IMMUNOGENETIC STUDIES OF PSEUDOALLELISM IN *DROSOPHILA* MELANOGASTER. II. ANTIGENIC EFFECTS OF THE VERMILION PSEUDOALLES¹

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A PREVIOUS study of the antigenic effects of the lozenge pseudoalleles in *D. melanogaster* (CHOVNIK and FOX 1953) yielded results sufficiently fruitful to encourage a similar study of the vermilion pseudoalleles. Extensive studies of the genetics and physiology of the vermilion mutants have been performed by M. M. GREEN (1949, 1952, 1954) and by P. B. SHAPARD (1954). All such mutants are recessive and are characterized by the almost complete absence of brown pigment in the eye. All accumulate non-protein tryptophane at a much higher level than wild type, all are non-autonomous in gynandromorphs with v^+ , and all produce brown pigment when supplied with exogenous kynurenine or formylkynurenine. They presumably suffer from the inability to convert tryptophane into formylkynurenine, which serves as the precursor of kynurenine in the synthesis of brown pigment. In spite of these similarities, they may be separated into two physiological categories on the basis of their phenotypes in the presence of the non-allelic, linked suppressor mutants (su^2-s and su^2-v). Some, as exemplified by v^1 , exhibit the wild type phenotype in the presence of either suppressor and are collectively designated as v^s , while others, as exemplified by v^{36f} , exhibit an unaltered phenotype and are collectively designated as v^u . The distinction is reported to be unequivocal, and accumulation of non-protein tryptophane is also suppressed in v^s mutants but not in v^u . A parallel separation is afforded by an additional physiological criterion: partial starvation of larvae results in the production of brown pigment in all v^s mutants but not in v^u . Finally, the separation of the mutants into two classes on the basis of physiology is apparently paralleled by a difference in mutational origin. Among the mutants studied, all of those which were X-ray induced were unsuppressed, while the spontaneous mutants (including v^1 and v^{36f}) fell into both physiological categories.

The pseudoallelism of vermilion mutants has been demonstrated in an elegant experiment performed by GREEN (1955). Crossing over between v^1 and v^{36f} was observed with low frequency, thus suggesting that they are mutants at two closely linked loci bearing the following relationships to the raspberry (1-32.8) and miniature (1-36.1) loci on the X chromosome:

$$\text{ras } 0.2v^1 \ v^{36f} \quad 3.1 \quad m.$$

In addition, these mutants exhibit the type of position effect characteristic of pseudoalleles in *Drosophila*: the "trans" heterozygote (v^1/v^{36f}) exhibits the mutant phenotype, while the "cis" heterozygote ($v^1v^{36f}/+$) is wild type.

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On the basis of the demonstration of pseudoallelism, and on the basis of the observations referred to above, GREEN suggests that all v^s mutants are alleles of v^1 and that all v^u mutants are alleles of v^{36f} . He further suggests that the two loci are also distinguishable on the basis of mutability, *i.e.* that all induced mutations involve the v^{36f} locus either as "point" mutations at that locus alone or as small rearrangements or "point" mutations at both loci. It should be noted, however, that definitive cross-over tests of the various mutants with v^1 and v^{36f} have not been reported.

From the point of view of theory, the implication of these suggestions is that the two vermilion loci are separable not only on the basis of crossing over, but also on the basis of their physiology and mutability. Extended to pseudoallelism in general and to the theory of the gene, the implication is that the gene defined as a breeding unit displays physiological and mutational unity as well (GREEN 1955).

The previous paper in this series (CHOVNICK and FOX 1953) suggested that an operational attitude toward the problems of pseudoallelism would insist on no unique relationship between the transmissional behavior of the genetic material and its physiological or mutational attributes. The apparent contradiction posed by pseudoallelic mutants, namely the fact that they are transmitted as non-alleles but exhibit the physiological properties of alleles, springs from such an insistence (GOLDSCHMIDT 1951) but disappears when viewed operationally. In the present instance, operationism would restrain the contrary expectation that a series of mutants which are transmitted as alleles at one pseudoallelic locus need be similarly separable from mutants at the other pseudoallelic loci on the basis of physiological or mutational criteria.

The present paper contains evidence bearing on this point. It also discusses the question of the origin of pseudoalleles, the mechanisms of their physiological action, and the problem of the position effect which they exhibit in light of the antigenic effects of the vermilion pseudoalleles.

STOCKS

Three vermilion mutants were used in this investigation: v^1 , v^{36f} , and v^{48a} . The first two have been adequately described (BRIDGES and BREHME 1944); v^{48a} first appeared in a single male from a mating of an X-rayed male with an unirradiated attached-X female, and possesses apparently normal salivary chromosomes (FOX 1948). It is characterized by the production of an antigenic component ("antigen 2") which is not produced by either of two different wild alleles (FOX 1949b; FOX and WHITE 1953). GREEN (1953) reports that it is a v^u mutant, and that no crossovers between it and v^1 were observed among 40,000 offspring (GREEN 1954 and personal communication).

Mutant stocks containing v^1 , v^{36f} , v^{48a} , and v^1v^{36f} were kindly provided by M. M. GREEN. For purposes of antigenic analysis it was necessary that the mutants be placed on a coisogenic background. The background chosen was that of the isogenic Oregon R (Series I) stock used in the analysis of the lozenge pseudoalleles (CHOVNICK and FOX 1953). This stock had originally been rendered isogenic by JACK SCHULTZ and had been maintained thereafter through 112 generations of brother-sister matings at the time of the present derivation.

In preparation for the derivation, each of the single mutants and the double mutant combination were inserted into a marked X chromosome: $y\ ras^2\ v\ m\ f$. The derivation itself was accomplished by inserting the vermilion segment of each of the X chromosomes mentioned above into the X chromosome of the Oregon R stock, and by replacing autosomal chromosomes with those of Oregon R. In detail, $y\ ras^2\ v\ m\ f$ females were crossed with $In(2L)Cy$, Cy , $In(2R)Cy$, $cn^2/In(2LR)Pm$, ds^{33k} ; $H/In(3R)Mo$, $Sb\ sr$ males, producing $y\ ras^2\ v\ m\ f/Y$; $Cy/+$; $Sb/+$ males which were mated to females from the isogenic Oregon R stock. Females from this mating having the genotype $y\ ras^2\ v\ m\ f/+$; $Cy/+$; $Sb/+$ were mated in turn with Oregon R males. Crossover male offspring of either $y\ ras^2\ v/Y$ or $v\ m\ f/Y$ genotype were recovered. Using the former as an example, one such male was crossed with Oregon R females, yielding $y\ ras^2\ v/+$ females which were mated with Oregon R males. Crossover v/Y males were recovered. One such male was mated with Oregon R females, yielding $v/+$ daughters. These were mated with their father to give v/Y and v/v males and females, which were then used to establish isogenic vermilion stocks.

Four such stocks were established; three single-mutant stocks (v^1 , v^{36f} , and v^{48a}), and the double mutant (v^1v^{36f}). Each of these stocks possessed Y, second, and third chromosomes of isogenic Oregon R origin, and a vermilion segment inserted into an Oregon R X chromosome. Together with the Oregon R wild stock, they made available five coisogenic stocks differing with respect to the vermilion pseudoalleles. From these, equal numbers of homozygous females and hemizygous males could be collected as desired. Heterozygous females were produced as needed by crosses between the stocks.

The following qualifications of coisogenicity should be mentioned: (1) Aside from the differences with respect to the vermilion loci, the X chromosomes of the stocks may differ with respect to a segment no longer than 0.2 crossover units to the left and 3.1 units to the right of the vermilions. (2) Heterogeneity of autosomes (2 and 3) may have resulted from crossing over between Oregon R chromosomes and "marker" chromosomes during the derivation, in spite of the inversions present in the latter. (3) The fourth chromosome was not controlled during the derivation. Repeated outcross to Oregon R renders it probable, however, that the mutant stocks possess Oregon R fourth chromosomes. (4) Spontaneous mutation, without visible effect, could not be controlled during or after the derivation.

RESULTS

Suppression and larval starvation tests

To demonstrate that the mutants in our stocks behaved the same as those tested by GREEN, suppression tests were performed with su^{u-v} (1–0.0+). The method used was essentially the same as that used by GREEN (1952). In large experiments, $su^{u-v}\ v^1$ males were wild in phenotype, while newly emerged $su^{u-v}\ v^{36f}$ and $su^{u-v}\ v^{48a}$ males were vermilion. Thus, the mutants behave the same in our stocks as in GREEN's: v^1 is suppressed, and v^{36f} and v^{48a} are unsuppressed. In addition, it was observed that the double mutant combination, v^1v^{36f} , is unsuppressed.

The results reported below place v^{48a} in an important position, and an additional check was performed by means of the larval starvation test. Again, the method used

TABLE 1
Allelism of v^{48a}

Mating	Number of offspring	Non-vermilion offspring
$ras^2 v^{36f} m/v^{48a}; Cy/+; Sb/+ \times ras^2 v^{36f} m/Y$	78,934	$\begin{cases} 1\ ras, Sb \\ 1\ ras \end{cases}$
$ras^2 v^1 m/v^{48a}; Cy/+; Sb/+ \times ras^2 v^1 m/Y$	32,734	0
$ras^2 v^1 m/v^{48a}; Cy/+; Ubx/+ \times ras^2 v^1 m/Y^*$	40,000	0

* Data from GREEN (1954 and personal communication).

was the same as that used by GREEN (1954). Confirming his report, there is no production of brown pigment when v^{48a} is subjected to partial larval starvation.

The allelic status of v^{48a}

The test of allelism of v^{48a} with v^{36f} was performed by mating previously prepared $ras^2 v^{36f} m; Cy/Pm, ds^{33k}; H/In(3R)Mo, Sb sr$ females with v^{48a} males, to yield $ras^2 v^{36f} m/v^{48a}; Cy/+; In(3R)Mo, Sb sr/+$ females. These were then crossed with $ras^2 v^{36f} m$ males and the offspring were counted and classified. Crossovers between v^{36f} and v^{48a} would yield non-vermilion offspring; miniature if v^{48a} were to the right of v^{36f} , and raspberry if it were to the left of v^{36f} . The presence of inversions in the second and third chromosomes of the heterozygous females enhances crossing over between the X chromosomes; ras^2 and m guard against mistaking spontaneous mutants for crossovers; Cy and Sb , along with ras^2 and m , guard against contamination. Similar crosses were performed using v^1 instead of v^{36f} to test the allelism of v^{48a} with v^1 . The results of these tests, together with similar results reported by GREEN, are contained in table 1.

In the tests with v^{36f} , two raspberry males were observed among 78,934 counted offspring. These were demonstrated to be genotypically ras^2 by the results of matings with $ras^2 v m$ and wild females. They show that v^{48a} is pseudoallelic to v^{36f} , occupying a locus to the left of that of v^{36f} . In the tests for allelism involving v^{48a} and v^1 , no crossovers were recovered among 32,734 offspring. If the results of GREEN are added to ours, no crossovers have been observed among approximately 73,000 offspring. There is thus no evidence that v^{48a} is anything but an allele of v^1 , and the fact that they are both located to the left of v^{36f} is in agreement with this conclusion.

Immunological analysis

Methods used in the preparation of antigens and antisera and in the performance of immunological tests are given in previous publications (CHOVNICK and FOX 1953; FOX 1949b; FOX and WHITE 1953; FOX 1949a). Antigens were prepared by homogenizing approximately equal numbers of males and females of each of the coisogenic stocks (Oregon R, v^1 , v^{36f} , v^{48a} , and $v^1 v^{36f}$) and females of each of the two heterozygous genotypes, v^1/v^{36f} and $v^1 v^{36f}/+$. Rabbit antisera were prepared to each of these antigenic preparations. Antisera to each of the first five antigenic preparations were absorbed with each of these five antigens and each absorption was then tested by means of modified precipitin-ring tests with all seven antigens. Antisera to the two hetero-

TABLE 2
Analysis of anti- v^1 serum

Absorbed by	Tested with						
	Ore-R	v^1	v^{36f}	v^{48a}	v^1v^{36f}	v^1/v^{36f}	$v^1v^{36f}/+$
Unabsorbed	+	+	+	+	+	+	+
Oregon R	—	+	+	+	+	+	+
v^1	—	—	—	—	—	—	—
v^{36f}	—	—	—	—	—	—	—
v^{48a}	—	—	—	—	—	—	—
v^1v^{36f}	—	—	—	—	—	—	—

Antiserum, normal serum, and antigen controls all negative.

zygotes were absorbed by each of the seven antigens and each absorption was then tested with all seven antigens. Antigen, antiserum, and normal serum controls were included in all tests. The tests were semi-quantitative in nature, similar to those presented in previous publications, although only qualitative summaries of the results are given: the symbol + indicates a definite reaction of any strength, while — indicates the lack of reaction. The differences reported are distinctly qualitative, and in each case absorptions with excess heterologous antigen did not inhibit the reactions with homologous antigen.

The results obtained with anti- v^1 serum are summarized in table 2. Before absorption, such serum reacts with all antigenic preparations with equal titer. Most of the antigens are therefore similar in all genotypes; these will be designated residual antigens. Following absorption with Oregon R antigens, differences among the genotypes begin to appear. At the proper level, such absorption removes from the serum all antibodies against components which Oregon R shares with v^1 , but the serum still reacts with the antigens of the six mutant genotypes. This indicates that v^1 possesses an antigenic component (simple or complex) which is not possessed by Oregon R but which is also present in the other mutant genotypes.

The specificity of the serum is made evident by its exhaustion by absorption with v^1 . It is similarly exhausted by absorption with each of the other mutant genotypes, indicating that v^1 possesses no antigenic components not also possessed by these other genotypes. Thus, anti- v^1 serum evidently contains antibodies specific to an antigenic component common to all six of the vermilion genotypes but not present in Oregon R. This component will be referred to as antigen V-1.

Table 3 contains the results obtained with anti- v^{36f} serum. The reactions with unabsorbed serum confirm the existence of residual antigens. The conclusion reached above that the vermilion genotypes contain at least one antigenic component (V-1) not possessed by Oregon R is also confirmed, since absorption by Oregon R inactivates the serum only with respect to Oregon R. In addition, absorption by v^1 yields results indicating the presence of anti- v^{36f} serum of antibodies to an antigenic component other than V-1, common to v^{36f} , v^{48a} , v^1v^{36f} , and the heterozygotes, but not present in either Oregon R or v^1 . Thus, a second antigenic component, V-2, is identified.

TABLE 3
Analysis of anti- v^{36f} , anti- v^{48a} (Type 1), anti- v^1/v^{36f} , and anti- $v^1v^{36f}/+$ sera

Absorbed by	Tested with						
	Ore-R	v^1	v^{36f}	v^{48a}	v^1v^{36f}	v^1/v^{36f}	$v^1v^{36f}/+$
Unabsorbed	+	+	+	+	+	+	+
Oregon R	—	+	+	+	+	+	+
v^1	—	—	+	+	+	+	+
v^{36f}	—	—	—	—	—	—	—
v^{48a}	—	—	—	—	—	—	—
v^1v^{36f}	—	—	—	—	—	—	—

Antiserum, normal serum, and antigen controls all negative.

It should be remarked that not all antisera exhibit such specificity. Thus, among anti- v^1 sera some are non-specific, but those which exhibit specificity yield the results contained in table 2. Such experiences are frequent when working with complex antigenic mixtures (IRWIN 1949; FOX and WHITE 1953), and the occurrence of non-specific antisera does not contradict the existence of specificity when it is demonstrated. Indeed, it is frequently observed that an antiserum may contain antibodies to some but not all of the components in an antigenic mixture, and that different sera may be specific for different components.

Such is the case among anti- v^{48a} sera. Those which exhibit specificity are of two types, designated 1 and 2. Table 3 contains the results obtained with anti- v^{48a} sera of the first type; the specificities exhibited by this type of serum are identical with those exhibited by anti- v^{36f} serum. Such antiserum thus yields confirmatory evidence of the existence of antigens V-1 and V-2 as well as residual antigens.

The second type of anti- v^{48a} serum yields evidence of an additional component, but contains no antibodies to antigens V-1 or V-2 (Table 4). This is indicated by the fact that absorption with Oregon R completely inactivates the serum, while absorption by v^1 or v^{36f} does not inactivate it with respect to v^{48a} and v^1v^{36f} . Apparently v^{48a} possesses an antigenic component, designated V-3, which is also present in v^1v^{36f} and in Oregon R.

The conclusion that v^{48a} and v^1v^{36f} possess antigen V-3 is based on two observations;

TABLE 4
Analysis of anti- v^{48a} (Type 2) and anti- v^1v^{36f} sera

Absorbed by	Tested with						
	Ore-R	v^1	v^{36f}	v^{48a}	v^1v^{36f}	v^1/v^{36f}	$v^1v^{36f}/+$
Unabsorbed	+	+	+	+	+	+	+
Oregon R	—	—	—	—	—	—	—
v^1	—	—	—	+	+	—	—
v^{36f}	—	—	—	+	+	—	—
v^{48a}	—	—	—	—	—	—	—
v^1v^{36f}	—	—	—	—	—	—	—

Antiserum, normal serum, and antigen controls all negative.

TABLE 5
Analysis of anti-Oregon R serum

Absorbed by	Tested with						
	Ore-R	v^1	v^{36f}	v^{48a}	$v^{1v^{36f}}$	v^1/v^{36f}	$v^1v^{36f}/+$
Unabsorbed	+	+	+	+	+	+	+
Oregon R	—	—	—	—	—	—	—
v^1	—	—	—	—	—	—	—
v^{36f}	—	—	—	—	—	—	—
v^{48a}	—	—	—	—	—	—	—
$v^{1v^{36f}}$	—	—	—	—	—	—	—

Antiserum, normal serum, and antigen controls all negative.

both completely inactivate the serum when used in absorptions, and both react with the serum after it has been absorbed by v^1 or v^{36f} . Oregon R, on the other hand, exhausts the serum when used in absorptions but does not react with serum previously absorbed with v^1 or v^{36f} . Oregon R, in other words, possesses V-3 specificity, but in a form incapable of precipitating V-3 antibodies even though uniting with them. The result, in immunological terminology, is inhibition.

Confirmation of the existence of antigen V-3 is obtained from the analysis of anti- v^1v^{36f} serum (table 4). Such antiserum reacts in a manner identical with that of Type 2 anti- v^{48a} sera. V-3 antibodies are present, and they are precipitated by the corresponding antigen in v^{48a} and $v^{1v^{36f}}$. Oregon R, however, inhibits these antibodies without precipitation; V-3 specificity is therefore present, but in a different form than in v^{48a} and $v^{1v^{36f}}$.

Further information concerning the nature of V-3 in Oregon R is obtained from the analysis of anti-Oregon R serum (table 5). None of several such antisera have contained antibodies to antigens other than residual antigens. Antibodies to antigens V-1 and V-2 are not expected, since these antigens have been demonstrated not to be present in Oregon R. Beyond this, there is no evidence that Oregon R contains any other specific antigenic components. Antigen V-3, as it is present in Oregon R, is therefore not only incapable of precipitating homologous antibody, but is also incapable of inducing antibody formation. While it is present in the form of a complete antigen in v^{48a} and $v^{1v^{36f}}$, it behaves like a hapten in Oregon R.

LANDSTEINER (1945) defines haptens as "... specific protein-free substances, although reactive in vitro, induced no, or only slight antibody response. For serologically active substances of this sort, in contradistinction to the protein antigens which possesses both properties, the term hapten has been proposed..." The serological behavior of V-3 in Oregon R corresponds with this definition. Preliminary tests indicate that, in Oregon R, V-3 is heat-stable and nondialyzable. These tests have not been extensive, however, and further evidence concerning the chemical nature of the V-3 substance in Oregon R is not yet available.

Cis and trans heterozygotes for v^1 and v^{36f} have thus far been demonstrated both to possess antigens V-1 and V-2. Therefore, no position effect has yet been found. Differences between the two heterozygous genotypes may exist, however, with

TABLE 6
Summary of antigenic distribution

Genotype	Antigenic Components			
	V-1	V-2	V-3	Residual
Oregon R	—	—	Haptenic	+
v^1	+	—	—	+
v^{36f}	+	+	—	+
v^{48a}	+	+	+	+
v^1v^{36f}	+	+	+	+
v^1/v^{36f}	+	+	—	+
$v^1v^{36f}/+$	+	+	—	+

respect to antigens not possessed by Oregon R or the homozygous and hemizygous mutants. Such differences would be disclosed in the analysis of antisera against the heterozygotes (anti- v^1/v^{36f} and anti- $v^1v^{36f}/+$). Both kinds of antisera react in the manner contained in table 3. The reactions summarized therein are indicative of the presence of antigens V-1 and V-2, as well as residual antigens, in both kinds of heterozygote. No differences between the two heterozygotes are indicated, and therefore no position effect on antigens.

The antigenic relationships of the analyzed genotypes are summarized in table 6.

DISCUSSION

As was the case with respect to the antigenic effects of the lozenge pseudoalleles, the results of the present investigation are consistent with the suggestion that pseudoalleles arise by a process of genic replication along the chromosome, accompanied or followed by complementary physiological differentiation (BRIDGES 1935; LEWIS 1951). The similarities in antigenic effects which have been encountered among pseudoallelic mutants suggest duplication; the differences suggest partial differentiation.

It was suggested, in connection with the lozenge analysis, that the physiological differentiation of pseudoalleles could take three forms: (1) With slight changes in substrate specificity pseudoallelic loci could become associated with successive steps in a sequence of reactions. This suggestion is incorporated in the physiological hypothesis applied to pseudoallelism by LEWIS (1951) and by PONTECORVO (1950). (2) Pseudoallelic loci could be modified in such a manner as to convert the same substrate or substrates into one or more products, similar in some respects and different in others. Such differentiation would reflect more directly the duplicate origin of the loci than would differentiation of the first type. (3) Each locus could be concerned with the transformation of two or more substrates, and differentiation could take the form of a gradual shift in "substrate spectrum." As in the previous case, such differentiation would be an easy consequence of origin by duplication. The antigenic effects of the lozenge pseudoalleles were such as to be explainable by means of simple models based on any of these three suggestions. In the present instance, the complexity of the antigenic effects render it extremely unlikely that they are produced by

the association of the two vermilion loci with successive steps in a linear sequence of reactions. At any rate, we have not been able to construct a model of this sort that would be consistent with the results without numerous *ad hoc* assumptions. Simple models based on the second and third suggestions will explain the present results. Since data are not yet available to distinguish between these latter models, it does not seem profitable to specify them in greater detail at the present time. Antigenic analysis of the unexamined genotypes should provide more definitive information.

However, the inconsistency of the present data with the possibility that the two vermilion loci are concerned with successive steps in a chain of reactions deserves additional comment. As indicated, that possibility is an integral part of the LEWIS hypothesis of the physiology of pseudoallelism. A second feature of the hypothesis is that the action of pseudoallelic loci is restricted to the immediate vicinity of the chromosome. Since the antigenic effects of the lozenge pseudoalleles suggested interallelic interaction at either or both of two loci (Iz^{46} and Iz^{Bs}), immunogenetic studies of pseudoallelism have yielded evidence difficult to reconcile with both aspects of the hypothesis. It is entirely possible, of course, that the processes of antigen formation are entirely unrelated to those concerned with the morphological and pigmentary effects that form the basis of the hypothesis. Little is known about the physiological mechanisms responsible for the morphological effects of the lozenge mutants. CHOVNICK (personal communication) suggests that an affect on the development of hypodermal derivatives may be involved, but no obvious connection with the antigenic effects exists. No gross effects on amino acid metabolism are detectable by means of paper chromatography of free amino acids. (FOX 1954). It has been suggested (NOLTE 1952) that the effects of eye color mutants are associated with protein metabolism, but the connection with antigenic effect may nevertheless be remote. The tryptophane content of proteins in vermilion is not detectably different from that of wild (GREEN 1949) in spite of the antigenic differences. The two models outlined above suggest either that tryptophane is the common substrate for the production of eye pigment and antigenic effect but that these are produced along different pathways, or that tryptophane is only one of several substrates utilized by the vermilion loci and not the substrate of antigenic effect.

An additional circumstance suggests that the antigenic effects of pseudoalleles are associated with different processes than the morphological effects, namely the failure to demonstrate a position effect on antigenic specificity in heterozygotes of both the lozenge and the vermilion mutants. These observations demonstrate that position effect is not a necessary physiological property of "position pseudoalleles" at all phenotypic levels. The suggestion of physiological unrelatedness of the antigenic and morphological effects does not completely resolve the difficulties for the LEWIS hypothesis, however, for a study of the effects of the lozenge pseudoalleles on tarsal claws, where a position effect is observed, has yielded evidence posing similar difficulties (CHOVNICK personal communication).

Considering the antigenic effects of the vermilion pseudoalleles in greater detail, it is to be noted that more than one antigen may be associated with a single gene. A similar observation was made in connection with the lozenge mutants, and recently in connection with the antigens determined by alleles at the H-2 locus in the mouse

(HOECKER, COUNCE, and SMITH 1954). The bearing of such observations on questions regarding the Rh and other similar systems in man and other species will be the subject of a separate communication. In this connection, the antigenic component previously (FOX 1949b; FOX and WHITE 1953) associated with v^{48a} and designated "antigen 2" is now shown to consist of at least three different components (V-1, V-2, and V-3). This resolution does not affect any previous conclusions. It should also be noted that antigen V-3 is present in v^{48a} and v^1v^{36f} , but not in v^1 or v^{36f} . Thus, the double mutant is capable of producing an antigenic component which is not produced by either of its mutant genes separately, but which is produced by a different mutant at one of the two loci. In the double mutant the effect must therefore be the result of interaction of the two mutant genes. Since this is the case, V-3 must be added to the list of antigens in *Drosophila* which are produced by genic interaction, a list which previously had included "antigen 1" and the lozenge antigen "L-3" (FOX 1949b; FOX and WHITE 1953; CHOVNICK and FOX 1953).

Some insight into the nature of the interaction between v^1 and v^{36f} , and some further insight into the physiological and mutational properties of the two loci, is afforded by the observation that V-3 is present in Oregon R in what appears to be a haptenic form. If this is a true hapten a possible mechanism explaining its occurrence, as well as the presence of V-3 in v^{48a} and v^1v^{36f} , is the following: the wild alleles of both loci are necessary for the production of the V-3 hapten; v^1 and v^{36f} involve complementary mutational changes in the sense that neither alone can support the production of the hapten but that both together can; these same complementary changes confer upon the two mutants the neomorphic property of participating together in bestowing upon the hapten complete antigenicity, possibly by coupling it with protein; v^{48a} involves a mutational change at its locus which does not prevent it from interacting with the wild allele at the other locus to produce the hapten, but which does confer upon it the neomorphic property of converting the hapten to complete antigen. The suggestion that genic action may involve the production of hapten-like prosthetic groups was first made some years ago by WRIGHT (1941) and has been applied more recently by EPHRUSSI (1950) to a case of cytoplasmic inheritance in yeast. The principal feature of the present suggestion is that the two vermilion loci appear to act as a single physiological unit in the production of V-3 specificity.

Returning to the problem outlined in the introduction, the demonstration that v^{48a} is transmitted as an allele of v^1 but not of v^{36f} has immediate importance. Re-stated, the problem is created by the fundamental characteristic of pseudoallelism: pseudoallelic mutants are mutants which are transmitted as non-alleles since they exhibit a low order of recombination (crossing over), but which exhibit the physiological characteristics usually associated with allelism (similar or identical phenotypes in homozygotes and interaction in heterozygotes) (McCLINTOCK 1944; LEWIS 1945). The usual interpretation given to such observations, and the one adopted here, is that such mutants occupy distinct but adjacent and duplicate loci on homologous chromosomes. The question is whether pseudoallelic loci are distinct physiologically and mutationally as well as in transmission. Further examination of this question as regards the vermilion mutants is facilitated by a tabulation of their physiological attributes in single mutant genotypes (table 7).

TABLE 7
Physiological attributes of vermilion mutants

Physiological attribute	v^1	v^{36f}	v^{48a}
Tryptophane accumulation	+	+	+
Response to kynurenine	+	+	+
Genetic suppression	+	—	—
Larval starvation	+	—	—
Antigen V-1	+	+	+
Antigen V-2	—	+	+
Antigen V-3	—	—	+

The features of this summary are such as to make apparent the complexity of the physiological relationships among these pseudoallelic mutants. Each mutant is characterized by its own constellation of physiological effects. The two mutants which are transmitted as alleles (v^1 and v^{48a}) show no greater physiological similarity than is exhibited by non-alleles. Indeed, v^{48a} shares more attributes with the non-allele v^{36f} than with its allele v^1 . There is no single attribute nor any combination of attributes which distinguish the mutants at one locus from those at the other. In short, the two loci are not physiologically distinct.

Nor, is the situation less complex with respect to mutation. The suggestion that X-ray induced mutants always involve an alteration at the v^{36f} locus is contradicted by the allelic status of v^{48a} . Thus, the two loci are not separable on the basis of this aspect of mutability.

The centrality of v^{48a} in these considerations is, of course, recognized. It is possible that some of its special attributes, such as its association with antigen V-3, are a consequence of its origin by X-ray induction. There is, however, neither genetic nor cytological evidence that it is anything but a "point mutation" at the v^1 locus. On the other hand, it is possible that it is not an X-ray induced mutant even though it arose in an irradiated X chromosome. This would have implications for the argument concerning mutability but would not affect the physiological considerations.

The lack of physiological distinctiveness among pseudoallelic loci has been taken to require a revision of genetic theory (GOLDSCHMIDT 1951; PONTECORVO 1950; DEMEREC 1955); we prefer to suggest that the investigation of transmission, of physiology, and of mutation involves a different set of operations in each case, and that there is consequently no need to expect that the units which are thereby established will correspond (BRIDGMAN 1932; WRIGHT 1954). There is, in addition, difficulty in establishing an operational basis for the definition of a physiological unit. In the present case, for example, the whole pseudoallelic segment appears to function as a physiological unit at one level, but at a second level the individual loci exhibit physiological unity, while at a third level each locus appears to consist of two or more physiological units. It does not help, as has been suggested (LEWIS 1954, 1955), to propose that at the most fundamental level each of the genes of a pseudoallelic series acts as an agent controlling a single, specific reaction. In the present case again, there is disparity between the antigenic and pigmentary effects but there is no way of

knowing which most directly reflects "primary" gene action. In short, it is impossible to specify at the present time the operational criteria which would identify the primary level of gene action. When such criteria are available it will be possible to establish the unit of physiological action, and the question of its relationship to the unit of transmission may then be subjected to critical examination. Since similar difficulties exist in the definition of the unit of mutation, it would appear that the most satisfactory definition of the gene is still that of transmission, namely that of inseparability by crossing over and rearrangement.

SUMMARY

Immunogenetic analysis of the effects of three vermilion mutants has been performed by the use of coisogenic stocks. The X-ray induced mutant v^{48a} exhibits crossing over with the spontaneous mutant v^{36f} , but not with the spontaneous mutant v^1 ; the two latter are known to exhibit crossing over. Suppression tests with su^{v-v} reveal that v^{48a} , like v^{36f} , is unsuppressed, and larval starvation tests demonstrate an additional physiological similarity of v^{48a} and v^{36f} . In addition to residual antigens, which are present in all genotypes, three specific antigenic components have been identified: V-1, which is associated with v^1 , v^{36f} , v^{48a} , and v^1v^{36f} ; V-2, associated with v^{36f} , v^{48a} , and v^1v^{36f} ; and V-3, associated with v^{48a} and v^1v^{36f} , and present in the wild genotype in apparently haptenic form. The "cis" and "trans" heterozygotes for v^1 and v^{36f} possess antigens V-1 and V-2; there is no position effect on antigenic specificity. The implications of these results for the origin and physiology of pseudoallelism, and for gene-antigen relationships, are discussed. It is noted that while the pseudoallelic vermilion loci exhibit transmissional distinctness, there is neither physiological nor mutational distinctness. An operational interpretation is suggested.

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