A PHYSIOLOGICAL STUDY OF THE VERMILION EYE COLOR MUTANTS OF DROSOPHILA MELANOGASTER[1]

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THE vermilion eye color mutant *Drosophila melanogaster* was one of the mutants used by BEADLE, EPHRUSSI, TATUM and their collaborators in their early attempts to interpret the biochemical effects of genes (Cf. Review by EPHRUSSI 1942). More recent studies on vermilion mutants (GREEN 1949, 1952, 1954) made it seem worthwhile to pursue further the study of the physiological effects of these mutants.

The sex-linked, recessive vermilion (v) eye color mutants of *D. melanogaster* are characterized phenotypically by a lack of brown eye pigment under the usual genetic and environmental conditions. GREEN (1952) distinguished two series of vermilion mutants by their differential interaction with a nonallelic X chromosome mutant, suppressor of vermilion (*su-v*). One group of v mutants approaches wild type in the production of brown eye pigment when combined with *su-v*. This series may be designated v^s, indicating that it is suppressible by *su-v*. The other series may be designated v^u, since it is unsuppressed by *su-v*. GREEN (1954) reported crossing over between a v^s allele (v^1) and a v^u allele (v^{36f}) indicating that they are pseudoallelic to each other. BARISH and FOX (1956) report that the v^u mutant, v^{48a}, is allelic to v^1 and pseudoallelic to v^{36f}.

STURTEVANT (1920) demonstrated by the use of gynandromorphs that the $v(v^1 = v^s)$ mutant was nonautonomous in development; i.e., flies which have genetically v eye tissue, but also contain v^+ tissue, may have phenotypically v^+ eyes. Transplantation of optic discs from v larvae into wild type larvae (EPHRUSSI and BEADLE 1935) confirmed the nonautonomous development of v with respect to wild type tissue. GREEN (1952) established developmental nonautonomy of the v^u mutants (v^{36f}, v^{51a}, v^{51b}, v^{51c}) with respect to wild type tissue in gynandromorphs. Studies by TATUM (1939), TATUM and HAAGEN-SMIT (1941), BUTENANDT, WEIDEL and BECKER (1940) and KIKKAWA (1941) established that genetically v flies will produce brown eye pigment if supplied with the tryptophan metabolite, kynurenine, either by injection or in the larval food.

GREEN (1949) found that large quantities of nonprotein tryptophan are accumulated by flies carrying v^1. Either kynurenine or its precursor, formylkynurenine, was shown to act as a precursor for brown eye pigment for both $v^1(v^s)$

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and the v^u mutants, (v^{36f} and v^{51c}), by GREEN (1952). It has been established that in rat liver formylkynurenine is an intermediate in the conversion of tryptophan to kynurenine (KNOX and MEHLER 1950; MEHLER and KNOX 1950). It appears from the data of GREEN (1949, 1952) that in both classes of v mutants the block in brown eye pigment formation is prior to the formation of formylkynurenine, and, in the case of v^1, the block occurs between tryptophan and formylkynurenine. KNOX and MEHLER (1950) postulated two steps in the conversion of tryptophan to formylkynurenine. It appears that the two steps are catalyzed by the same enzyme in successive ferric-ferrous states (KNOX 1952, 1954) as a peroxidase and an oxidase. An intermediate between tryptophan and formylkynurenine has not been found (MEHLER 1955).

The evidence reported below supports the idea that both v^s and v^u mutants are blocked in the immediate utilization of tryptophan. Further differences between the two series of v mutants in their interaction with the suppressor mutant and with certain environmental situations are demonstrated. A brief report of these results has been made (SHAPARD 1954b).

MATERIALS AND METHODS

The mutants used in most of the experiments were the *D. melanogaster* mutants, $v^1(v^s)$, $v^{36f}(v^u)$ and $su^2\text{-}s(su\text{-}v)$. Except where otherwise indicated these were combined with the autosomal recessive eye color mutant brown (*bw*) which blocks red eye pigment formation. The combination of the *bw* mutant with a v mutant results in the production of a nearly white eye, making it possible to determine more accurately the quantity of brown eye pigment which may be produced under experimental conditions. Three other v mutants: $v^2(v^s)$, $v^k(v^s)$ and $v^{51c}(v^u)$ were used in some experiments. An allele of $su^2\text{-}s$, $su^{51c}\text{-}v$, was used also. Cinnabar (*cn*), an autosomal recessive eye color mutant which fails to produce brown eye pigment and which accumulates kynurenine (KIKKAWA 1941) was used as a control in some tests. The sex-linked recessive mutants yellow (y and y^2), singed (sn^3), forked (f) and furrowed (fw) were present in some stocks. Most of the above mutants are described in BRIDGES and BREHME (1944). $su^{51c}\text{-}v$ arose spontaneously in a y^2 v f stock at The University of Texas. v^k was obtained by AUERBACH from irradiated larvae; its suppression was shown by GREEN (personal communication). v^{51c} also was obtained in an X-ray experiment (GREEN 1952). No attempt was made to develop isogenic stocks. Certain special *D. melanogaster* stocks and stocks of other species will be described where appropriate.

Flies prepared by the following freeze-dry technique were used in biochemical and feeding experiments. Flies were raised on regular corn meal, molasses, agar, dry brewer's yeast medium seeded with live yeast (this will be referred to as regular medium) or on the same medium supplemented with 1 mgm DL-tryptophan/ml medium (referred to as tryptophan medium). Twenty females mated to 20 or more males were allowed to lay eggs for 24 hours or less on heavily yeasted bottles. After the parents were removed, "kleenex" soaked in a suspension of live

yeast was added to the bottles. Temperatures were maintained in a range of 23–25°C. Adult flies were collected 0–2 hours after emergence and the bottles from which they were collected were discarded 10–11 days after the beginning of the egg-laying period. The freshly collected flies were frozen in a bath of methyl cellosolve and solid CO_2. They were kept in the bath at least one hour, transferred to a vacuum desiccator containing "drierite" ($CaSO_4$) and dried *in vacuo* for 24 hours or more, then stored in the vacuum desiccator until used.

Analysis for nonprotein tryptophan was performed on weighed samples of dried flies which had been pulverized in an agate mortar. Protein was precipitated with a ten percent solution of trichloracetic acid. The mixture was filtered, and tryptophan was determined on the filtrate by the HORN and JONES (1945) modification of the MAY-ROSE *p*-dimethylaminobenzaldehyde method at a wave length of 600 millimicrons on a Coleman Junior spectrophotometer, Model 6a. Tryptophan in this paper always refers to nonprotein tryptophan. A standard curve was made with L-tryptophan in aqueous solution. The range from 0.02–0.04 mgm tryptophan is most sensitive. Where it is indicated that a determination is outside the range of accuracy of the test, the amount of material used had a quantity of tryptophan outside the range 0.02–0.04 mgm but within the range 0.00–0.06 mgm for which an optical density *vs.* L-tryptophan curve was determined. Paper chromatography provides further evidence that the substance which is accumulated by *v* flies is indeed tryptophan. It does not separate from L-tryptophan when chromatographed by either ascending or circular techniques with 4 butanol:5 water:1 acetic acid solvent and stains the purple of tryptophan when stained with Erlich's reagent prepared by the method of SMITH (1953).

EXPERIMENTAL RESULTS

Accumulation of nonprotein tryptophan by v^s *and* v^u *mutants*

Tryptophan is high in both v^s and v^u mutants as compared to v^+ flies (Tables 1 and 2). An increased accumulation of tryptophan by both classes of mutants was observed in flies raised on the medium supplemented with tryptophan. The quantity of tryptophan in v^+ flies does not appear to be affected by the amount of tryptophan in the medium. A comparison with GREEN's 1949 report of 0.236 mgm tryptophan/gm dried flies for v^+; *bw* and 1.389 mgm/gm for $v(v^1)$ suggests that his medium may have had a higher tryptophan content.

Determinations made on flies raised at the same time, and under similar culture conditions (Table 1), indicate that v^1; *bw* flies accumulate slightly less tryptophan than do flies of the v^{36f}; *bw* genotype. This might be expected since the v^{36f} mutant and other v^u mutants combined with the *bw* mutant actually have white eyes. The v^1 mutant and other v^s mutants when combined with the *bw* mutant produce eyes with a slight brownish tinge indicating that the block in the production of brown eye pigment is not complete. However, except in experiment "j" on regular medium, this difference is small. The data with respect to flies of the genotypes v^2 *fw*(v^s) and *y* sn^3 v^{51c}; *bw* (v^u) (Table 2) do not allow a generalization that v^s mutants accumulate less tryptophan than do v^u mutants. The

TABLE 1

Nonprotein tryptophan composition of adult flies homozygous or heterozygous for v *mutants* (v^1 *or* v^{36f}) *or for the* v^+ *gene*

		Regular medium			Tryptophan supplement			
			mgm tryptophan*			mgm tryptophan		
Genotype	Sex	Ex.†	Avg.‡	Range	Ex.	Avg.	Range	Eye color§
v^+; *bw*	♂ ♀	h	0.268(3)	0.266–0.270	h	0.237(3)	0.229–0.245	5.0
	♂ ♀	a	0.239(2)	0.237–0.240	f	0.268(2)	0.247–0.288	5.0
v^1; *bw*	♂ ♀	h	0.733(2)	0.717–0.748	h	0.900(2)	0.898–0.901	0.0+
	♀ ♀	j	0.757(2)	0.752–0.762	i	1.008(2)	0.974–1.042	0.0+
v^{36f}; *bw*	♂ ♀	h	0.768(2)	0.755–0.780	h	0.920(2)	0.885–0.954	0.0
	♀ ♀	j	0.944(2)	0.925–0.962	i	1.045(2)	1.041–1.048	0.0
v^1/v^+; *bw*	♀ ♀	c	0.206(2)	0.201–0.211				5.0
v^1/v^{36f}; *bw*	♀ ♀	c	0.593(2)	0.577–0.608				0.0

* Tryptophan is expressed as mgm/gm dried flies.
† Ex. = Experiment; all determinations in the same experiment were made on flies raised at the same time on the same batch of medium except in experiment h in which flies were raised simultaneously on regular medium and on tryptophan supplemented medium.
‡ Number in brackets following average value represents number of determinations.
§ Eye color is based on the scale of TATUM and BEADLE (1939) where v^+; *bw* = 5, *v*; *bw* = 0.

TABLE 2

Nonprotein tryptophan composition of adult flies of the mutants v^2 *and* v^{51c} *and of their heterozygous combinations with* v^1 *and* v^{36f} *mutants*

	Regular medium				Tryptophan supplement			
			mgm tryptophan*				mgm tryptophan	
Genotype	Sex	Ex.†	Avg.‡	Range	Sex	Ex.	Avg.	Range
v^2 *fw*	♂ ♀	e	0.803(3)	0.770–0.854	♀ ♀	i	0.916(1)	.....
y sn^3 v^{51c}; *bw*	♂ ♀	e	0.583(2)	0.578–0.587	♀ ♀	i	0.838(2)	0.828–0.847
v^{36f}/y sn^3 v^{51c}; *bw*	♀ ♀	c	0.666(2)	0.578–0.783				
v^1/v^2 *fw*; *bw*/+	♀ ♀	e	0.843(2)	0.829–0.856				

* Tryptophan is expressed as mgm/gm dried flies.
† Ex. = Experiment; all determinations in the same experiment were made on flies raised at the same time on the same batch of medium.
‡ Number in brackets following average represents number of determinations.

values for the v^2 and v^{51c} mutants may be affected by the background genotype, and real conclusions on such small differences could only be derived from analysis of a series of co-isogenic stocks differing only in their *v* alleles. The mutant allele, v^1, is recessive to its wild type allele in tryptophan accumulation as it is in eye color effect (Table 1). The heterozygote, v^1/v^{36f}; *bw*, accumulates tryptophan at a level comparable to either homozygote (Table 1).

Developmental autonomy of v^1 *and* v^{36f} *mutants in relation to each other*

The developmental autonomy of v^1 and v^{36f} mutants in relation to each other was tested by constituting gynandromorphs in which the female tissue was v^1/v^{36f}, and the male tissue was either v^1 or v^{36f} in genotype. The production of

gynandromorphs with relative ease was made possible by deriving stocks which carried the mutants to be tested along with the third chromosome eye color mutant claret nondisjunctional (ca^{nd}). The mutant, ca^{nd}, kindly made available to us by DR. E. B. LEWIS of the California Institute of Technology, is associated with a greatly increased frequency of somatic elimination of the maternal X chromosome (LEWIS and GENCARELLA 1952). A light, clear orange eye is observed in *v*; *ca* flies as opposed to the deep opaque orange of *v* mutants. Crosses were made as follows:

(a) v^{36f}; ca^{nd} ♀♀ × $y\ v^{1}\ f$; ca^{nd} ♂♂

(b) v^{1}; ca^{nd} ♀♀ × $y\ sn^{3}\ v^{36f}$; ca^{nd} ♂♂

When the maternal X chromosome was eliminated, gynandromorphs were obtained in which the female tissue had the markers *y* and *f* in cross "a" and the markers *y* and sn^{3} in cross "b."

i.e., (a) Female tissue = $v^{36f}/y\ v^{1}\ f$; ca^{nd} Male tissue = $y\ v^{1}\ f$; ca^{nd}

(b) Female tissue = $v^{1}/y\ sn^{3}\ v^{36f}$; ca^{nd} Male tissue = $y\ sn^{3}\ v^{36f}$; ca^{nd}

Seven gynandromorphs from cross "a" were observed. Three of these were "bilateral gynandromorphs," i.e., all external tissue on one side of the body was *y f* male, and the other side was $y^{+}\ f^{+}$ female. Seven gynandromorphs were also observed from cross "b." One was a "bilateral gynandromorph"; another had head and thorax $y^{+}\ sn^{+}$ female, abdomen $y\ sn^{3}$ male; a third had the head and one side of the thorax $y^{+}\ sn^{+}$ female, and the remainder of the body $y\ sn^{3}$ male. The eyes of all of the gynandromorphs observed from both crosses "a" and "b" were *v*; ca^{nd} in phenotype, i.e., there was no production of brown eye pigment which would result in eyes which approached the v^{+}; ca^{nd} phenotype.

Several alternative explanations of these data are possible, but of importance here is the fact that they do not give evidence of a diffusible substance produced in either v^{1} or v^{36f} tissue that is utilizable by the heterozygote v^{1}/v^{36f} in the production of brown eye pigment.

Transplantation experiments (GREEN and H. W. LEWIS unpublished) indicate developmental autonomy of v^{1} and v^{36f} mutants in relation to each other. That is, reciprocal transplants of optic discs from v^{1} larvae into v^{36f} larvae and from v^{36f} larvae into v^{1} larvae failed to cause brown eye pigment production in either host or implant.

Feeding supplements to v^{s} *and* v^{u} *larvae*

Feedback experiments: BEADLE and LAW (1938) reported that *v*; *bw* larvae (60–84 hours after egg laying) fed wild type pupae (immersed in boiling water for 30–40 seconds, crushed and added to food) produced some brown eye pigment. Effects were more clear in the absence of agar than in its presence.

In the experiments to be reported here, reciprocal tests were made feeding pupae of each of several test genotypes to larvae of each genotype. The test stocks were of the following genotypes: (v^{1}; *bw*), (v^{36f}; *bw*), ($y\ sn^{3}\ v^{51c}$; *bw*) and (*cn bw*). At least 150 pupae were immersed in boiling water for 30–45 seconds, then crushed and mixed with two ml of 20 percent dry brewer's yeast suspension (supplemented with 0.02 ml propionic acid and 50 gamma streptomycin)

and five test larvae (65–79 hours after egg laying) were placed on the medium; all tests were run in duplicate. The control medium was the same food with no pupae added. The emerging *cn bw* flies all had white eyes. Flies homozygous for v^1, v^{36f} or v^{51c} mutant alleles all produced brown eye pigment when fed *cn* pupae but had white eyes when raised on the control medium or when fed the same or any other *v* mutant pupae. Similar results were obtained by feeding lyophilized adults to test larvae. Two hundred and fifty mgm of dried flies which had been ground in an agate mortar were added to 2 ml of the yeast suspension. Transfer of larvae to the experimental food was made 58–71 hours after egg laying. Flies of the *cn bw* genotype were used as controls, and v^1 and v^{36f} mutants were tested. Both v^1; *bw* and v^{36f}; *bw* flies developed brown eye pigment if the larvae were fed on *cn bw* adults, but not if they were fed on either type of *v* adults. Thus feeding either pupae or adults gives no evidence that either class of *v* mutants accumulates a substance which the other class can utilize in the production of brown eye pigment. Lyophilized su^2-*s* v^{36f}; *bw* adults fed to v^1; *bw* larvae appeared to cause the production of a small amount of brown eye pigment; however, the effect was so slight as to be of doubtful significance.

Feeding tryptophan analogs: It remains possible that if the immediate product of trytophan metabolism could be obtained from some other source, either biological or chemical, it might behave as a precursor to brown eye pigment if fed to *v* larvae of one or both types. DR. BERNHARD WITKOP (United States Public Health Service) has kindly supplied us with a series of tryptophan analogs (DL-2-phenyltryptophan, DL-2-carbethoxytryptophan, 5(OH)tryptophan, 7(OH)-tryptophan), none of which was thought to be the actual immediate product of tryptophan metabolism, but all of which were tested for possible activity as a precursor of brown eye pigment in the mutant flies. The methods used were those which GREEN (1952) used for feeding kynurenine and formylkynurenine. Both kynurenine and formylkynurenine produce a color intermediate between the *v*; *bw* phenotype and the v^+; *bw* phenotype when fed to v^{36f}; *bw* or v^1; *bw* flies at a concentration of 1 mgm/2 ml medium (GREEN 1952). None of the tryptophan analogs tested at a concentration of 1.0 − 1.2 mgm/2 ml medium caused the production of brown eye pigment in the flies tested: (v^1; *bw*), (v^{36f}; *bw*) and (*cn bw*). Feeding of tryptophan is reported in a later section.

Accumulation of tryptophan by v^s *and* v^u *mutants combined with the* su-v *mutant*

Tryptophan accumulation by the two classes of *v* mutants in combination with the suppressor mutant was determined in order to ascertain whether the suppressor differentiated the two classes on this level as well as on the level of eye color phenotype. GREEN (1949) has shown that the *su-v* mutant reduces the accumulation of tryptophan by the v^1 mutant. Tryptophan accumulation in the two mutant classes and the heterozygotes between them in combination with the homozygous or heterozygous *su-v* genes is recorded in Tables 3 and 4.

The mutant classes v^s and v^u are clearly differentiated by the quantity of tryptophan which they accumulate when combined with the homozygous *su-v*

TABLE 3

Nonprotein tryptophan composition of adult flies of v *mutants combined with homozygous or heterozygous* su-v *mutant*

	Regular medium				Tryptophan supplement				
			mgm tryptophan*				mgm tryptophan		
Genotype	Sex	Ex.	Avg.	Range	Sex	Ex.	Avg.	Range	Eye color
su²-s v¹; bw	♀♀	j	0.405(2)	0.395–0.414	♀♀	i	0.469(2)	0.460–0.477	3.0
	♂♀	a	0.372(2)	0.368–0.372					
su²-s v²; bw	♂♀	e	0.368(3)	0.349–0.380	♀♀	i	0.381(2)	0.367–0.395	3.5
su²-s v¹/su²-s v²; bw	♀♀	e	0.339(2)	0.326–0.352					
v¹/su²-s v¹; bw	♀♀	j	0.685(2)	0.685–0.685	♀♀	i	0.776(2)	0.759–0.793	0.7
v¹/su²-s v²; bw	♀♀	e	0.675(2)	0.655–0.694					
v² fw/su²-s v¹					♀♀	i	0.855(2)	0.805–0.904	. .
su²-s v³⁶ᶠ; bw	♀♀	j	0.773(2)	0.757–0.788	♀♀	i	0.884(2)	0.852–0.915	0.0
	♂♀	e	0.902(2)	0.890–0.914					0.0
su²-s v⁵¹ᶜ f; bw	♂♂	a	0.778(1)	. . . . .					0.0
	♂♂	b	0.762(1)	. . . . .					0.0
su²-s v⁵¹ᶜ					♀♀	i	0.822(2)	0.807–0.836	. .
su²-s v⁵¹ᶜ/su²-s v³⁶ᶠ	♀♀	e	0.813(2)	0.762–0.863					. .
v³⁶ᶠ/su²-s v³⁶ᶠ; bw	♀♀	j	0.788(2)	0.784–0.792	♀♀	i	0.985(2)	0.975–0.994	0.0

* See footnotes of Table 1 for abbreviations.

TABLE 4

Nonprotein tryptophan composition of adult v^s/v^u *heterozygous females with homozygous or heterozygous* su-v

	Regular medium			Tryptophan supplement			
	mgm tryptophan*			mgm tryptophan			
Genotype	Ex.	Avg.	Range	Ex.	Avg.	Range	Eye color
su²-s v¹/su²-s v³⁶ᶠ; bw	j	0.574(2)	0.572–0.575	i	0.598(2)	0.595–0.601	1.5
	e	0.485(2)	0.478–0.492				1.5
su²-s v¹/su²-s v⁵¹ᶜ				i	0.701(2)	0.672–0.730	. .
su²-s v³⁶ᶠ/su²-s v²; bw				i	0.717(2)	0.714–0.720	1.5
v¹/su²-s v³⁶ᶠ; bw	j	0.753(2)	0.752–0.754	i	0.910(2)	0.900–0.920	0.0⁺
v³⁶ᶠ/su²-s v¹; bw	j	0.746(2)	0.730–0.762	i	0.914(2)	0.909–0.918	0.0⁺–0.1
y sn³ v⁵¹ᶜ/su²-s v¹; bw				i	0.838(2)	0.818–0.857	0.0⁺
v³⁶ᶠ/su²-s v²; bw				i	0.857(2)	0.839–0.875	0.0⁺
v¹/su²-s v⁵¹ᶜ; bw				i	0.947(2)	0.927–0.966	0.0⁺
v² fw/su²-s v³⁶ᶠ; bw/+				i	0.916(2)	0.872–0.960	. .

* See footnotes of Table 1 for abbreviations.

mutant (Table 3). Both v^1 and v^2, the v^s mutants tested, accumulate much less tryptophan when combined with the *su-v* mutant (Cf. Tables 1 and 2 with Table 3). However, v^u mutants (v^{36f} and v^{51c}) continue to accumulate a high level of tryptophan in the presence of the homozygous suppressor (Table 3). Where there are strictly comparable data, as in experiments j and i, there appears to be a little less tryptophan in flies of the genotype *su²-s* v^{36f}; *bw* than in

v^{36f}; *bw* flies (Cf. Tables 1 and 3). This could be indicative of a quantitative rather than a qualitative difference between v^s and v^u mutants in their interaction with the suppressor, or it may indicate an effect of the suppressor on tryptophan utilization in addition to its interaction with the v^s mutants.

It is clear from Table 3 that the suppressor behaves as an incompletely dominant gene in its effects on the v^s mutant, both with regard to eye color and to accumulation of tryptophan. Also, the homozygous suppressor mutant has a clear effect on the heterozygote v^s/v^u (Table 4). Thus the combined effect of the *su-v* mutant with the v^s mutants depends both on the dosage of v^s alleles and on the dosage of the *su-v* alleles. The data from experiment i (Table 4) indicate a slight effect of the suppressor mutant in heterozygous condition on v^s/v^u flies as might be expected from the dosage relationships.

Further information on dosage relationships was obtained through the use of a deficiency for the suppressor. Since only a small number of flies was obtained in this manner, only the effect on pigment production was considered. If flies are constituted which carry the X chromosome of T(1;2)*Bld* (BRIDGES and BREHME 1944) and a normal second chromosome, they are deficient for the tip of the X chromosome including the loci of *y* and *su-v*. The alleles v^1 and v^{36f} were each introduced into the X chromosome of T(1;2)*Bld*, and flies of the following genotypes were constituted. The number of flies obtained and their average eye color (Based on the scale of TATUM and BEADLE 1939) are also indicated.

Genotype	Number flies	Average color
$\frac{\text{X from T(1;2)}Bld,\ v^1\ ;\ bw}{y^2\ su^{51c}\text{-}v\ v^1\ f}$	23	3.1
$\frac{\text{X from T(1;2)}Bld,\ v^1\ ;\ bw}{y^2\ su^{51c}\text{-}v\ v^{36f}}$	96	1.2
$\frac{\text{X from T(1;2)}\ Bld,\ v^{36f}\ ;\ bw}{y^2\ su^{51c}\text{-}v\ v^{36f}}$	17	1.7
$\frac{\text{X from T(1;2)}\ Bld,\ v^{36f}\ ;\ bw}{y^2\ su^{51c}\text{-}v\ v^{36f}}$	38	0.0

These data indicate that a deficiency for suppressor in combination with one suppressor gene gives essentially the same eye color effect as does the homozygous suppressor mutant (Cf. Tables 3 and 4). Thus, it appears that the absence of su^+-*v* allows the production of brown eye pigment in a v^s mutant.

Starvation

Another technique used to differentiate the v^s and v^u mutants is the "starvation effect." BEADLE, TATUM and CLANCY (1939) observed that if *v; bw* larvae were removed from complete medium to a medium containing only 0.5–1.0 percent dry brewer's yeast at 60–70 hours after egg laying, the level of brown eye pigment in the emerging adults was increased over that of the controls on

complete food. TATUM and BEADLE (1939) reported that the most effective and efficient method for the production of the "starvation effect" was to transfer sterile eggs to a medium of 0.5 percent dry brewer's yeast in 1.5 percent agar and to allow the larvae to develop at 25°C. Under these conditions, there was a prolongation of larval life such that the time from egg laying to emergence of the adult was increased from the normal nine days to 11–13 days. A color of 2.5–3.5 on a scale where *v; bw* = 0, *bw* = 5 was observed in the emerging *v; bw* flies.

The "starvation" medium used in experiments to be reported here was 0.5 percent yeast in 1.5 percent agar; the control medium was 12 percent yeast in 1.5 percent agar. Flies were allowed to oviposit on freshly yeasted vials of regular medium. Eggs were washed with distilled water and 70 percent alcohol; larvae, with distilled water. Ten eggs or larvae (52–66 hours after egg laying) were placed in each vial containing 2 ml of "starvation" or control medium. An increase of 2–4 days in the time from egg laying to emergence of adults was observed on the "starvation" medium as compared to the control medium whether eggs or larvae were used. At least two vials (in most cases three) were made of each genotype on each medium. Males and females of (v^1; *bw*), (v^{36f}; *bw*) and (*y* sn^3 v^{51c}; *bw*) and heterozygous females (v^1/v^{36f}; *bw*), (*y* sn^3 v^{51c}/v^1; *bw*) and (v^{36f}/y sn^3 v^{51c}; *bw*) were tested both as eggs and larvae. *bw* and *cn bw* were also used in the experiments using eggs. Visual comparisons were made of eye colors which were classified on the scale of TATUM and BEADLE (1939). The eye color of v^1; *bw* was classified 0.0+ on the control medium and 2.0–3.5 on "starvation" medium. No difference was observed between "starvation" and control flies of any of the other homozygotes. The v^1 mutant is then distinguished from the v^{36f} and v^{51c} mutants by the environmental condition of partial starvation of larvae as well as by the genetic suppressor mutant. There appeared to be a very slight effect of "starvation" on the v^1/v^{36f}; *bw* females but it was so small as to be of doubtful significance, and the other heterozygotes did not show any change in eye color in response to "starvation." BARISH and FOX (1956) report that v^{48a} (a v^u mutant) is not affected by "starvation." We recently investigated another v^s mutant, v^k, and it responded in the same manner as v^1; i.e., it developed an eye color of 2.0–3.0 on "starvation" medium though it was almost white on the control medium. Flies of the genotype ras^2 v^1 v^{36f} *m f* (obtained from M. M. GREEN who had recovered it from a cross-over between v^1 and v^{36f} in an attached-X female, GREEN 1954) were also tested, and no evidence for a starvation effect was found for this combination of vermilion alleles.

The effect of larval "starvation" on tryptophan accumulation by the mutants was also studied. Attempts were made to raise the flies in bottles in which at least 300 eggs had been placed on 50 ml "starvation" medium. However, though the number of eggs per unit of food was greater than that used in the vials (ten eggs/2 ml "starvation" medium), and the flies were not collected at less than 13 days after egg laying, the brown eye pigment of the v^1; *bw* flies was only

slightly increased (the color was about 1.0 as opposed to 2.5–3.5 in the experiments run in vials). The genotypes studied were: (*bw*), (*v*^{1}; *bw*), (*v*^{36f}; *bw*) and (*v*^{1}/*v*^{36f}; *bw*). The tryptophan in mgm/gm dried flies is essentially the same for all genotypes as in experiments involving regular medium (Cf. Tables 5 and 1). Also it is clear that either on a mgm/gm dried flies basis or on a mgm/1000 flies basis, the tryptophan accumulation is very nearly the same in *v*^{1}; *bw* as in *v*^{36f}; *bw* flies. It was not clear from these experiments whether starvation failed to differentiate between *v*^{1} and *v*^{36f} with regard to tryptophan accumulation, or whether we had failed to attain "starvation" conditions when raising the flies in bottles rather than vials.

Therefore, a series of new experiments was begun, culturing the flies in vials. These were performed in a different laboratory (during the tenure of a Postdoctoral fellowship from the National Institutes of Health at The University of Texas, Department of Zoology), and for reasons still not clear, the adults would not emerge on the "starvation medium" which had been used in the previous experiments (and which was the same as that used by TATUM and BEADLE (1939)). We therefore derived a different medium containing 0.5 gm Fleischmann dry brewer's yeast and 0.5 gm Difco Bacto yeast extract (water soluble fraction of autolyzed yeast) in 1.5 percent agar. This was supplemented with streptomycin and propionic acid in the same manner as the original medium. This medium gave a clear-cut starvation effect (color of *v*^{1}; *bw* adults emerging on it was 2.5–3.0), and a high percentage of adults emerged. A control medium was used consisting of the same ingredients plus two percent sucrose. TATUM and BEADLE (1939) had shown that the addition of two percent sucrose to the "starvation" medium resulted in the same delay in larval development but completely inhibited the effect on eye pigment production. The sterilization procedure used in these experiments was to transfer eggs from vials of regular medium (not seeded with live yeast) to sterile water in watch glasses lined with filter paper. The agar was washed from the eggs and they were then transferred to a mercuric chloride solution (0.5 gm $HgCl_2$, 6.5 gm NaCl, 1.25 ml conc. HCl, 250 ml ethyl alcohol brought up to 1000 ml with sterile H_2O) contained in sterile watch glasses in petri dishes. The eggs remained in the $HgCl_2$ solution 20–30

TABLE 5

Nonprotein tryptophan composition of D. melanogaster *mutants raised on "starvation" medium in half pint bottles*

		Tryptophan mgm/gm dried flies		Tryptophan mgm/1000 flies	
Genotype	Sex	Avg	Range	(Avg.)	Eye color
bw	♂ ♀	0.236(2)	0.214–0.258	0.047(2)	5.0
v^{1}; *bw*	♂ ♀	0.738(3)	0.709–0.769	0.146(3)	1.0
v^{36f}; *bw*	♂ ♀	0.759(2)	0.757–0.760	0.155(2)	0.0
v^{1}/*v*^{36f}; *bw*	♀ ♀	0.831(2)	0.829–0.832	0.154(2)	0.0*

* See Table 1 for abbreviations.

minutes; the solution was then siphoned off and the eggs washed with two or three changes of 70 percent ethyl alcohol. The eggs were left in the last change of alcohol for 20–30 minutes, then transferred in lots of 20 by means of a sterile needle to "starvation" or "sucrose" vials containing 4 ml of medium. The sucrose did not completely inhibit eye pigment formation in v^1; *bw* under these circumstances; however, the color was around 1.0 while it was 2.5–3.0 on the "starvation" medium. The amount of material which we were able to get under these circumstances allowed only a small number of assays (Table 6), but it seems clear that the effect of "starvation" must be quite different from that of the genetic suppressor since there is no evidence for a decrease in tryptophan accumulation when brown pigment is produced. The results on sucrose medium were based on quite small samples, and the significance of the apparent reduction in tryptophan accumulation by both mutants is not clear.

Feeding tryptophan

VALADERES and CHARCONNET (1950) report that by varying the quantity of tryptophan in a synthetic medium, the quantity of brown eye pigment produced by *D. melanogaster* may be altered. Genetically v^+ eyes may be caused to approach the *v* phenotype by limiting the amount of tryptophan, while increasing tryptophan in the medium causes genetically *v* eyes to approach v^+ eyes in phenotype. An attempt was made to repeat the latter results in order that they might possibly be used as one method of differentiating the two classes of *v* mutants. A synthetic medium was not used in our experiments. A control medium was used consisting of 12 percent dry brewer's yeast in a 1.5 percent agar base. Mold and bacterial growth were inhibited by 0.01 ml propionic acid and 25 gamma streptomycin/ml medium. For experimental purposes the 12 percent yeast medium was supplemented with 4 mgm, 2 mgm, or 1 mgm DL-tryptophan or 2 mgm or 1 mgm L-tryptophan/ml medium. Eggs as well as larvae of various ages of (*cn bw*), (v^1; *bw*) and (v^{36f}; *bw*) genotypes were transferred to the test and control media (ten eggs or larvae/vial containing 2 ml medium). No effect on brown eye pigment in flies of any of these genotypes was found at any of the concentrations tested. VALADERES and CHARCONNET

TABLE 6

Nonprotein tryptophan composition of D. melanogaster *mutants raised on "starvation" and "sucrose" media (Expts. performed at Univ. of Texas, see text)*

	Starvation medium			Sucrose medium		
	mgm tryptophan*			mgm tryptophan		
Genotype	Avg.	Range	Eye color	Avg.	Range	Eye color
v^+; *bw*	0.204(1)	.....	5.0	0.204(1)	.....	5.0
v^1; *bw*	0.878(2)	0.815–0.941	2.5–3.0	0.563(1)	.....	0.8–1.5
v^{36f}; *bw*	1.090(2)	1.075–1.104	0.0	0.729(2)	0.690–0.768	0.0

* See footnote of Table 1 for abbreviations.

report positive effects on *v*; *bw* flies at concentrations of 1.0–1.5 mgm L-tryptophan/ml medium.

The results of VALADERES and CHARCONNET might be expected as a "starvation effect." They use the medium of "SCHULTZ *et coll.*" (presumably SCHULZ, ST. LAWRENCE and NEWMEYER 1946) which causes a lengthening of larval life (approximately 8–9 days from egg laying to pupation) of about the same amount characteristic of the conditions producing the "starvation effect."

Comparison of homologous mutants in other species

The sex-linked mutant vermilion (*v*) of *D. simulans* accumulates tryptophan at a level much higher than do the wild type individuals of the same species (Table 7) and it does not respond to conditions of larval "starvation" by producing brown eye pigment. The evidence points to a tentative homology of the *v* mutant of *D. simulans* to the v^u mutants of *D. melanogaster*.

Two mutants of *D. virilis* which fail to produce brown eye pigment under normal conditions have characteristics similar to the *v* mutants of *D. melanogaster*. These are the sex-linked vermilion mutant (*v*) and the autosomal mutant cardinal (*cd*). Optic discs of both *v* and *cd* develop nonautonomously when implanted into wild type or scarlet larvae (PRICE 1949). The mutant scarlet is apparently homologous to the *cn* mutant of *D. melanogaster* (PRICE 1949; GREEN 1949). Both *v* and *cd* mutants produce brown eye pigment when fed kynurenine (PRICE 1949; GREEN 1952) or formylkynurenine (GREEN 1952). GREEN (1949) has shown that the *v* mutant of *D. virilis* accumulates about four times as much nonprotein tryptophan as do wild type or eosinoid flies. The mutant eosinoid (*es*) appears to be homologous to the *bw* mutant of *D. melanogaster* (MORI 1937).

The mutants *v* and *cd* of *D. virilis* were combined with the *es* mutant in the studies to be reported here. It may be seen from Table 7 that the *cd* mutant, as well as the *v* mutant, accumulates a high level of tryptophan. The two mutants were also compared in regard to their response to larval "starvation." The *cd* mutant produces more brown eye pigment on "starvation" medium than on the control medium, but no response of the *v* mutant was observed. GREEN (1955) suggests that *v* of *D. virilis* may be homologous to v^u of *D. melanogaster* and *cd* of *D. virilis* to v^s of *D. melanogaster*. He presents evidence indicating that *cd* will respond to the suppressor of vermilion from *D. melanogaster* when eye discs of *cd* are transplanted into hosts carrying the suppressor and an unsuppressible *v* mutant. The *v* mutant of *D. virilis* did not respond to the *D. melanogaster* suppressor.

The accumulation of tryptophan in the meal moth, *Ephestia kuhniella*, (Table 7) is also of interest in regard to interspecific gene homologies. Our value for nonprotein tryptophan in wild type moths can only be considered to be very low since such a value was outside the range of accuracy of the test. However, the values for the *a/a* mutant, which fails to produce brown eye pigment, are within the range of accuracy, and it is clear that the *a/a* individuals accumulate

TABLE 7

Nonprotein tryptophan composition and starvation response of mutants of other Drosophila species and of Ephestia kuhniella

Species	Genotype	mgm tryptophan* Avg.	mgm tryptophan* Range	Starvation response
D. simulans	+	0.304(2)	0.290–0.318	. .
	v	0.783(3)	0.705–0.823	0
D. virilis	*es*	0.143†(2)	0.133–0.143	. .
	v^{40d}; *es*	0.722(3)	0.650–0.961	0
	cd; *es*	0.632(3)	0.612–0.648	+
	v^{40d}/+; *cd*/+	0.194(2)	0.191–0.196	. .
Ephestia kuhniella	+	0.067(1)†	. . . . .	. .
	a/*a*	0.346(2)	0.329–0.364	0(CASPARI 1943)

* See footnotes of Table 1 for abbreviations.
† Outside range of accuracy of test.
The lyophilized adults of Ephestia were kindly provided by DR. M. M. GREEN.

a much higher level of nonprotein tryptophan than do wild type moths. CASPARI and RICHARDS (1948) report a value of 0.04 mgm nonprotein tryptophan/gm wet wt. larvae for wild type and 0.10 mgm/gm for *a*/*a* larvae. CHEN and KUHN (1956), using paper chromatography, detected nonprotein tryptophan in several developmental stages of *a* mutant moths, but not in white eye(*wa*) mutants or wild type moths studied at the same developmental stages. Protein tryptophan is also higher in *a* mutants than in wild type (CASPARI 1946; CASPARI and RICHARDS 1948; BUTENANDT and ALBRECHT 1952); however, this may be a secondary effect of nonprotein tryptophan accumulation. It seems probable that the primary actions of a^+ and *a* of Ephestia are homologous to those of v^+ and *v* of *D. melanogaster*. If they are homologous, it is interesting to speculate whether *a* is a v^u or v^s type mutant. CASPARI (1943) found that the *a* mutant did not produce brown eye pigment when the larvae were subjected to starvation conditions, possibly indicating an homology to the v^u mutant. The *a* mutant has recently been studied by KUHN and EGELHAAF (1958), and they were able to show that it lacked the ability to oxidize tryptophan *in vitro*. They were able to demonstrate the ability of wild type tissues to carry out this process. This is of particular interest since it is the first demonstration of the *in vitro* oxidation of tryptophan in insects. It may make possible further studies of the enzyme in Drosophila. Many workers have previously attempted to obtain such enzyme activity in wild type Drosophila but have failed up to now.

DISCUSSION

The results reported here establish certain similarities and differences between v^s and v^u mutants which may be considered in formulating a tentative theory of their mode of action and their interaction with the suppressor mutant.

Phenotypically both v^s and v^u flies differ from wild type individuals in their

failure to produce brown eye pigment. Both develop nonautonomously with respect to wild type tissue in gynandromorphs (STURTEVANT 1920; GREEN 1952) and produce brown eye pigment when fed kynurenine (BUTENANDT, WEIDEL, and BECKER 1940; GREEN 1952) or formylkynurenine (GREEN 1952). Mutants of both classes accumulate tryptophan at a level several times that of v^+ flies. A trace of brown eye pigment is produced by v^s flies, and it may be that they accumulate slightly less tryptophan than do v^u flies. There is no evidence that either class of v mutants accumulates a diffusible substance which the other class of mutants can utilize in the production of brown eye pigment. Both cause a block in the conversion of tryptophan to formylkynurenine, and the evidence so far accumulated is compatible with the idea that both mutants block the tryptophan peroxidase-oxidase system in the immediate conversion of tryptophan to the intermediate compound.

Mutants of the two classes, v^s and v^u, differ in their response to a nonallelic suppressor gene. Flies of the v^s type produce brown eye pigment in the presence of the suppressor mutant (GREEN 1952), and there is a concomitant decrease in accumulation of non-protein tryptophan (GREEN 1949). However, the suppressor mutant causes no eye color change in v^u mutants (GREEN 1952) and has little, if any, effect on accumulation of tryptophan by v^u mutants. Flies heterozygous for the suppressor and a deficiency for suppressor produce eye pigment in the same amount as those homozygous for suppressor. The "starvation effect" also differentiates the two classes of v mutants; v^s (v^1 and v^k) flies produce brown eye pigment under conditions of partial starvation of larvae, while flies of the v^u (v^{36f}, v^{48a}, v^{51c}) type fail to produce brown eye pigment under the same conditions. When v^{36f} and v^1 are on the same chromosome, both the suppressor (GREEN 1954) and "starvation" fail to affect the production of brown eye pigment.

The suppressor mutant which interacts with the v^s mutants to allow the production of brown eye pigment also interacts with several dissimilar nonallelic mutants causing them to approach wild type in phenotype (GREEN 1954). One such mutant is purple (*pr*) which causes a reduction in red eye pigment and an increase in brown eye pigment (NOLTE 1955), develops autonomously with respect to wild type tissue (BEADLE and EPHRUSSI 1936), does not accumulate nonprotein tryptophan and does not approach wild type in phenotype when subjected to larval "starvation" (SHAPARD 1954a). The same suppressor mutant also suppresses the mutant effect of sable (BRIDGES 1932), a temperature sensitive sex-linked mutant which causes abnormally black body and is nonautonomous (E. B. LEWIS personal communication). H. W. LEWIS (1955) and H. W. LEWIS and H. S. LEWIS (1958) have shown that both the sable mutant and suppressor of sable affect tyrosinase activity. Another mutant which is suppressed is the autosomal mutant speck (*sp*) (BRIDGES and BREHME 1944; GREEN 1954) which causes a darkening of body color and a black speck at the base of the wings.

The actual effect of the v^s and v^u mutant genes and of the suppressor mutant on the tryptophan peroxidase-oxidase system can be determined only through a study of these enzymes in wild type individuals and in the v mutant with and

without the suppressor mutant. It has not been possible so far to obtain an active tryptophan oxidizing preparation from Drosophila, so these studies have not been made. KUHN and EGELHAAF (1958) have obtained a tryptophan oxidizing enzyme from Ephestia and have demonstrated that the *a* mutant of Ephestia lacks tryptophan oxidizing activity *in vitro*. GLASSMAN (1956) demonstrated activity for kynurenine formamidase (the enzyme which converts N′-formylkynurenine to kynurenine) in cell free extracts of both wild type and various mutant lines of *D. melanogaster* including both v^s and v^u mutant types. This is further evidence that the enzyme system affected by the v mutants must be the one which affects the conversion of tryptophan to formylkynurenine. GLASSMAN and many other workers including the present author have failed to obtain tryptophan oxidizing activity in *in vitro* preparations of Drosophila tissues. It is hoped that the techniques of KUHN and EGELHAAF (1958) may make it possible to obtain this activity in Drosophila and thus to investigate the effects of these mutants on this enzyme system.

We can say little about the action of the v^u mutants except that the block in tryptophan metabolism is so effective that we have not found a means of overcoming it (we can cause the production of brown eye pigment by feeding tryptophan metabolites, but presumably this does not overcome the block in tryptophan oxidation).

However, with regard to v^s mutants and the suppressor, we have some basis for speculation. The v^s mutants appear to have an incomplete block since a small amount of brown eye pigment is produced. This block may be partially overcome in at least two ways: (1) genetic suppression which causes the production of brown eye pigment and a reduction in the accumulation of tryptophan and (2) larval "starvation" which causes a production of brown eye pigment and of v^+ hormone (BEADLE, TATUM, and CLANCY 1938); therefore, it presumably acts on the production of kynurenine, but does not noticeably reduce the accumulation of tryp*ophan. At least one other mutant (*pr*) which is affected by the suppressor is not affected by starvation under the same conditions as those under which v^s is caused to produce brown eye pigment. A deficiency for the suppressor gene appears to be as effective as a mutation to the suppressor allele. This would indicate that a substance produced when su^+-v is present interacts in some way with a substance produced in the presence of v^s to block tryptophan metabolism. In the absence of this su^+-v substance, tryptophan is oxidized though at a lower rate than in wild type flies. Apparently, the heterozygote, su^+-v/*su*-v, produces less of this substance since such flies are intermediate between the two homozygotes. The evidence, so far, indicates that starvation may affect a later step in the reaction sequence, perhaps blocking a side reaction, thus allowing more of any tryptophan metabolites that are produced to be diverted to the production of brown eye pigment.

SUMMARY

The suppressed (v^s) and unsuppressed (v^u) vermilion eye color mutants of *Drosophila melanogaster* have been further studied and found to differ in several

physiological and biochemical characteristics. Both v^s and v^u mutants fail to produce brown eye pigment under normal genetic and environmental conditions, and both accumulate nonprotein tryptophan. Feeding tests, gynandromorphs between the two mutants, and transplantation experiments fail to indicate any difference between the two mutant classes.

The semidominant nonallelic suppressor gene (*su-v*) suppresses both the eye color phenotype and the accumulation of nonprotein tryptophan in v^s mutants but has no apparent effect on either trait in v^u mutants. The eye color effect of the v^s mutants may also be overcome by partial starvation of larvae; nonprotein tryptophan accumulation does not appear to be affected by "starvation." There is no observable effect of "starvation" on the v^u mutants.

Analogous mutants in other species of Drosophila and in *Ephestia kuhniella* are shown to correspond in their physiological characteristics to the two classes of *v* mutants of *D. melanogaster*.

The significance of these results for an interpretation of the action of the *v* mutants and of the suppressor is discussed. It is concluded that both v^s and v^u mutants probably block the conversion of tryptophan to formylkynurenine, but that the nature of the block produced by the two classes of mutants is different. The suppressor probably has a "negative" effect, i.e., the su^+-*v* substance is not produced and v^s flies are able to metabolize some tryptophan in the absence of this substance. Starvation may affect a later step in the reaction sequence.

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