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Chapter 15

*Specificity of Gene Effects**

If an attempt were made to survey all the possible ramifications suggested by the title of this paper, it should include much of the published work in genetics. It is of course a truism to all students of genetics to state that some sort of differential specificity towards the end product must exist between allelic genes or their effects could not be studied. It would be very interesting as a part of this discussion to attempt to trace the change in concepts held by various workers during these past fifty years concerning the nature and paths of action of the gene. However, beyond a few remarks, such considerations are hardly within the scope of this chapter.

Since the effects of genes can be recognized only if there are differences in the end product, it is quite natural that the differences in the experimental material first subjected to genetic analyses should have been those which were visible, as differences in form, color, etc. Although the pendulum has swung somewhat away from intensive investigations of such hereditary characteristics, it should be emphasized that by their use the underlying mechanisms of heredity have been elucidated.

Major attention was given by most of the investigators during the first quarter of this century to the effects of respective genes upon individual hereditary characters. In some quarters there was an oversimplification in the interpretation of the relation of the gene to the character affected by it. Gradually, however, the concept has become clearer that the majority of hereditary characters—even many of those which had previously appeared to be most simply inherited—are affected by many genes.

An early observation of gene specificity, too long neglected by all but a few geneticists, was that made by Garrod in 1909 (see 1923 edition of *Inborn*

* Paper No. 433 from the Department of Genetics, University of Wisconsin.

Errors of Metabolism) on the inability of some humans to break down homogentisic acid (2,5-dihydroxyphenylacetic acid), resulting in the disease known as alcaptonuria. Observations reported by Gross (1914) indicated that this affliction was due to the lack of a ferment (enzyme) in the serum of alcaptonurics, whereas the enzyme capable of catalyzing the breakdown of homogentisic acid was demonstrable in the serum of normal individuals. As Beadle (1945) has stated, no clearer example exists today that "a single gene substitution results in the absence or inactivity of a specific enzyme and that this in turn leads to the failure of a particular biochemical reaction." (The writer distinctly remembers that, while he was a student in a class in physiological chemistry, the instructor paid considerable attention to the chemical explanation of alcaptonuria, but none at all to its hereditary nature.)

Another example of gene specificity and also of gene dosage is that of yellow endosperm in corn and the content of vitamin A reported by Mangelsdorf and Fraps (1931). Their study showed that the amount of vitamin A in the endosperm of white corn was almost negligible, but that the presence in the endosperm of one, two, or three genes for yellow pigmentation was accompanied by corresponding increases in the amounts of the vitamin.

GENE EFFECTS IN A SERIES OF REACTIONS

There are numerous examples which have shown that many genes contribute to the development of a heritable character. Thus, in corn there are many genes which affect the development of chlorophyll. Each recessive allele, when homozygous, allows the formation of only partial pigmentation, or in extreme cases no pigmentation at all, and the seedlings are albino. It is generally believed that the majority, if not all, of these different genes for albinism affect different steps in the process of chlorophyll development. A breakdown of the process at any one of these steps results in albinism of the seedling. Haldane (1942) has likened the complexity of such a synthetic process to the activity of an equal number of students as there are genes, "engaged on different stages of a complicated synthesis under the direction of a professor, except that attempts to locate the professor have so far failed. Or we may compare them to modern workers on a conveyor belt, rather than skilled craftsmen each of whom produces a finished article."

One of the earliest examples of the physiological bases of the specificities which are the final gene products is that of the chemical analyses of genetic variations in flower color. These studies were carried out in England by several workers. See reviews by Beadle (1945), Beale (1941), Haldane (1942), Lawrence and Price (1940) for the general results and references to specific papers.

Mention will be made here of only one of the many investigations which have defined in chemical terms the hereditary differences in pigmentation. Anthocyanin is one of the five types of pigments concerned in flower color,

and its presence or absence in several species is genetically determined. One way in which anthocyanin may be modified is by the degree of oxidation of the prime ring. According to Beale (1941) in the two genera Lathyrus and Streptocarpus, the hydroxyl group is at position 4′ in the pelargonidin type, at positions 3′ and 4′ in the cyanidin types, and at 3′, 4′, and 5′ in the delphinidin types. The more oxidized pigments are usually dominant to the less oxidized types. Thus flowers with genes *AB* and *Ab* will be of the delphinidin type of pigment, those with *aB* of the cyanidin type, and those with *ab* of the pelargonidin type.

These and other extensive chemical studies on the anthocyanin pigments genetically modified in various ways are dramatic examples of the specificities of gene effects. The analogy drawn above between the various genes and students working on a complicated synthesis becomes a little more clear in relation to flower pigments, since considerable information is available as to what some of the genes accomplish.

A further example of the effect of many genes upon a character is that of eye color in *Drosophila melanogaster*. Between twenty-five and thirty genes are known to modify the brownish-red color of the wild-type eye. There appear to be two independent pigments, brown and red, concerned in the development of the wild-type eye, each of these being affected by specific genes. Certain components of the brown pigment are diffusible from one part of the body to another, and hence are more readily subjected than others to chemical analyses.

The details of these analyses are presented in other review articles (Beadle, 1945; Ephrussi, 1942a, 1942b). Briefly, dietary tryptophan is converted to alpha-oxytryptophan by a reaction controlled by the wild-type allele of the vermilion gene (*v*). This substance is oxidized further to kynurenine (the so-called v^+ substance). By virtue of the activity of the normal allele of the cinnabar gene, kynurenine is further oxidized to the cn^+ substance, which may be the chromogen of the brown pigment (Kikkawa, 1941). The production of either brown or red eye pigment can be blocked by genes at the white eye locus, thus indicating that such genes act on a common precursor of the red and brown pigments.

Mention should be made of the relation between the original designation of certain of the genes for eye color and their presently known effects. Thus, the eyes of flies with the mutant alleles *bw bw* are brown. But it is now known that this pair of alleles, instead of being concerned with the production of brown pigment, restricts the development of red pigment and thus we see only the brown color. Similarly, the four gene pairs whose mutants modify the red coloration do so by virtue of their effect on the brown pigment, not upon the red.

Wheldale (1910) proposed four decades ago that genetic characters were the resultant of a series of reactions, and that if a break in the chain occurred,

the series of steps would have proceeded only to that point. Following the initial work in Neurospora by Beadle and Tatum (1941) on mutants which blocked certain metabolic processes, this type of approach has expanded enormously and profitably. Attention can be called here to but one very significant example of this kind of experimental study in microorganisms. A report by Srb and Horowitz (1944) shows clearly how many genes act in the synthesis of arginine. Of fifteen mutant strains studied, there were seven different steps represented in the synthesis of arginine. One of the forms grew only if arginine was supplied. Two others required either arginine or citrulline, and these two strains were genetically different. Four other strains, genetically different from the first three strains and from each other, would grow if arginine, citrulline, or ornithine were provided. For a diagrammatic representation of these steps, see Beadle (1945).

DIRECT EFFECTS OF GENES

The preceding examples are but a few of the many which could be cited to illustrate the gene specificities in the development of a genetic character which involves the successive activities of many genes. Are there any genetic characters which may be the immediate products of the causative genes? An example almost unique in higher plants is that of the waxy gene in corn (Collins, 1909) in its effects upon the starch of the pollen grain and the endosperm reserves. As is well known, the starch granules in the pollen grains bearing the waxy gene are stained reddish-brown with iodine, as are the endosperm reserves of waxy seeds, in contrast to the typical blue reaction of the starch granules of non-waxy pollen and of the endosperm reserves of non-waxy seeds. Following studies of the physiological effects of the waxy gene, Brink (1929) proposed that this gene has its effect on the enzyme amylase which functions directly in the synthesis of starch.

Another class of hereditary characters which in some respects appears to satisfy some of the criteria for a direct effect of the causative genes is that of the antigenic characters of the red blood cells of animals. With only rare exceptions, to be considered later in more detail, each of the known antigenic substances has appeared in the cells of an individual only if one or both parents also possessed it. If there is but a single pair of contrasting characters, each is expressed in the heterozygote. Further, the cells which give rise to the hematopoietic tissue from which the red blood corpuscles are derived are laid down shortly after the first division of the fertilized egg. The possibility cannot be excluded, of course, that there is a chain of reactions within each cell leading to the formation of the antigen, but no block in such a chain of reactions has yet been observed. There are two statements concerning the cellular antigens which are of interest: (1) the antigenic substance must be located at or near the surface of the cell in order to be detectable, and (2) there is no known effect of the environment upon them.

We should avoid misunderstanding about the meaning of the terms commonly used in immunological literature. For example, the word antigen was originally defined as any substance which, when introduced parenterally into an animal, would invoke the production of antibodies. This definition would now be extended to include any substance which will react visibly with an antibody. And an antibody would be defined as a constituent of the serum which reacts with an antigen in any of several ways. The circle of reasoning here is obvious. However, insofar as chemical studies of various antigens have contributed to an understanding of their specificities, the specificities have always been associated with structural differences of the antigenic substances. On the other hand, the reasons underlying the specificities in reactivity of the antibodies are almost completely unknown, although it is known that the antibodies are intimately associated with the globulins of the serum, and in fact may constitute the gamma globulins of the serum.

CELLULAR ANTIGENS IN HUMANS

As our first example of these antigenic substances, let us consider the well known and extensively studied O, A, B, and AB antigenic characters, or blood groups, of human cells. Following their discovery by Landsteiner (1900, 1901), it soon became clear that these substances were gene controlled. At the present time, the theory of three allelic genes, as postulated by Bernstein (1924) on statistical grounds, is generally accepted. The two other theories proposed for their inheritance—independent and linked genes, respectively—are fully discussed by Wiener (1943). Landsteiner noted that the serum of certain individuals would agglutinate (clump) the cells of other individuals, and from this observation the reciprocal relationship between the presence and absence of each antigen and its specific antibody has been elucidated.

Group	A or B Antigen on the Cells	Antibody of the Serum
O.....	None	Anti-A, Anti-B
A.....	A	Anti-B
B.....	B	Anti-A
AB.....	AB	None

It may readily be seen that the presence of an antigen, as A, on the cells is accompanied by the presence of the antibody (anti-B) for the contrasting antigen, as B, in the serum, and vice versa. If both antigenic characters are found on the cells, as in AB individuals, the serum contains no antibodies. While if neither A nor B is present on the cells, the serum contains both anti-A and anti-B.

These phenomena pose the question whether the genes producing the cellular substances also have an effect on the antibodies of the serum. That is, does the gene which is responsible for the O antigen (which is definitely an entity but is less reactive than A and B) also effect both anti-A and anti-B

in the serum—while in individuals with substance A, only anti-B is found; in those with B, only anti-A is present; and in AB individuals the effects of the respective genes on the antibodies are somehow neutralized?

Before attempting to answer this question, it will be advisable to review the present knowledge of the chemistry of the A and B substances of human cells. See Kabat (1949).

These antigens (blood groups, cellular characters, antigenic factors, etc.) are found in nearly all the fluids and tissues of the human body. They also are widely distributed throughout the animal kingdom. The A substance or an A-like substance has been found, for example, in hog gastric mucosa, in the fourth stomach (abomasum) of the cow, and in swine pepsin, while both A and B substances have been noted in the saliva and stomachs of horses. Following chemical fractionations, principally of horse saliva and hog gastric mucosa, various investigators have obtained preparations with activity related to the A substance. These preparations have been largely polysaccharide in nature. In addition to the polysaccharides, even in the purest preparations, some workers have noted traces of amino acids.

At present, while it appears that both the A and B substances of human cells may be classed as nitrogenous polysaccharides, no information is available as to the structural differences between them. Our knowledge of such specificities rests entirely upon the technics of immunology, that is, by the interaction of either naturally occurring antibodies (as anti-A and anti-B), or immune antibodies, with the respective substances A and B.

The antigenic substances A and B of human cells are complex polysaccharides, while the antibodies are modified globulins, or are found in serum protein very closely related to the globulin fraction. If the gene which effects antigen A is responsible also for the B antibody, and that for antigen B for the A antibody, it would seem that here is a clear-cut case of pleiotropic effects of the respective genes. This explanation runs into difficulties in AB individuals which, on this proposal, should have both kinds of antibodies but actually have none. In contrast, a current explanation of the reciprocal presence of the antigenic substance of the cells and the antibody for the contrasting substances is that the antibodies for both substances (A and B) are normal constituents of human serum. Production of the antibodies would then be controlled by a gene or genes at another locus than that having to do with the cellular substances, *if* genes were involved in their production. If an individual carries the gene for A, and hence has A substances widely distributed throughout his body, the A antibodies are presumed to be absorbed from the serum, and of course the B antibodies are left. Also, an individual with the B substance would absorb the B antibodies, and the antibodies to A would remain, while both anti-A and anti-B would be absorbed in an AB individual. Other hypotheses are given by Wiener (1943). Unfortunately, no experimental test of the correctness of this or other hypotheses is likely.

Landsteiner and Levine (1927) announced the discovery in human cells of a new pair of contrasting antigens, called M and N. These were detectable only by the use of immune sera produced in rabbits, as was another antigenic factor called P. The heritability of the M and N substances is adequately explained by the assumption of a single pair of allelic genes, and the substance P appears to be dominant to its absence.

Another antigenic factor in human blood which has aroused wide interest is the recently discovered Rh substance, or complex, as it might be termed. In 1940, Landsteiner and Wiener (1940) reported that a new antibody, derived from a rabbit immunized with the erythrocytes of a rhesus monkey, was reactive with the cells of about 85 per cent of the white population of New York. They gave the name Rh (a contraction of rhesus) to this agglutinable property of human cells. As Boyd (1945) aptly states:

> The technic of testing for the new factor was difficult, the best available serums were weak, and had it not been for a remarkable series of discoveries which followed in the next few months, the Rh factor might have aroused no more interest than its practically still-born brethren. . . .

The Rh factor was shown to be involved in previously unexplained complications following transfusions (Wiener and Peters, 1940), but is most widely known for its role as the etiologic agent in the majority of cases of hemolytic disease of the newborn. The proposal was first made by Levine and Stetson (1939) that an antigen in the fetus, foreign to the mother and presumably transmitted by the father, could pass through the placenta and immunize the mother. Later studies implicated the Rh factor as the foreign antigen, and showed that the antibodies developed in the mother may pass back through the placenta and affect the red blood cells of the fetus, before or following birth. Although the majority of cases of hemolytic disease of the newborn may be justly ascribed to Rh incompatibility between the father and mother, there is no satisfactory explanation as to why only about one in forty of such potentially dangerous combinations leads to morbidity.

There exist several subgroups, or subtypes, of the Rh complex, and investigations as to their respective specificities occupy the center of interest of many workers at the present writing. There are two schools of thought as to the mode of inheritance of these subgroups, which also involves the terminology to be used in their identification (see Strandskov, 1948, 1949, for leading references). One explanation is that the various subtypes are manifestations of a series of multiple allelic genes, the other that they are the result of the action of respective genes at three different but closely linked loci. It is not within the province of this chapter to discuss the arguments for and against these two proposals. However, it should be stated that the genetic results under either explanation are essentially the same.

One of the most pertinent statements which can be made about these various antigenic substances of the erythrocytes is that they are detectable

no matter in what gene complex they may occur. That is, other genes than the causative ones have no measurable influence upon their expression. A possible exception to this statement might be proposed for the A and N characters, respectively, since each is somewhat less readily agglutinated when in the heterozygote, AB and MN, than when either occurs singly.

THE HYBRID SUBSTANCE IN SPECIES HYBRIDS

Until the early part of this century, most of the workers in immunology had reached the conclusion that the specificities obtained in immunological reactions were primarily if not entirely concerned with proteins. Therefore, the finding by Heidelberger and Avery (1923, 1924) that the immunological specificities of the pneumococcal types were dependent upon polysaccharides was indeed a forward step in our understanding of the chemical nature of biological specificity. It is a pleasure to acknowledge that this work of Heidelberger and Avery convinced the writer that immunological technics should be a useful tool in studying genetic phenomena. Also, although at that time pollen differing in gene content seemed (and still does) to be promising experimental material, the species and species hybrids in pigeons and doves produced by the late L. J. Cole were tailor-made for further studies.

Pigeon-Dove Hybrids

The first step was to determine whether the cells of one species could be distinguished from those of the other. In brief, all the comparisons by immunological technics, between any pair of species of pigeons and doves, have resulted in the ability to distinguish the cells of any species from those of another, and to show that each species possessed antigenic substances in common with another species, as well as those peculiar to itself—those species specific. A dozen or more kinds of species hybrids have been obtained in the laboratory, and in general, each kind of hybrid has contained in its cells all or nearly all of the cellular substances of both parental species. One such species hybrid is that obtained from a mating between males of an Asiatic species, the Pearlneck (*Streptopelia chinensis*) and the domesticated Ring dove females (*St. risoria*). The corpuscles of these hybrids contained all the substances common to each parental species, but did not contain quite all the specific substances of either parental species. Further, the cells of these hybrids did possess a complex of antigenic substances not found in the cells of the parents. These relationships are presented in Table 15.1 and are given in diagrammatic form in Figure 15.1. This new antigen has been called the "hybrid substance," and it has been present in every hybrid produced between these two species.

Upon repeatedly backcrossing these species hybrids and selected backcross hybrids to Ring dove, ten antigenic substances which differentiate Pearlneck from Ring dove have been isolated as probable units. That is, a

backcross bird carrying any one of these unit substances, when mated to a Ring dove, has produced approximately equal proportions of progeny with, and without, the particular substance in their blood cells. These substances peculiar to Pearlneck, as compared with Ring dove, have been called d-1, d-2, d-3, d-4, d-5, d-6, d-7, d-8, d-11, and d-12. Each of these is distinct from the others (Irwin, 1939) both genetically and immunologically. Thus it appears that a gene or genes on each of ten of the thirty-odd pairs of chromo-

TABLE 15.1

ANTIGENIC RELATIONSHIPS OF THE BLOOD CELLS OF PEARLNECK, RING DOVES, AND THEIR HYBRIDS

Immune Serum	Absorbed by Cells of	Agglutination Titers with Cells of		
		Pearlneck	Ring Dove	F_1
Pearlneck		23040	23040	23040
Pearlneck	Ring dove	11520	0	11520
Pearlneck	F_1	90	0	0
Ring dove		15360	15360	15360
Ring dove	Pearlneck	0	3840+	3840+
Ring dove	F_1	0	180	0
F_1		15360	15360	15360
F_1	Pearlneck	0	3840+	3840+
F_1	Ring dove	7680	0	7680
F_1	Pearlneck and Ring dove	0	0	360+

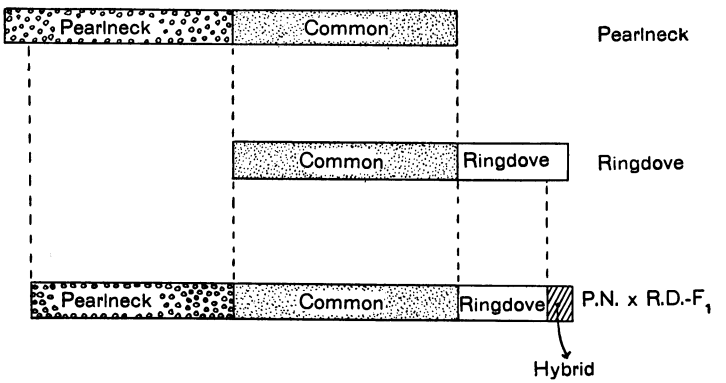

FIG. 15.1—Diagrammatic representation of the antigenic relationships of the Pearlneck, Ring dove, and their hybrids.

somes of Pearlneck produce effects on cellular antigens which differentiate Pearlneck from Ring dove. Although the cellular substances particular to Ring dove, in contrast to Pearlneck, have not been obtained as units, the available evidence indicates strongly that a gene or genes on nine or ten chromosomes of Ring dove produce antigenic effects which differentiate that species from Pearlneck.

The question may well be raised as to what this recital of antigenic characters in man and doves, which in general illustrates gene specificity in the production of cellular antigens, has to do with the general topic of heterosis. The so-called *hybrid substance* has one word (hybrid) in common with the term hybrid vigor, and suggests a possible relationship of the two terms.

The hybrid substance seemingly represents a departure from the hypothesized direct action of a gene on the antigenic substance, in that it appears to result from the interaction of two or more genes in the species hybrids to produce some antigenic substance different from any detectable in either parent. With but one exception proposed by Thomsen (1936) in chickens, and for which another explanation will be considered shortly, a hybrid substance has thus far been found only in species hybrids.

Mention should be made of the technics required for the detection of the hybrid substance. Briefly, if an antiserum prepared against the cells of an individual, whether a species hybrid or not, would be absorbed by the cells of both its parents and would then react with the cells of the individual, but not with the cells of either parent, there would be evidence of a different antigenic substance in the homologous cells—those used in the immunization. (If an antigen were recessive, it would be present in the heterozygote, and presumably could absorb its specific antibody.)

Domestic Fowl Hybrids

As stated above, Thomsen (1936) reported that within each of two families of chickens there was a different antigenic substance present than was found in the parents. Attempts in our laboratory by Mrs. Ruth Briles to duplicate this finding were without success, but a very interesting and quite unexpected observation was made which may be the explanation of Thomsen's finding. If an antigenic substance were present in an individual different from that possessed by either parent, immunization of either parent (as #1) with the cells of this individual might engender antibodies against the new substance. Absorption of such an antiserum by the cells of the other parent (as #2) should remove all antibodies except those formed against the new or hybrid substance, and such a reagent should be reactive only with the cells containing the new substance. This was the procedure followed by Thomsen, except that his tables do not show that the cells of the two parents were used as negative controls in the tests made after the various absorptions.

Immunizations of each of the parents of a family of chickens against the

cells of one of the offspring, or the pooled cells of two or more, were made by Mrs. Briles. Following the absorption of the antiserum obtained from either parent by the cells of the other, it was noted that the absorbed antiserum was at least weakly reactive with the cells of the individual from which the antiserum was obtained. That is, such an antiserum would not react (agglutinate) with its own cells before absorption with the cells of the mate, but after such absorption it definitely would agglutinate the cells of the individual from which it was derived.

To use a concrete example, bird R614 (containing B_1 antigen) was immunized with the washed cells of R2043, to produce B_3 antibodies (Briles, McGibbon, and Irwin, 1951). After this antiserum from R614 was mixed for absorption with the washed cells of R622 (having B_3 antigen in its cells and having been immunized to produce B_1 antibodies), all cells containing the B_1 antigen were reactive with it, including those of R614 itself. Thus it appears that the antibodies to B_1 which were circulating in the serum of R622 were also attached to the surface of the red blood cells and were transferred to the antiserum from R614 during the absorption process. It was possible to demonstrate that, after washing the cells of R622 in saline, the saline contained antibodies, even after nine successive washings. Hence, unless the cells of both parents were used as controls in comparable tests for the presence of a hybrid substance, agglutination of any cells could be explained as due to a transfer of antibody from the blood cells to an antiserum. Unfortunately, such controls are not given in Thomsen's paper, and the possibility cannot be eliminated that the reactions obtained by him were due simply to segregation within the various families of an antigenic character of one of the parents. This possibility was mentioned by Thomsen (1936), but was not considered applicable to his experiments.

Hybrid Substances

Returning to the hybrid substance for which there is definite evidence, it should first be stated that such a substance has not been found in all kinds of species hybrids, as may be seen from the data given in Table 15.2. It has been reported from our laboratory in hybrids between Pearlneck and Ring dove, the pigeon (*Columba livia*) and Ring dove, the Mallard (*Anas platyrhynchos*) and Muscovy duck (*Cairina moschata*), but not in the hybrids between the triangular spotted pigeon (*C. guinea*) and *livia*. Irwin (1947) gives the specific references to pertinent articles.

A hybrid substance has been detected but not previously reported in hybrids from matings between the Philippine turtle dove (*St. dussumieri*) and Ring dove, the dwarf turtle dove (*St. humilis*) and Ring dove, the Oriental turtle dove (*St. orientalis*) and Ring dove, and the band tail pigeon (*C. fasciata*) and *livia*. No such substance has been observed in the hybrids between the Senegal dove (*St. senegalensis*) and Ring dove, an African dove

(*St. semitorquata*) and Ring dove, the Senegal dove and the Cape turtle dove (*St. capicola*), the spot wing pigeon (*C. maculosa*) and *livia*, and between the Grayson dove (*Zenaidura graysoni*) and the common mourning dove (*Zen. macroura*). It is possible that a hybrid substance does exist in these latter species hybrids, but the same technics by which it was observed in the other species hybrids failed to demonstrate its presence in them.

Three different fractions of the hybrid substance have been demonstrated in the hybrids between Pearlneck and Ring dove (Irwin and Cumley, 1945), by virtue of a frequent association of each fraction with one or more antigens

TABLE 15.2

TESTS FOR HYBRID SUBSTANCES IN THE CELLS OF VARIOUS SPECIES HYBRIDS

Antiserum to	Absorbed by Cells of		Reactions of Parental and Hybrid Cells with the Respective Reagents		
	Parent 1	Parent 2	Parent 1	Parent 2	Hybrid
F_1—Pearlneck × Ring dove	Pearlneck	Ring dove	0	0	++
F_1—*C. livia* × Ring dove	*livia*	Ring dove	0	0	++
F_1—*St. dussumieri* × Ring dove	*dussumieri*	Ring dove	0	0	+
F_1—*St. humilis* × Ring dove	*humilis*	Ring dove	0	0	+
F_1—*St. orientalis* × Ring dove	*orientalis*	Ring dove	0	0	+
F_1—*C. fasciata* × *livia*	*C. fasciata*	*livia*	0	0	+
F_1—Mallard × Muscovy	Mallard	Muscovy	0	0	++
F_1—*St. senegalensis* × Ring dove	*St. senegalensis*	Ring dove	0	0	0
F_1—*St. semitorquata* × Ring dove	*St. semitorquata*	Ring dove	0	0	0
F_1—Senegal × *St. capicola*	Senegal	*St. capicola*	0	0	0
F_1—*C. maculosa* × *livia*	*C. maculosa*	*livia*	0	0	0
F_1—*C. guinea* × *livia*	*C. guinea*	*livia*	0	0	0
F_1—*Zenaidura graysoni* × *Zen. macroura*	*Zen. graysoni*	*Zen. macroura*	0	0	0

peculiar to Pearlneck. Thus one fraction called dx-A was always associated in the backcross hybrids with the d-11 substance, dx-B seemingly was loosely linked with the d-1 character and with certain others as well—thereby providing strong evidence that on several chromosomes of Pearlneck there are duplicate or repeat genes—and dx-C was always associated with the d-4 antigen. The pertinent reactions which show these specificities are given in Table 15.3 and are represented diagrammatically in Figure 15.2.

Because of the constant association of the dx-A and dx-C fractions with the d-11 and d-4 substances, respectively, one cannot be certain that these two fractions, although antigenically distinct from the d-1 and d-11 specific characters, are not simply a new specificity conferred upon the specific characters by some sort of rearrangement of the specific substances following the

interaction of the causative genes. This question cannot be completely answered until either a genetic separation has been observed, as between the dx-A and d-11, or the chemical separation into two distinct substances has been done. On the other hand, the dx-B fraction has been separated from each of the species specific characters to which it presumably is loosely linked, thereby showing that this fraction of the hybrid substance is an antigenic entity.

The reagent which interacts with the hybrid substance (hybrid antiserum

TABLE 15.3

TESTS FOR SIMILARITIES AND DIFFERENCES OF THE COMPONENTS OF THE "HYBRID SUBSTANCE" OF THE SPECIES HYBRID BETWEEN PEARLNECK AND RING DOVE

Cells	Reactions of Different Cells with Anti-F_1 Serum							
	Absorbed by Cells of Both Pearlneck and Ring Dove	Absorbed by the Cells of Both Pearlneck and Ring Dove, in Combination with Others as Listed						
		d-1 (dx-B)	d-4 (dx-C)	d-11 (dx-A)	$F_1 \frac{P.N.}{R.D.}$	$F_1 \frac{Pgn}{R.D.}$	Sen.	Aus. cstd.
Pearlneck	0	0	0	0	0	0	0	0
Ring dove	0	0	0	0	0	0	0	0
F_1—P.N./R.D.	++	++	++	+	0	++	++	++
d-1 (dx-B)	+	0	+	+	0	+	+	±
d-4 (dx-C)	+	?	0	±	0	±	±	±
d-11 (dx-A)	++	++	++	0	0	+	++	++
F_1—Pgn/R.D.	+	+	+	0	0	0	+	+
Senegal	+	0	0	0	0	0	0	±
Australian crested	±	±	±	0	0	0	±	0
Column	2	3	4	5	6	7	8	9

Symbols: ++ = marked agglutination; + = agglutination; ± = definite but weak agglutination; ? = doubtful reaction; 0 = no agglutination—at the first dilution of the serum cell mixture.

absorbed by the cells of both Pearlneck and Ring dove) will also agglutinate the cells of various species. Thus in the genus Streptopelia, there were five species (*capicola*, *dussumieri*, *humilis*, *orientalis*, and *senegalensis*) other than Pearlneck and Ring dove whose cells were reactive, and one (*semitorquata*) with nonreactive cells. Within the genus Columba, the cells of one species (*rufina*) likewise reacted with this reagent, but those of seven other species (*fasciata*, *flavirostris*, *guinea*, *livia*, *maculosa*, *palumbus*, and *picazura*) did not. And of twelve species tested in other genera within the Columbidae, only three species from Australia (Australian crested dove, or *Ocyphaps lophotes*, the bronze wing dove or *Phaps chalcoptera*, and the brush bronze wing dove, or *Phaps elegans*) possessed reactive cells. In Table 15.3, the Senegal cells are representative of the parallel reactions of the five species of the Streptopelia,

as are those of the Australian crested of the equivalent reactivities of the three Australian species.

Although the reagent for the hybrid substance did not agglutinate the cells of *livia*, it invariably clumped those of the hybrids between pigeon (*livia*) and Ring dove. As previously reported (Irwin and Cole, 1936), these hybrids also contain a hybrid substance. Because of the cross reactions existing between these two hybrid substances, a certain degree of similarity can be assumed. That the fraction in the hybrid substance of the F_1-Pearlneck $\times$

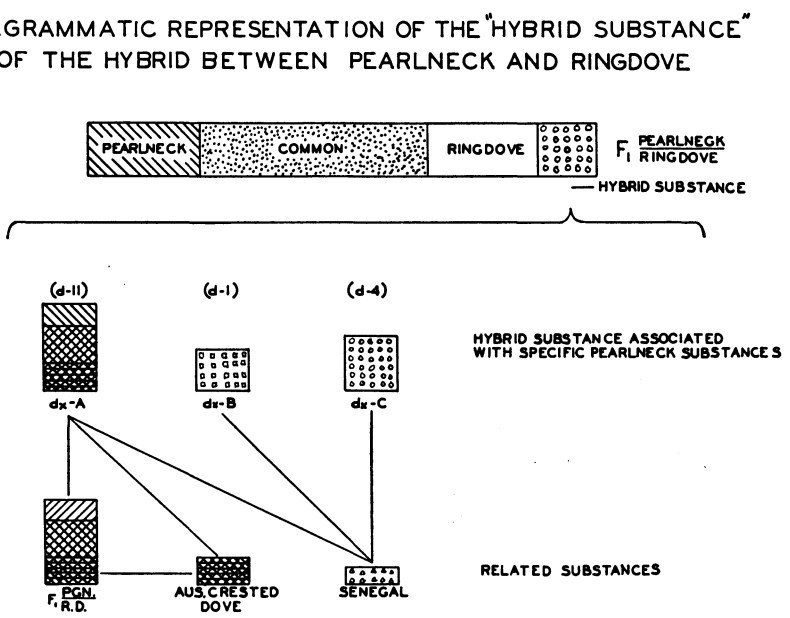

FIG. 15.2 —The separation into constituent parts of the *hybrid substance* of the species hybrid between Pearlneck and Ring dove.

Ring dove, which is primarily if not entirely responsible for the cross reactions, is dx-A may be deduced from Table 15.3, in that this fraction (associated with d-11) is the only one which will exhaust the antibodies from the reagent for the cells of the pigeon-Ring dove hybrid (column 5). Also, in unpublished tests the reagent for the hybrid substance of the pigeon-Ring dove hybrid (anti-hybrid serum absorbed by the cells of pigeon and Ring dove) did not react with Pearlneck cells, but reacted strongly with d-11 cells, presumably by virtue of their content of the dx-A fraction, and not definitely with cells carrying dx-B or dx-C. If the dx-A hybrid substance of the species hybrids between Pearlneck and Ring dove were partially or largely a rearrangement of an antigenic substance, in this case d-11, which is species specific to Pearlneck—since the Ring dove is a common parent of the two kinds

of species hybrids—that specific substance (d-11) should be detectable in the cells of *livia*.

To date, reasonably extensive tests (unpublished) have not shown that the cells of *livia* contain more than a trace of an antigenic substance related to the d-11 of Pearlneck. Whatever the relationship of the genes in Pearlneck (associated with those on a chromosome effecting the d-11 specific substance) and *livia*, respectively, which presumably by interaction with a gene or genes from Ring dove in the two species hybrids effect a common fraction of the two hybrid substances, they are not associated with genes which produce similar antigenic patterns in the two species. On these grounds, it would seem unlikely that the hybrid substances in these two kinds of species hybrids are merely a different arrangement of a species specific antigen.

The question is pertinent as to whether such reactivities in the cells of these other species, as Senegal and Australian crested, are themselves an indication of antigenic response to gene interaction within each species, or the more direct product of a gene. This cannot be answered directly. But, as given in Table 15.3, the fact that absorption of the reagent for the hybrid substance by fractions dx-A, dx-B, or dx-C removes the antibodies for the cells of Senegal indicates that there is some common constituent of these three fractions related to, if not identical with, a reactive substance in Senegal cells. However, only the dx-A fraction removes the antibodies for the cells of the Australian crested dove. Further, absorption by the cells of the pigeon-Ring dove hybrid also removes the antibodies from this reagent for the cells of the Australian crested dove.

The hypothetical explanations could be advanced, (1) that the antigenic substances in Senegal and the Australian crested dove, themselves being distinct, but both related to the hybrid substance in Pearlneck-Ring dove hybrids, are the result of a genic interaction. But there is no evidence for such an assumption. Also, (2) the argument could be advanced that the relationship between these substances in Senegal and Australian crested, and in the respective species hybrids, is fortuitous, simulating the occurrence of the Forssman antigen in many species of animals and plants, including bacteria (Boyd, 1943). That is, the antigenic substances involved (related in some manner to the hybrid substance) may be gene controlled in each of the related species, since indistinguishable substances to those of Senegal were found in four other species of *Streptopelia*, *capicola*, *dussumieri*, *humilis*, and *orientalis*, and to those of the Australian crested dove in two species of another genus, *Phaps chalcoptera* and *Phaps elegans*, but the antigenic similarity to the hybrid substance is by virtue of some related antigenic component. Various ramifications of these and other explanations would be purely speculative.

The hybrid substance, as it has been observed in the cells of various species hybrids in birds, simulates for cellular antigens the expression of heterosis in plants and animals. That is, it appears as the resultant of an interaction be-

tween genes. One may well ask if there is any other manifestation of heterosis in these species hybrids and backcross hybrids. Extensive measurements of eight body characteristics, as over-all length, extent, width of tarsus, width of band, length of wing, beak, middle toe, and tail, were made over a period of years under the supervision of L. J. Cole. The differences in the averages of these various characteristics between the parental Pearlneck and Ring dove species, as yet unpublished, were statistically significant, and the averages of the measurements of these characteristics in the species hybrids showed them to be in general intermediate between those of the parental species. Thus there was no evidence of heterosis in any external characteristic of the species hybrids, and no correlation with the hybrid substance of the blood cells.

CELLULAR CHARACTERS WITHIN A SPECIES

The finding that one or more genes on each of nine or ten pairs of chromosomes of Pearlneck had effects on the species specific antigens of the blood cells of this species made plausible the belief that many more genes than commonly believed would have effects within a species making for individuality of the cellular patterns. Acting on this assumption, a series of exploratory tests were made in experimental animals, principally in cattle and chickens. For example, following the transfusion of the blood of a young cow into her dam, an antibody was obtained from the serum of the recipient which reacted (produced lysis of the reacting cells upon the addition of complement to the serum-cell mixture) with the cells of some individuals, but not with those of others. The reactive substance was called A.

The objective was to be able to detect each antigenic factor separately, according to the following criterion. The reactive cells from any individual should remove the antibodies from the reagent specific for those cells, when added in excess to the reagent. However, if there were antibodies in the reagent which recognized two or more distinct blood factors, any such absorption with cells containing only one such substance would remove only a part of the antibodies. Those remaining would still be reactive with all cells containing the substance corresponding to the unabsorbed antibody.

To this criterion was added that of genetics for a single character, using the gene-frequency method since controlled matings were not possible. A typical example of the analysis is that for substance A, as follows:

Type of Mating	Number of offspring	
	With Antigen A	Lacking Antigen A
A×A.....	217	23
A×−.....	76	51
−×−.....	0	41

These results illustrate the observation that an individual has any cellular character recognized to date only if one or both parents possessed it. Also, each behaved as if it were a dominant to its absence.

From further isoimmunizations in cattle, and from immunizations of rabbits, various antisera have been obtained which detect other antigenic factors of cattle cells. Each of these has been subjected to the criteria of both genetics and immunology for a single character, as described in reports by Ferguson (1941), Ferguson *et al.* (1942), and Stormont (1950). At present, about forty different reagents are regularly used in typing cattle cells.

Other Antigens in Cattle

As stated above, the first substance detected in cattle cells was named A. The next was called B, the next C, . . . Z. That called A′ implies no relationship to A, nor B′ to B, etc. Each of these antigenic factors is therefore recognized independently, and when subjected to an analysis of gene frequency, each has behaved as expected if effected by a single gene in comparison to its absence.

However, some definite associations have been noted among them. For example, Ferguson (1941) reported that the C and E factors were not independent, for only C occurred alone, whereas E was present always with C, and such cells therefore had CE. It was postulated that there were three allelic genes involved, one for the components C and E together, one for C alone, and a third for the absence of both C and E.

It was later noted by Stormont that certain additional antigenic factors appeared only if one or more other components also were present. For example, the substance B occurs alone, as does that called G. But a third factor called K has never been observed unless both B and G were also present. (A possible exception to this rule was noted shortly after these factors were first demonstrable, and a weak reaction at that test with the reagent for the G substance was probably incorrectly recorded.) This association of K with B and G has been noted in over eighteen hundred animals of more than six thousand tested. Hence the combination of the BGK factors has always occurred as a unit, and it has also behaved as a unit in the progeny of individuals possessing it. A compilation of some unpublished data has yielded the following information:

Type of Mating	Number of Offspring	
	With BGK	Without BGK
BGK×BGK.....	151	44
BGK×−.....	185	137
−×−.....	0	160

Notwithstanding the fact that B, G, and K are recognized separately by respective reagents, these data, and the observation that K has occurred only with both B and G, are strong evidence for the conclusion that B, G, and K in the cells behave as a unit.

Further, offspring of some individuals possessing B and G (BG) in their cells have given only two classes of offspring, those with B and those with G, as would be expected if the causative genes were alleles. But another type of BG individual has produced offspring of two quite different types—those with both B and G (BG) and those with neither, as if a gene producing B and

TABLE 15.4

THE DISTRIBUTION OF THE CONSTITUENTS OF THE "B" COMPLEXES IN THE OFFSPRING OF SELECTED SIRES

Sire	Antigenic Complex	Number of Offspring	Antigenic Complex	Number of Offspring
H-1	$B_{BGIO_1T_2A'}$	25	$B_{O_1Y_2A'}$	23
H-4	$B_{BO_2A'E'_3}$	35	$B_{O_3J'K'}$	31
H-5	$B_{BO_1Y_2D'}$	26	$B_{O_3J'K'}$	24
H-6	B_{BO_1}	15	$B_{GY_2E'_1}$	23
H-7	$B_{BGKE'_2}$	14	$B_{GY_2E'_1}$	15
H-11	$B_{O_1A'}$	31	$B_{O_3J'K'}$	23
H-19	$B_{GY_2E'_1}$	19	B_b	13
G-19	$B_{IE'_1}$	8	B_b	7

G together was allelic to one not effecting either B or G. These combinations of antigenic substances, as BG and BGK, have been called *antigenic complexes*.

There are two series of such complexes, called the B and C series, respectively. In the B series there are twenty-one of the forty-odd antigenic characters which are associated in various conbinations. At least seven of these may appear singly, as was described for B and G. The other fourteen have been found only in various antigenic complexes, each of which may be made up of from two to eight of the twenty-one characters. The majority of these twenty-one characters do not occur at random in a complex with each of the others. As was stated above, the character K has always been found with B and G, but it has never occurred with I, with which it appears as a contrasting substance. In contrast, either B or G may be present in a complex with I. No separation of the antigenic characters of a complex has ever been observed in the cells of the offspring of an individual possessing it. A few examples are listed in Table 15.4 from more complete data given in a paper by Stormont, Owen, and Irwin (1951). All present evidence makes it seem somewhat more reasonable to assume that each antigenic complex is produced by a single gene than by linked genes. The various antigenic complexes in each

of the two systems, or series, would then be produced by a series of multiple alleles. The possibility of pseudo-alleles cannot be eliminated, but for the present may be assumed not to be a complicating factor.

If the assumption be granted that a single gene controls an antigenic complex, as BGK, what explanation or explanations can be proposed for the different antigenic specificities of this and other complexes, and, in turn, what can be inferred from such an explanation as to the action of the causative gene?

Antigens of Pneumococci

By virtue of the ability to attach simple chemical compounds to proteins, thereby preparing conjugated antigens with specifically reacting components of known constitution, there has emerged from such studies the realization that a so-called single antigenic substance may engender a multiplicity of antibodies of varying specificities (see Landsteiner, 1945, for a critical review and references). A pertinent example of this sort may be found in the antigenic relationship existing between type III and type VIII pneumococci. Cross reactions between the respective antisera (produced in horses) and the two types of pneumococci have been observed, implying to them some sort of antigenic similarity.

As is well known, the specificities of the pneumococcal types depend upon the carbohydrates of the capsules (Heidelberger and Avery, 1923, 1924). Thus, the carbohydrate of type III has been found to be a polyaldobionic acid (Reeves and Goebel, 1941). The understanding of the structure of the polysaccharide of type VIII is not as complete as for type III, but about 60 per cent of the molecule of the carbohydrate of type VIII appears to be aldobionic acid. Cross reactivity may therefore be expected between the soluble specific substances of types III (S III) and VIII (S VIII), by virtue of the presence in each of multiples of the same aldobionic acid as a structural unit. It is probable that the serologically reactive unit in each of these two types is a larger portion of the polysaccharide molecule than a single chemical structural unit. Type S VIII also contains approximately two glucose molecules for every aldobionic acid residue, thereby presumably accounting for at least a part of the specificity of type VIII in contrast to type III. Thus it may be seen that serological cross reactions may be expected when the antigenic substances under comparison are closely related chemically. Also to be expected is the ability to distinguish between such substances, as was actually possible in the case of types III and VIII (Heidelberger, Kabat, and Meyer, 1942).

Genetic Significance

The above example may be combined with other findings in the field of immunochemistry to allow the statement that antigenic substances of related but not identical chemical constitution may—but sometimes do not—incite the production of cross reacting antibodies. From the serological point of

view, a pertinent question concerning these antigenic complexes in cattle is whether the cells which react with the B reagent, or with any other specific reagent, do so by virtue of the presence of a specific reacting substance in a single antigenic molecule, or otherwise? Does the complex BGK, for example, represent (1) three different and separate antigenic substances? Or does it represent (2) a single antigenic substance with (*a*) a possible common base and three more or less different reactive groups accounting for B, G, and K, respectively, or (*b*) a single substance capable of inciting many specificities of antibodies, of which those for B, G, and K represent only a part of the reactivities of the spectrum of antibodies which may be produced? A combination of (*a*) and (*b*) also may be a possibility.

At present, very little experimental evidence is available concerning the adequacy of any one or combination of the above possibilities to explain the antigenic relationships of the components of the antigenic complexes of cattle cells. Tests are under way to determine whether the reactive substance called B, for example, is the same in all cells in which it appears, whether singly or in an antigenic complex.

In terms of the action of the causative genes, apart from the possibilities of linkage and pseudo-allelism, the question seems to resolve itself around two main aspects: (1) Do the genes controlling an antigenic complex, as a single gene for BGK, have separate specificities for B, G, and K, or (2) does this gene produce a single substance with no such separate specificities, and the similarities between such a complex as BGK and BGIY, are due primarily if not entirely to the general similarities in their chemical structure. The writer is inclined to adopt a combination of these two possibilities as a current working hypothesis. No matter what may eventually prove to be the correct interpretation of antigenic structure of the complexes, and the action of the controlling genes, it appears that these studies have given some insight into the complexities of the gene products and perhaps also of the causative genes.

The studies of the specificities of the gene products—the antigens of the blood cells of cattle—and the resulting inferences of the structure of the genes themselves, may not be directly related to the over-all heterosis problem. Nevertheless the writer is convinced that somewhat comparable specificities might well be obtained in plants, in which attempts are currently in progress to measure various aspects of the genetic bases for heterosis. Just how useful an additional tool of this sort would be is only a guess.