ch-8/ch-8) only, or additionally in combinations as shown			Proposed antigenic specificity
		C^g/ch-8	C^l/se-8	
C^l/ch-8	+	0	0	**n**
C^l/se-8	+		0	**n**
C^g/ch-8	+	0		**n**
ch-8/se-8	+, 0			
C^g/ri-8	0			
C^g/se-8	0			
C /ri-8	0			

Symbols: See Table 1. Absorbing dilution = 1:10.

* No agglutination was observed with any other cell type listed in Materials and Methods; se-8/se-8 cells were not tested.

for the hybrid substance as did three antisera that had been stored at $-20° \pm 5°$F for several years. Miller (1956) had used one of these in testing for specificities of the hybrid substance, but at that time only two of the various kinds of hybrids used in the present tests were available. The data given in Table 6, therefore, represent only a partial duplication of those reported by Miller. In general, reactions duplicating those of Miller were obtained when the same types of cells were used.

The reactions given in Table 6 may be explained by assigning antigenic specificities to the different cell types as follows: specificity **o** to cells with hu-8/se-8, **op** to those of *C. rufina*. That is, another species (*rufina*) normally carries on its erythrocytes an antigenic constituent that is related to an interaction product of the *livia/risoria* hybrids. Other examples of such relationships have been noted in our laboratory, and some of them have been reported (Irwin & Cumley, 1945; Miller, 1956; Palm & Irwin, 1962). It will be noted that factor **p** is assigned to ch-8/ri-8 and ri-8/se-8 cells by virtue of their absorptive capacities which differed from their anticipated agglutinative potentials. Factor **q** is a constituent of ch-8/ri-8 cells, and also of ch-8/hu-8, ch-8/se-8, ri-8/se-8 and F_1 *capicola/senegalensis*, according to their absorbing capacities. An additional specificity **r** is required to explain the agglutination of cells with C^g/se-8 by reagents prepared by absorption with cells carrying factors **opq** (e.g. ch-8/ri-8). Also, another factor **s** is proposed for C^l/se-8 cells, and **t** for those of types C^g/ri-8 and C^l/ri-8, the two latter types being indistinguishable by the antibodies of this antiserum.

However, a differentiation of cells of types C^g/ri-8 and C^l/ri-8 has been noted with other antisera in these tests (Tables 3, 4 and 7) and by the use of antisera to ch-8/ri-8 cells (Underkofler & Irwin, 1965).

Table 6. *Absorption analysis of an antiserum (493F4) to the erythrocytes of F_1-*livia*/risoria *hybrids* (C^l/*ri-8*)*

Test cells*	Reactions with reagents prepared by absorption with the cells of *livia* (C^l/C^l) and *risoria* (ri-8/ri-8) only, or additionally in combinations as shown								Proposed antigenic specificities
		C^g/ri-8	C^g/se-8	C^l/se-8	ch-8/hu-8†	ch-8/ri-8‡	*rufina*	hu-8/se-8	
C^l/ri-8	+	0	+, 0	+, 0	+	+	+	+	**opqrst**
C^l/ch-8	0								—
C^l/se-8	+			0					**opqrs**
C^g/ch-8	0								—
C^g/ri-8	+	0	+, 0	0	+	+	+	+	**opqrs(t)**
C^g/se-8	+	0	0	0	+	+	+	+	**opqr**
ch-8/hu-8§	+		0		0	0	0	0	**o(q)**
ch-8/se-8	+		0		0	0	0	0	**o(q)**
ch-8/ri-8	+	0	0	0	0	0	+, 0	+, 0	**o(p)q**
hu-8/se-8§	+, 0							0	**o**
ri-8/se-8	+, 0					0			**(opq)**
C. rufina	+		0	0	+	0	0	+	**op**
F_1-*capicola*/*senegalensis*	+		0		+	0	0	+	**op(q)**
Antibodies remaining:		—	**st**	**t**	**prst**	**rst**	**grst**	**pqrst**	

Symbols: See Table 1. Absorbing dilution = 1·30. The specificities enclosed in parentheses are assigned on the basis of the reactions following absorptions by these cells.

* No agglutination was observed of other cell types given in Materials and Methods, except those listed in the table or footnotes.

† Parallel results were obtained by absorption with cells of type ch-8/se-8.

‡ Parallel results were obtained by absorption with cells of types ri-8/se-8 and F_1-*capicola*/*senegalensis*.

§ As is stated in the text, antigens hu-8 and or-8 are as yet indistinguishable. Any combination of hu-8 with ch-8 or se-8 has given results paralleling those of or-8 with ch-8 or se-8.

Another antiserum (186F7) to the cells of the *livia/risoria* hybrids (C^l/ri-8) contained antibodies only for the interaction antigen of C^l/ri-8 cells, and not for any other type of cell tested (Table 7). Absorption of this reagent by cells with C^g/ri-8, themselves non-reactive, did not remove antibodies for C^l/ri-8 cells. Specificity **u** is assigned to the homologous cells, and differentiates them from all other types.

The same antibody specific for factor **u** may be responsible for the differentiation of C^l/ri-8 cells in another antiserum to C^l/ri-8 cells (481F3), according to other reactions in Table 7. A single factor **v** is added to the list for ch-8/ri-8 cells, and specificity **w** is also implied to account for the difference between ch-8/ri-8 and C^g/ri-8 cells.

4. SUMMARY OF RESULTS

For purposes of comparison, the antigenic specificities assigned to the different types of cells reactive with one or more of the different reagents for the hybrid substance are summarized below.

Cell type	*Proposed antigenic specificities*
C^g/ch-8	**c n**
C^g/ri-8	**a cdefghi k opqrs(t)vw**
C^g/se-8	**a cde(f) ijk lm opqr**
C^l/ch-8	**abc n**
C^l/ri-8	**a cde(f) l opqrstuvw**
C^l/se-8	**abcde(f)gijkl nopqrs**
ch-8/ch-8	**i**
ch-8/hu-8	**o(q)**
ch-8/ri-8	**d o(p)q v**
ch-8/se-8	**ij o(q)**
hu-8/se-8	**o**
ri-8/se-8	**(o)(p)(q)**
C. rufina	**de(f) op**
F_1-$\frac{capicola}{senegalensis}$	**de op(q)**

Except for the factors assigned to the cells of the *livia/risoria* hybrids with C^g/ch-8 and C^l/ch-8, it can be readily seen that the cells of the other four kinds of hybrids (C^g/ri-8, C^g/se-8, C^l/ri-8, and C^l/se-8) were more like each other than any one of them resembled the other types of cells listed. If one compares the antigenic factors of the cells with C^g/ri-8 and C^l/ri-8, it is seen that they are alike in thirteen factors, C^g/ri-8 differs from C^l/ri-8 in four (**ghik**), and C^l/ri-8 from C^g/ri-8 in two (**lu**). Similarly, C^g/se-8 and C^l/se-8 cells also possess thirteen factors in common, C^g/se-8 differs from C^l/se-8 in one specificity (**m**), and C^l/se-8 from C^g/se-8 in four (**bgns**). The substitution of C^g for C^l in the *livia* parent, and of se-8 for ri-8 in the dove parent, has therefore resulted in definite changes in the respective antigenic specificities of the hybrid substances. Further, a comparison of the changes in

Table 7. *Absorption analyses of antisera (186F7 and 481F3) to the erythrocytes of* F_1-livia/risoria (C^l/*ri-8*)

	Reactions with reagents prepared by absorption with parental species cells (*livia* and *risoria*) only, or additionally in the combinations shown						
	Antiserum 186F7		Antiserum 481F3				Proposed antigenic specificities
Test cells*	—	C^g/ri-8	—	C^g/ri-8	C^g/se-8	ch-8/ri-8	
C^l/ri-8	+	+	+	+	+	+	**uvw**
C^l/ch-8	0		0				—
C^l/se-8	0		+				
C^g/ri-8	0	0	+	0	0	+, 0	**vw**
C^g/se-8	0		0				—
ch-8/ri-8	0		+	0	+	0	**v**
Antibodies remaining to specificity	**u**	**u**	**uvw**	**u**	**uvw**	**u w**	

Symbols: See Table 1. Absorbing dilution for 186F7 = 1:20; for 481F3 = 1:15.

* Cells of types C^g/ch-8, ch-8/ch-8 and se-8/se-8 were not tested with reagents from either antiserum. No agglutination of other cells listed in Materials and Methods was noted with the reagents for the hybrid substance of these antisera.

factors of the hybrid substance when se-8 is substituted for ri-8 in hybrids with C^g or C^l reveals the same kind of phenomenon. In contrast, the substitution of ch-8 for either ri-8 or se-8 in combination with C^g or C^l is noticeable more for a lack of reactivity with the other reagents than for common specificities, except with each other.

On the basis of the similarities for the reactivities of C^g/ch-8 and C^l/ch-8 cells in Tables 2, 5 and 6, it seems reasonable to propose that had C^g/ch-8 cells been available for testing, they would have reacted with the reagents of Table 1, and would have been assigned either specificity **a**, or **ab**. This would mean that C^g/ch-8 and C^l/ch-8 cells were indistinguishable, or nearly so. On theoretical grounds one would expect that the interaction antigens of these two types of cells should be different, as obtains for the four other types of hybrid cells. One must keep in mind that the attempt to determine the relationships of the different hybrid substances is completely dependent upon the response in immunization of the rabbits, and their variability in this respect is notorious (Kabat & Mayer, 1961).

The cross-reactivity between the hybrid substances of the F_1-*chinensis/risoria* and the F_1-*livia/risoria* has been known for many years, and experimental evidence that the ch-8/ri-8 heterozygote is the only one involved has been reported (Irwin & Cumley, 1945). Miller (1956) proposed that the cross-reactivity of these two hybrid substances could be explained by assuming that they shared two antigenic specificities, whereas in these tests with the different heterozygous cells available, and with antisera to different kinds of F_1-*livia/risoria*, four, possibly five antigenic factors have been demonstrated to be held in common by these two types of cells.

As was stated earlier, the appearance of specificity **i** on the cells of ch-8/ch-8 birds, but not on those of the heterozygotes with ch-8 (ch-8/hu-8 and ch-8/ri-8) except ch-8/se-8, is puzzling. Also, the presence of factors **o** and **p** as normal constituents of *rufina* cells may parallel the recent finding (Irwin, 1966) that some antigens which behave as unit characters within a species may actually be effected, at least in part, by interaction between genes on independent chromosomes.

The similarities and also the differences observed of the hybrid substances in the different combinations of the C system of *Columba* and the group-8 system of *Streptopelia*, and the cross-reactions, primarily with heterozygotes of group-8, provide strong evidence that the hybrid substances observed are interaction products of these two systems. Possibly the antigenic characters of these two systems are contrasting, and the causative genes therefore allelic. If so, the lack of cross-reactivity between the C^l antigen of *livia* and any member of group-8 of the *Streptopelia* species (Bryan & Irwin, 1961) could be explained on the basis that this constitutes an example of changes in genes—as recognized by their products—to the extent that a once existing similarity has been lost (Dobzhansky, 1941). The C^g of *guinea* has been cross-reactive only with the ch-8 of *chinensis*, not with hu-8, or-8, ri-8 or se-8 at the concentrations of antisera employed (Bryan & Irwin, 1961, and unpublished data). These relationships could be an example of the changes in formerly allelic genes so that the end-products of only two (in this case C^g and ch-8) are recognizably related.

5. DISCUSSION

Relatively recently, other examples of interaction products simulating if not paralleling the hybrid substance have been reported. Thus Cohen (1962) has demonstrated an interaction antigen on the erythrocytes of each of two of three heterozygotes in rabbits. The production of products of interaction of alleles and non-alleles in *Drosophila melanogaster* has been reported (see Barish & Fox, 1956, for references). Manwell, Baker & Childers (1963) found that the hemoglobins of three species hybrids in fish, out of six hybrids studied, were characterized by electrophoretic properties different from those of the parental species. Two loci with effects on esterases in maize, in which a new band during starch electrophoresis characterizes each heterozygote as compared with the respective homozygotes, have been noted by Schwartz (1960, 1964). Shaw (1965) has summarized the electrophoretic variants in enzymes of diploid organisms and listed the heterozygotes with hybrid enzymes. Thus, interaction products are not unique to species hybrids, nor to blood cells.

Various explanations have been advanced for the cause of the appearance of the hybrid substance on the blood cells (Irwin, 1932; Burnet & Fenner, 1948; Bryan, 1953; Miller, 1956; LaBar, 1964). A basic question is whether the appearance of the new antigenic substances is (*a*) a physical manifestation of an interaction of the antigens themselves, or (*b*) a product of gene interaction, presumably of gene products at some point in the chain of reactions between the causative genes and the end-products.

The explanation that the interaction of different antigens *per se* may impart changed or new antigenic specificities to one or the other, or both, of the antigens involved cannot be disregarded. However, it seems reasonable to expect that, if this is the correct explanation for the appearance of the various hybrid substances, many more examples of the phenomenon would be known than are now recognized. Their recognition has been a relatively rare event, even among the progeny resulting from species hybridization. Under this explanation the role of the genes in the different heterozygotes would be important, but passive.

If, on the other hand, the role of the genes is active rather than passive in the production of the hybrid substance, there still remains to be explained the mechanism of its production. The present evidence indicates that the antigenic characters with which the hybrid substance is associated are seemingly as completely expressed in the different heterozygotes as in the respective homozygotes. Thus the hybrid substance does not represent a substitution or replacement of any part of the usual end-product of the presumed interacting genes, but rather represents an additional antigenic product. There is, of course, the possibility that the interacting genes are not those that effect the antigens of the parental species recognizable in the heterozygotes, but are linked to them. If so, the linkage to date has been absolute. That is, the interaction may be between subunits of the causative genes and no separation has yet been observed.

It is unfortunate that information of the chemical nature of the specificities of

the cellular antigens is practically non-existent. It is often assumed that these are proteins. That the specificities need not be proteins is exemplified by the findings that the specificities of the ABH and Lewis blood-group substances of humans undoubtedly depend upon differences in carbohydrate structure (see Watkins, 1966, for an excellent summary and bibliography of the chemical, serological and genetical analyses). The concluding statement by Watkins (1966) is pertinent—'A large variety of structural patterns can be formed with a relatively limited number of sugar units, and if the role of many of the blood-group genes is the control of the arrangement of these carbohydrate building blocks it is possible to perceive how great diversity of the cell surface can be produced with considerable economy of means.'

If the cellular antigens associated with the hybrid substance, and the hybrid substance itself, were proteins, the proposal by Fincham (1966) that 'all those examples of hybrid antigens can very reasonably be explained as due to the formation of hybrid proteins by interaction of allelic polypeptide chains ...' would be pertinent. But if they are not proteins, as may very well be the case, some other kind of chemical event than interaction of polypeptide chains will obtain. Whether this will be (*a*) a rearrangement of existing groupings as might occur if the antigenic specificities depended upon carbohydrates, or (*b*) a formation of completely new entities is an open question.

SUMMARY

Following the transfer to the common pigeon (*Columba livia*) of a cellular antigen (C^g) from *C. guinea*, and to *Streptopelia risoria* of respective members of the group-8 system from *chinensis* and *senegalensis*, new combinations of these two antigenic systems were produced from matings of *livia* and *risoria*. The hybrid substances associated with the cells of each of the five new combinations of the cellular antigens were related to each other and to the usual F_1-*livia/risoria* cells but, with the possible exception of two, were definitely differentiated from each other. Cross-reactivities of the reagents for the hybrid substances were also observed, primarily with heterozygotes of the group-8 system of *Streptopelia*. Evidence is thus provided that the interaction takes place primarily if not entirely between the genes of the C-system of *Columba* and the group-8 system of *Streptopelia*.

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