INTERACTING GENE SYSTEMS: I. THE REGULATION OF TRYPTOPHAN PYRROLASE BY THE VERMILION-SUPPRESSOR OF VERMILION SYSTEM IN DROSOPHILA[1]

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Received January 23, 1969

THE many mutant vermilion (v, 1–33.0) alleles of *Drosophila melanogaster* differ from one another (a) in response to a suppressor gene (GREEN 1952, 1954), (b) in ability to synthesize kynurenine (v^+ hormone) and tryptophan pyrrolase (TP, L-tryptophan:oxygen oxidoreductase, EC 1.13.1.12, tryptophan oxygenase) when placed on a partial starvation diet (BEADLE, TATUM and CLANCY 1938, 1939; GREEN 1954; SHELTON, SIMMONS and BOWMAN 1967), (c) in genetic location within the locus (GREEN 1954; BARISH and FOX 1956) and (d) suppressible vermilion alleles (v^s) arise only spontaneously whereas all X-ray induced vermilion mutations that have been studied are unsuppressible (v^u) (GREEN 1954).

The suppressor gene, suppressor of sable or *su-s* is non allelic to vermilion (SCHULTZ and BRIDGES 1932) and linked with it on the *X* chromosome. *su-s* not only suppresses certain vermilion alleles but also the mutant loci sable (*s*, 1–43.0), purple (*pr*, 2–54.5) and speck (*sp*, 2–107.0). In the case of suppressible vermilion alleles it has been demonstrated that the suppressor can restore tryptophan pyrrolase activity to v^s flies completely devoid of this enzyme (BAGLIONI 1960; KAUFMAN 1962; MARZLUF 1965a). MARZLUF (1965a) has examined various characteristics of partially purified TP preparations from suppressed vermilion and wild-type flies. He observed that for the one mutant vermilion allele under consideration both the suppressed vermilion and wild-type TP have indistinguishable Michaelis-Menten constants, pH optima, rates of thermal inactivation, activation energy and inhibition by Cu^{++}. These results suggested to MARZLUF (1965a) that the suppressor gene allows the synthesis of a small amount of completely "normal" enzyme.

With three independently derived suppressor alleles and two suppressible vermilion alleles of separate origin, these results have been extended. They demonstrate that TP is aberrant in the case of one vermilion allele, and that this is due to a mutation in the vermilion locus. The data suggest that in the interacting vermilion–suppressor of vermilion gene system, v^+ is the structural gene for tryptophan pyrrolase.

[1] Supported by Public Health Service Predoctoral Genetics Training Grant NIH-5-T01-GM-00071-10 and NSF Grant GB-6110. Taken from a dissertation submitted to the University of Michigan in partial fulfillment of the requirements for the Ph.D. degree.

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Although there has been much speculation (BEADLE and TATUM 1941; PONTECORVO 1958; BAGLIONI 1960; MARZLUF 1965a), the mechanism by which the suppressor acts is still quite uncertain. Thus, a further objective of the present study was to test systematically the effects of various vermilion and suppressor allele combinations on the activity of tryptophan pyrrolase with the purpose of establishing the mode of *su-s* action. The findings will be evaluated according to the categories of suppressors as outlined by GORINI and BECKWITH (1966). Finally, a model to account for all properties of the vermilion and suppressor genes will be presented as a basis for further investigation.

MATERIALS AND METHODS

Stocks: Drosophila melanogaster were raised in half pint milk bottles using the standard corn meal-agar medium at 24.0° ± 0.5°C. Oregon R wild-type and the mutant strains su^2-*s* v^1, su^2-*s* v^k, su^2-*s*, su^3-*s* v^1, su^3*s* v^k, su^3-*s*, y^2 su^{51C15}-*v* v^1, y^2 su^{51C15}-*v* v^k and y^2 su^{51C15}-*v*;*pr* used throughout this study were either supplied directly by Dr. T. M. RIZKI or synthesized from the appropriate component stocks in his collection.

Enzyme purification: Drosophila tryptophan pyrrolase was partially purified from adult flies according to the procedure of MARZLUF (1965b) through the 43%–57% saturated ammonium sulfate precipitation. This increases the specific activity some 4-fold and removes substances of low molecular weight. In order to maintain similar final protein concentrations the precipitate was dissolved in 1/10 of the initial homogenizing volume in 0.005 M phosphate buffer, pH 7.4. Although such preparations retain appreciable activity when stored at —20°C for some months, experiments were done routinely on enzyme less than 3 weeks old.

Enzyme assay: Tryptophan pyrrolase was assayed according to the procedure of KAUFMAN (1962) as modified by MARZLUF (1965b) utilizing 2-mercaptoethanol as the activator. The reaction mixture was incubated at 37°C for 2 to 3 hrs in test tubes whose atmosphere was continuously saturated with oxygen (Puritan, U.S.P.) (RIZKI and RIZKI 1963). This precaution significantly reduces the assay variability. Control readings were obtained by omitting tryptophan from the reaction mixture and then adding it after the incubation period. The reaction was stopped by the addition of 3.0 ml of 6.7% trichloroacetic acid. Following centrifugation, kynurenine was determined in 3.0 ml aliquots of the supernatant fluid by the diazotization procedure of Bratton and Marshall (1939). The optical density of the samples was determined at 560 mμ after they had stood for 2 hrs at 24.5° ± 0.5°C. To insure the highest degree of repeatability the following precautions were observed. Solutions of 2-mercaptoethanol and sodium nitrite (for diazotization) were prepared fresh daily. L-tryptophan was prepared according to KNOX (1955). N-1-Naphthylethylenediamine dihydrochloride (Lot No. 26A, Eastman Kodak) which couples with the diazotized kynurenine was prepared fresh every thirty days and stored in a brown stoppered bottle at 2°C. Under these conditions an optical density change of 0.100 at 560 mμ is equivalent to 0.25 micromoles of L-kynurenine per ml and is linear up to an optical density of 1.0.

pH optimum: In these experiments the pH of the incubation medium was varied with phosphate buffer, pH 6.2–7.4, or Tris (tris(hydroxymethyl) aminomethane) buffer pH 7.8–8.5. These buffers in no way interfered with the colorimetric assay. The pH of the complete reaction mixture was measured just prior to assay.

Thermal inactivation: For studies of heat inactivation of tryptophan pyrrolase, test tubes containing an aliquot of the enzyme preparation were incubated in a water bath at 55°C for 2 to 10 min, removed at appropriate intervals and swirled in an ice bath for 30 sec. The precipitated protein was removed by centrifugation and the supernatant was assayed in the usual manner. Since the protein concentration affects the rate of denaturation (MARZLUF 1965a) this parameter was kept constant at 15 mg/ml.

Assay of tryptophan pyrrolase activity in various strains: Drosophila melanogaster adults (± 24 hrs old) were collected by first clearing the culture bottle and then collecting all those

adults that hatched 48 hrs thereafter. The animals were lightly etherized, weighed and then homogenized in an all-glass homogenizer with four volumes of 0.14 M KCl containing 0.0025 M NaOH at 0°C. The homogenate was centrifuged at 30,000 × *g* for 15 min at 0°C and the supernatant carefully removed for use as the source of enzyme. TP activity was then assayed as described above.

Fractionation of v^k *for the material responsible for the super-additive effect:* This material was partially purified in a manner identical to the extraction of wild-type *Drosophila melanogaster* tryptophan pyrrolase through the 43%–57% saturated ammonium sulfate precipitation (MARZLUF 1965b) except that no protamine sulfate was used.

RESULTS

pH optimum: The effect of pH on the activity of partially purified tryptophan pyrrolase from wild type and various suppressed-vermilion mutants is compared in Figure 1. In general, the shape of the curves is similar for each suppressed-

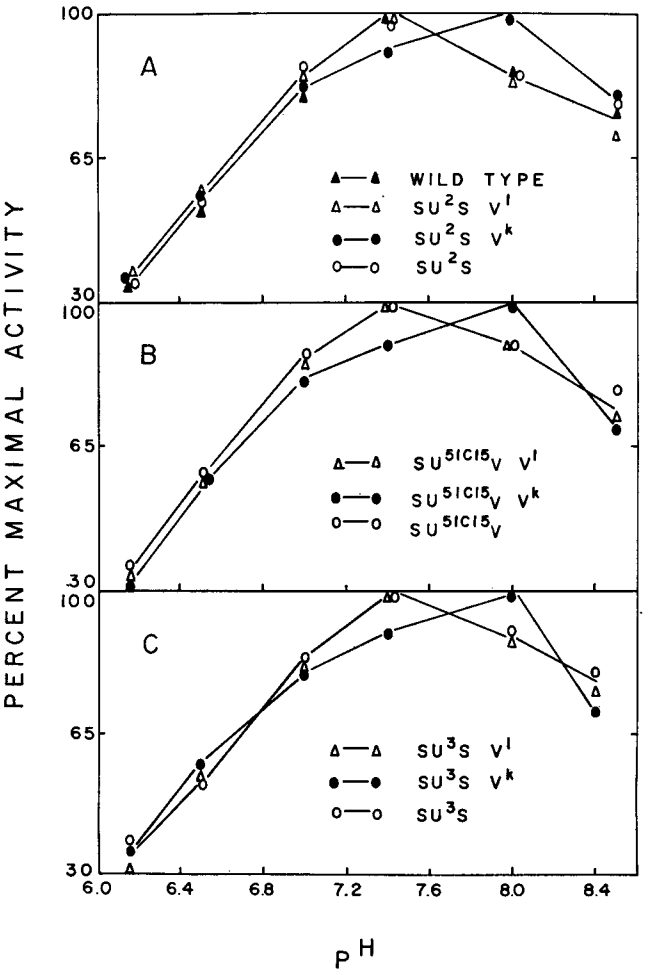

FIGURE 1.—The pH dependence of wild-type and suppressed-vermilion tryptophan pyrrolase.

vermilion allele, independent of the particular suppressor gene in combination with it. That is, suppressed v^+ and v^1, like the wild type, all have a pH optimum at 7.4 while the pH optimum for suppressed v^k is 8.0, whether the suppressor allele is *su*^{2}-*s*, *su*^{3}-*s* or *su*^{51C15}-*v*. The data illustrated in Figure 1 are typical and have been repeated at least twice from independently extracted tryptophan pyrrolase preparations.

Kinetics: As might be expected on the basis of the pH data, the Michaelis-Menten constant (K_m) of tryptophan pyrrolase extracted from suppressed v^k flies differs from the enzymes extracted from wild type, suppressed v^+ or suppressed v^1. The K_m's were determined by linear regression from the LINEWEAVER-BURK plot (1934) as illustrated in Figure 2. Table 1 summarizes this difference for K_m's determined either at pH 7.4 or 8.0. The mean K_m for all suppressed v^k tryptophan pyrrolase compared to all other strains examined differs not only in magnitude at a given pH but also in decreasing at the higher pH instead of increasing.

Thermal inactivation: As is evident from Figure 3, no significant differences can be established among the mutant strains with respect to their stability at 55°C within the limits of experimental reproducibility (10%). The enzyme, whether extracted from wild type, *su*^{2}-*s* v^1 or *su*^{2}-*s* v^k, is also protected against heat denaturation by tryptophan at pH 7.4 with no detectable differences.

Tryptophan pyrrolase activity among suppressed-vermilion mutants: The suppressibility of several vermilion alleles, as determined by restoration of tryptophan pyrrolase activity in the presence of the *su*^{2}-*s* mutation, has been examined using freshly hatched adults (Table 2). The *v* alleles are all of different origin (v^1,v^k and v^{36f} are spontaneous; v^{48a} and v^{51c} are X ray induced). The v^s (sup-

TABLE 1

The K_m's of Drosophila tryptophan pyrrolase at pH 7.4 and 8.0 in wild type, suppressed v+ *and suppressed* v *strains*

Genotype	$K_m \times 10^{-3}$ M (mean ± SE) (*N*)* pH 7.4		pH 8.0	
Oregon-R, wild type	1.53 ± 0.15	(3)	2.93 ± 0.05	(3)
su^2-*s*	1.54 ± 0.10	(2)	2.98	(1)
su^3-*s*	1.61 ± 0.10	(2)	3.03 ± 0.12	(2)
y^2 *su*^{51C15}-*v*;*pr*	1.48 ± 0.16	(2)	3.00 ± 0.08	(2)
su^2-*s* v^1	1.55 ± 0.12	(3)	2.90 ± 0.06	(3)
su^3-*s* v^1	1.50 ± 0.05	(2)	3.03	(1)
y^2 *su*^{51C15}-*v* v^1	1.52 ± 0.05	(3)	2.99 ± 0.11	(3)
su^2-*s* v^k	2.16 ± 0.16	(5)	1.25 ± 0.10	(3)
su^3-*s* v^k	1.98 ± 0.10	(2)	1.50 ± 0.12	(2)
y^2 *su*^{51C15}-*v* v^k	2.00 ± 0.03	(2)	1.53 ± 0.10	(3)
mean, suppressed v^k	2.05 ± 0.08	(9)	1.43 ± 0.07	(8)
mean, suppressed v^1	1.52 ± 0.07	(10)	2.97 ± 0.10	(7)
mean, suppressed v^+	1.54 ± 0.12	(6)	3.00 ± 0.10	(5)

* *N* is the number of independently extracted tryptophan pyrrolase preparations on which the mean value is based.

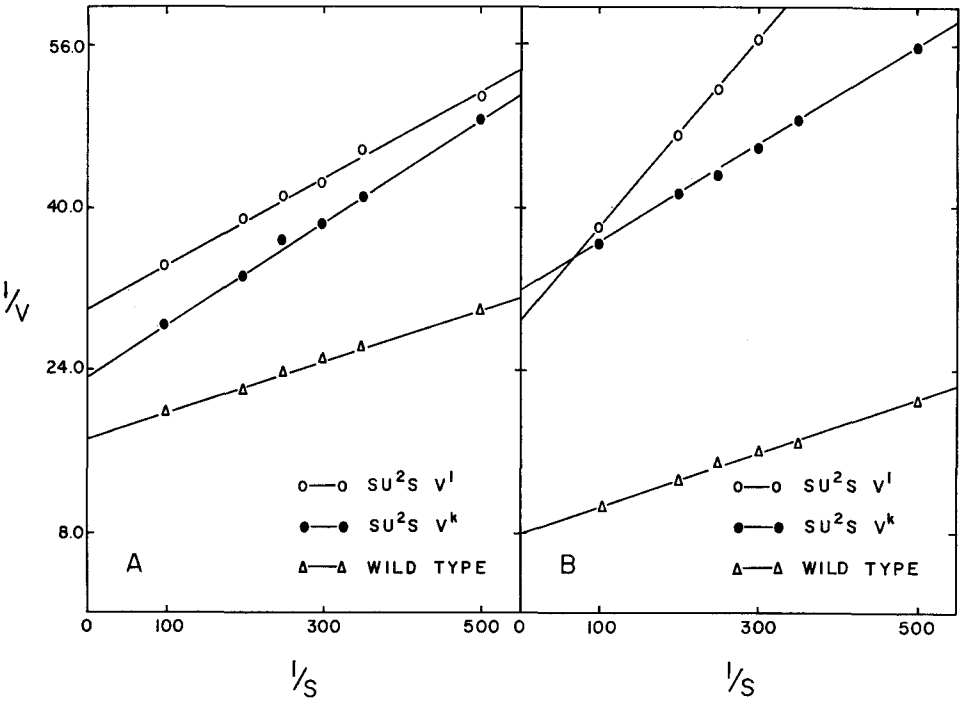

FIGURE 2.—Kinetics of tryptophan pyrrolase from various strains. A typical Lineweaver-Burk plot of suppressed-vermilion and wild-type tryptophan pyrrolase at pH 7.4 (A) and 8.0 (B), where S = M L-tryptophan and V = μM kynurenine formed/2 hrs.

pressible) alleles can be ranked in order of their suppressibility with $v^k > v^1 > v^{36f}$ while v^{48a} and v^{51c} are unsuppressible (v^u). GREEN (1954) classified the v^{36f} allele as v^u because su^2-s v^{36f} flies have vermilion eyes. However, such flies do indeed possess tryptophan pyrrolase activity (4.4% of wild type) which can be concentrated by ammonium sulfate precipitation (43%–57% saturation; see MARZLUF, 1965b). RIZKI and RIZKI (1964) have reported the presence of kynurenine in the fat body of su^2-s v^{36f} larvae. Thus, v^{36f} must now be classified as a v^s allele. Although some vermilion mutants exhibit slight tryptophan pyrrolase activity it is doubtful that this is real since MARZLUF (1965a) was unable to concentrate enzyme activity with ammonium sulfate precipitation and because this level of activity corresponds to very low optical density measurements. Nor is it possible to observe complementation between v^k and other vermilion alleles. It should also be noted that tryptophan pyrrolase is dosage compensated since males and females have essentially equivalent specific activities, both in wild type and in su^2-s v^k genotypes. This is in agreement with KAUFMAN (1962).

The effect different suppressor alleles have on tryptophan pyrrolase activity: The suppressibility of v^1 and v^k alleles in combination with three suppressor alleles, all of independent origin (su^2-s spontaneous, su^3-s and su^{51c15}-v X ray) has also been determined (Table 3). The evidence indicates that the degree to

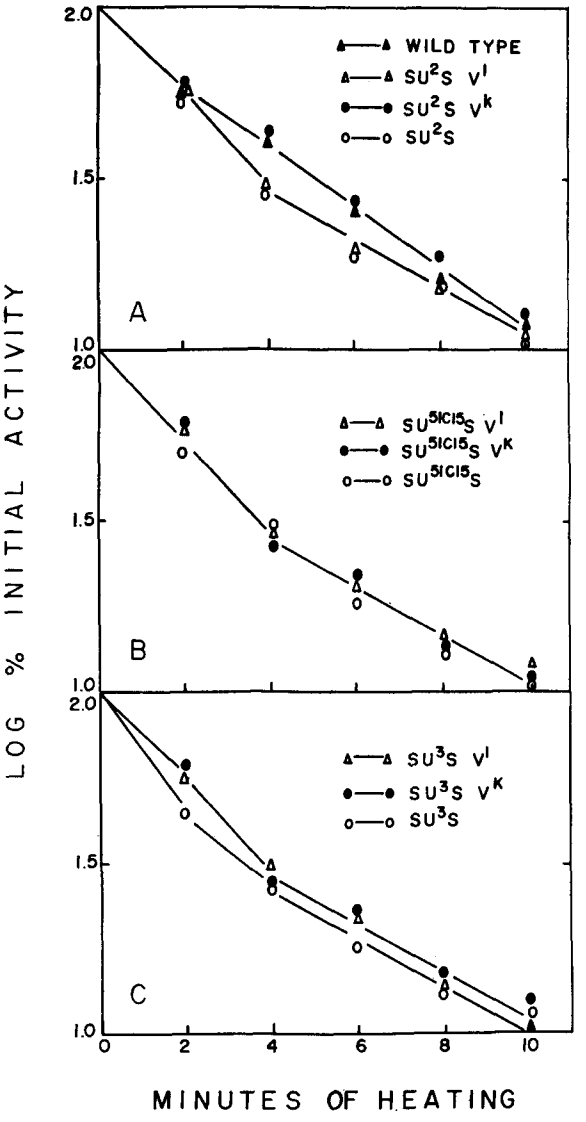

FIGURE 3.—The thermal inactivation of Drosophila tryptophan pyrrolase in the various strains as indicated.

which tryptophan pyrrolase is restored depends wholly on the particular vermilion allele, since three independent suppressor alleles have the same effects on two different mutant vermilion alleles. (The slight differences among suppressed-v^k levels of TP specific activity are not statistically significant.) The suppressor gene evidently acts only qualitatively, providing the appropriate environment for the restoration of enzymic activity. That suppressed-v^+ flies possess tryptophan pyrrolase activities equivalent to the wild type does not lend support to the

TABLE 2

Tryptophan pyrrolase activity among various suppressed-vermilion alleles

Genotype*	TP specific activity† mean ± SE (*N*)	Percent wild-type activity
Oregon-R, wild type	0.570 ± 0.020 (3)	100.0
Oregon-R, ♀ ♀	0.540 ± 0.020 (6)	94.6
Oregon-R, ♂ ♂	0.600 ± 0.020 (6)	105.0
su²-s v^k	0.117 ± 0.010 (3)	20.5
su²-s v^k, ♀ ♀	0.110 ± 0.020 (3)	19.3
su²-s v^k, ♂ ♂	0.120 ± 0.030 (3)	21.0
su²-s v¹	0.050 ± 0.010 (4)	8.8
su²-s v^36f	0.025 ± 0.010 (3)	4.4
su²s v^48a	0 (3)	0
su²-s v^51c	0 ± 0.006 (3)	< 1.0
v^k	0 ± 0.004 (3)	< 1.0
v¹	0 ± 0.004 (3)	< 1.0
v^36f	0.004 ± 0.004 (3)	< 1.0
v^48a	0 ± 0.006 (3)	< 1.0
v^51c	0.004 ± 0.004 (3)	< 1.0
v^k/v¹, ♀ ♀	0 ± 0.002 (3)	< 1.0
v^k/v^36f, ♀ ♀	0.002 ± 0.002 (3)	< 1.0
v^k/v^48a, ♀ ♀	0 (3)	0
v^k/v^51c, ♀ ♀	0 (2)	0

* Unless otherwise indicated determinations were made on a mixture of male and female adult flies, ± 24 hrs old.
† μM kynurenine/2 hrs/g wet weight.

hypothesis of MARZLUF (1965a) that the suppressor is a regulatory gene and that the suppressible vermilion alleles are altered operators having increased affinity for the repressor. The marked difference in enzyme activity in the case of one strain (*y² su^51c15-v; pr*) is reproducible.

TABLE 3

The effect of three independently derived suppressor alleles on the tryptophan pyrrolase activity in v^1 *and* v^k *mutants*

Genotype*	TP specific activity† mean ± SE (*N*)	Percent wild-type activity
su²-s v^k	0.117 ± 0.010 (3)	20.5
su³-s v^k	0.080 ± 0.017 (3)	14.0
y² su^51c15-v v^k	0.098 ± 0.004 (3)	17.2
su²-s v¹	0.050 ± 0.010 (4)	8.8
su³-s v¹	0.055 ± 0.010 (3)	9.7
y² su^51c15-v v¹	0.050 ± 0.008 (4)	8.8
su²-s	0.580 ± 0.010 (3)	101.9
su³-s	0.600 ± 0.030 (3)	105.2
y² su^51c15-v;pr	0.750 ± 0.040 (6)	131.6

* Determinations were made on a mixture of male and female adult flies, ± 24 hrs old.
† μM kynurenine/2 hrs/g wet weight.

TABLE 4

The interaction between wild type and mutant vermilion alleles in vivo *and* in vitro

Genotype*	TP Specific activity† mean ± SE (*N*)	Percent wild type activity	Extracts mixed‡	TP Specific activity† mean ± SE (*N*)	Percent wild-type activity
v^+/v^+	0.570 ± 0.020 (3)	100.0	$v^+ + v^+$	0.575 ± 0.028 (4)	100.0
v^+/v^k	0.442 ± 0.020 (3)	77.5	$v^+ + v^k$	0.417 ± 0.025 (6)	73.2
v^+/v^1	0.383 ± 0.024 (3)	67.2	$v^+ + v^1$	0.384 ± 0.013 (4)	67.4
v^+/v^{36f}	0.358 ± 0.012 (4)	62.8	$v^+ + v^{36f}$	0.358 ± 0.008 (3)	62.8
v^+/v^{51c}	0.308 ± 0.024 (4)	54.0	$v^+ + v^{51c}$	0.335 ± 0 (3)	58.8
v^+/v^{48a}	0.292 ± 0.017 (4)	51.2	$v^+ + v^{48a}$	0.300 ± 0.010 (3)	52.6
v^k/v^k	0.000 ± 0.006 (3)	< 1	$v^k + v^k$	0.000 ± 0.007 (3)	< 1

* Adult females (± 24 hrs old) of the genotypes indicated were obtained from the appropriate cross, separated and assayed in the usual manner.

† μM kynurenine/2 hrs/g wet weight.

‡ Extracts were prepared from females of the indicated genotype and equal aliquots were mixed together and assayed in the usual manner.

Interaction between wild type and mutant vermilion alleles in vivo *and* in vitro: Since the vermilion mutants tested do not complement (Table 1; GREEN 1954; BARISH and FOX 1956) it was expected that the specific activity of the enzyme in v^+/v heterozygotes should behave in an additive fashion: that is, they should yield half the enzymic activity of v^+/v^+ flies. However, certain vermilion mutants behave in a super-additive manner when heterozygous with the v^+ allele (Table 4). There are two highly significant features of these data: (1) That the minimum amount of tryptophan pyrrolase activity observed in these heterozygotes is never less than half the wild-type activity and (2) that the amount of excess enzymic activity contributed by a vermilion mutation is strictly related to the suppressibility of that allele (compare Table 4 with Table 2).

All of the *in vivo* observations on heterozygotes are closely paralleled by mixing extracts of homozygotes (Table 4). This *in vitro* system also provides a basis for characterizing the material produced by the mutant vermilion allele responsible for this super-additivity. Since the greatest effect is achieved with v^k, this mutant was chosen for further study.

As illustrated in Figure 4, the ratio of v^+ to v^k protein in crude extracts required to produce the maximal increase for the super-additive effect in v^+ tryptophan pyrrolase activity is 1:1. Table 5 also demonstrates that the v^k material responsible for this super-additive effect is thermolabile, ammonium sulfate-precipitable and non dialyzable. It is probable, therefore, that this v^k material is a protein.

The effect of the suppressor gene on the interaction between wild-type and mutant vermilion alleles: When the tryptophan pyrrolase activities from flies of the genotype su^s-s v^+/su^s-s v are compared to that of v^+/v (from Table 4), a curious observation becomes apparent (Table 6). The TP activities of suppressed and unsuppressed v^+/v^k and v^+/v^{36f} heterozygotes are equivalent. However, a similar comparison for v^+v^1, v^+/v^{51c} and v^+/v^{48a} heterozygotes reveals approximately 20% less enzymic activity in the suppressed condition: v^1 no longer

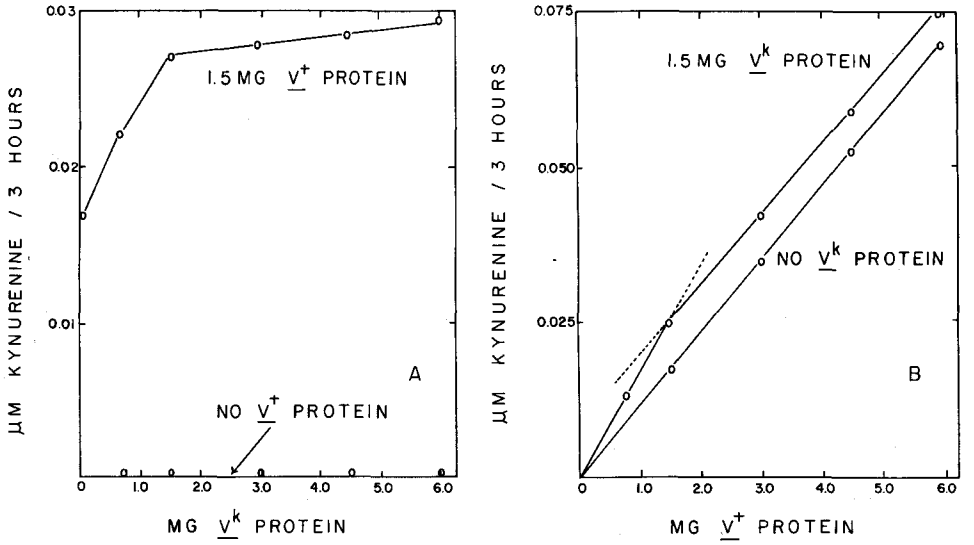

FIGURE 4.—Examination of the *in vitro* system that is capable of producing maximal super-additive tryptophan pyrrolase activity by mixing crude extracts from v^+ (wild type) and v^k adult females (± 24 hrs old). (A) A varying amount of v^k extract added to a constant amount of v^+ extract. (B) A varying amount of v^+ extract mixed with a constant amount of v^k extract. Protein was determined by the Biuret reaction.

exhibits a super-additive effect with v^+ while v^{51c} and v^{48a} show evidence of a less than additive interaction with the v^+ allele.

The complete recessiveness of su-s: Since vermilion flies lack the brown eye pigment and brown eye (*bw*, 2–104.5) flies lack the red pigment, a *v;bw* fly will

TABLE 5

Properties of the v^k *material responsible for the super-additive* in vitro *effect with wild-type extracts*

Composition of *in vitro* mixture*	TP activity μM kynurenine/3 hrs
wild type	0.017
v^k	0
wild type + v^k	0.026
wild type + heated† v^k	0.016
wild type + 43–57% ammonium sulfate precipitate‡ of v^k	0.028
43–57% ammonium sulfate precipitate of v^k	0
wild type + dialyzed§ ammonium sulfate precipitate of v^k	0.025

* 0.1 ml of each indicated extract was added to the standard assay incubation medium.
† A v^k extract was placed in a boiling water bath for 3 min and then immediately cooled by swirling in ice.
‡ See MATERIALS and METHODS.
§ 1.0 ml was dialyzed against 4 one liter changes of 0.1 M phosphate buffer, pH 7.4, for 24 hrs at 0°C.

TABLE 6

The effect of the suppressor gene on the interaction between wild type and mutant vermilion alleles

Genotype*	TP Specific activity† mean ± SE (N)	Percent wild-type activity	Genotype	Percent wild-type activity
su²-s v+/su²-s v+	0.600 ± 0.033 (3)	105.3	*v+/v+*	100.0
su²-s v+/su²-s v^k	0.458 ± 0.020 (4)	80.4	*v+/v^k*	77.5
su²-s v+/su²-s v¹	0.286 ± 0.020 (6)	50.2	*v+/v¹*	67.2
su²-s v+/su²-s v^36f	0.373 ± 0.008 (4)	65.4	*v+/v^36f*	62.8
su²-s v+/su²-s v^51c	0.200 ± 0.020 (6)	35.1	*v+/v^51c*	54.0
su²-s v+/su²-s v^48a	0.190 ± 0.020 (4)	33.3	*v+/v^48a*	51.2

* Adult females (± 24 hrs old) of the genotypes indicated were obtained from the appropriate cross, separated and assayed in the usual manner.
† μM kynurenine/2 hrs/g wet weight.

have white eyes. BAGLIONI (1960), on the basis of a slight amount of brown eye pigment in *su²-s v¹/su²-s+ v¹;bw* females concluded that the suppressor was not completely recessive. It is difficult, however, to judge the activity of tryptophan pyrrolase on the basis of eye pigmentation alone. Thus, females of the genotype, *su²-s v^k/su²-s+ v^k*, were selected and found to possess a specific activity of 0.006 ± 0.004 (7) μM kynurenine/2 hrs/g wet weight as compared to that of the *v^k* mutant alone which was 0 ± 0.006 (3) μM kynurenine/2 hrs/g wet weight. Therefore, the mutant suppressor gene appears to be completely recessive to its wild-type allele.

DISCUSSION

Tryptophan pyrrolase from suppressed *v^k* flies possesses an altered K_m and pH optimum, with respect to the wild-type and suppressed-*v+* genotypes (Table 1; Figure 1). This suggests that of the two genes (*v* and *su-s*) which may interact to control tryptophan pyrrolase activity, *v+* is the structural gene for this enzyme in *Drosophila melanogaster*. The central problem, then, is to determine the mechanism by which *su-s* restores function to certain mutant tryptophan pyrrolase structural genes.

GORINI and BECKWITH (1966) distinguish suppressors with respect to their mode of action as being either (a) indirect or metabolic suppressors, which circumvent, rather than repair the genetic lesion, (b) intragenic suppressors or second site reversions, or (c) informational suppressors, which alter the meaning of a mutant codon and repair the primary mutation. The foregoing data eliminate two of these three major categories of suppression. *su-s* is not an intragenic suppressor, since it is nonallelic to the vermilion locus (SCHULTZ and BRIDGES 1932). Five lines of evidence suggest that *su-s* is not an informational suppressor. (1) The fact that *su-s* is completely recessive tends to negate the idea that the suppressor acts either at the level of translation or transcription. (2) Since certain vermilion mutations yield super-additive tryptophan pyrrolase activities with the *v+* allele *in vivo* and *in vitro* (Table 4; Figure 4), and in a manner strictly

related to the suppressibility of the vermilion allele (Tables 2 and 4), it is clear that v^s mutants produce a potentially functional protein-like product (Table 5). Furthermore, the polarity in the degree of suppressibility of vermilion alleles, $v^k > v^1 > v^{36f} > v^{48a} = v^{51c}$ does not correlate with that order of v^1 and v^{48a} mutants mapping to the left of v^{36f} (BARISH and FOX 1956). Therefore, *su-s* cannot be a nonsense suppressor nor can the suppressible vermilion mutants be nonsense mutations. (3) The relatively high level of suppression (20% of the wild-type tryptophan pyrrolase activity as in the case of su^2-s v^k) would seem to generate a degree of codon missense incompatible with the viability of any complex multicellular organism since the highest level of bacterial missense suppression is only 7% (BRODY and YANOFSKY 1963). (4) RITOSSA, ATWOOD and SPIEGELMAN (1966) have shown a multiplicity of 13 templates per haploid genome for the transcription of each of the approximately 60 tRNA species. In Drosophila missense suppression *via* a mutant tRNA would be most inefficient. (5) When v^s larvae are subjected to partial starvation the brown eye pigment is restored by producing the v^+ hormone, kynurenine (BEADLE, TATUM and CLANCY 1938, 1939; GREEN 1954), as well as tryptophan pyrrolase activity (SHELTON, SIMMONS and BOWMAN 1967); v^u larvae do not produce brown eye pigment when placed on such a diet (GREEN 1954). Moreover, only carbohydrates and related substances can inhibit this starvation effect in v^s mutants (TATUM and BEADLE 1939). These latter observations are extremely difficult to reconcile with any sort of informational suppression and indicate that *su-s* is an indirect or metabolic suppressor. Indeed, the data presented in this study are most easily understood from this point of view.

The observations demonstrating super-additive tryptophan pyrrolase activities in certain v^+/v heterozygotes *in vivo* and *in vitro* (Tables 4 and 5; Figure 4) may be interpreted in terms of a hypothesis of interaction among subunits of an aggregate enzyme, a homomultimer composed (in the simplest case) of two identical subunits. The homomultimeric nature of tryptophan pyrrolase is proposed on the basis of the fact that the vermilion mutants do not complement with respect to eye color (Table 1; GREEN 1954; BARISH and FOX 1956); thus, the vermilion gene behaves as a single structural gene. It is proposed, then, that the subunit polypeptide products of tryptophan pyrrolase produced by the v^+ and v alleles, $P_v{}^+$ and P_v, respectively, are free to dimerize randomly with the restriction that P_v subunits are unable to aggregate with one another due to local misfolding as the result of mutation (CRICK and ORGEL 1964). It is assumed that both subunits are produced in equal number and that dimer formation is strongly favored. At equilibrium, then, all dimers will be in the form of $P_v{}^+P_v$. It is further assumed that each monomer has at least one catalytic site, but is not active unless dimerization occurs (this accounts for the inactivity of vermilion homozygotes). Thus, never less than 50% of the wild-type tryptophan pyrrolase activity will be observed as in the case of unsuppressible vermilion alleles (Table 4). The super-additive value in the case of a v^+/v^s heterozygote, however, is due to the activity of the catalytic site of the $P_v{}^s$ (polypeptide produced by a v^s allele) monomer which has been partially corrected through local refolding induced by interacting with the $P_v{}^+$ subunit of the dimer. It is also important to note that the super-

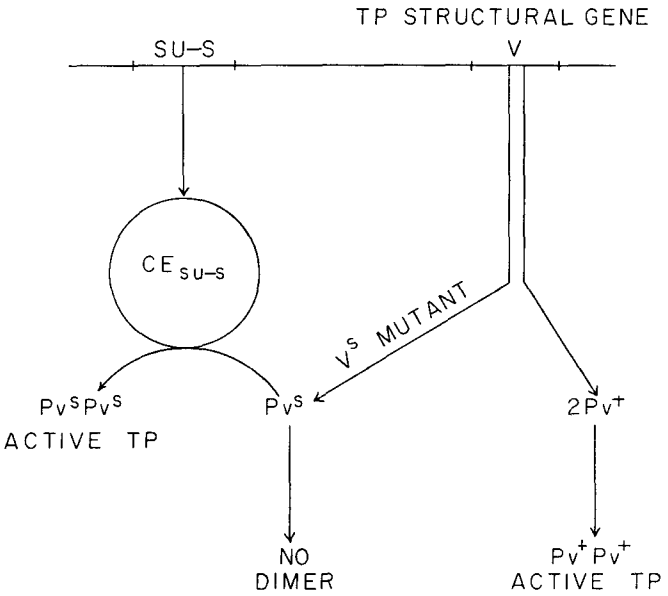

FIGURE 5.—A schematic representation of the mode of action of the suppressor of vermilion. Drosophila tryptophan pyrrolase is a homomultimer composed (in the simplest case) of two polypeptide subunits ($P_{v^+}P_{v^+}$), whose structure is defined by the $v+$ locus, which must dimerize in order to form an active enzyme, TP. The various suppressible vermilion (v^s) mutations produce subunits (P_{v^s}) which possess local misfolding as a result of mutation and are no longer able to dimerize and are thus inactive. The function of the suppressor of vermilion, *su-s*, is to produce a particular cellular environment (CE_{su-s}) in which P_{v^s} subunits can be more easily refolded. Hence, dimers are formed capable of tryptophan pyrrolase activity.

additive behavior of a mutant vermilion allele is correlated with its suppressibility (Tables 2 and 4). However, in the presence of the suppressor certain vermilion mutations interact with v^+ to yield reduced TP activities (Table 6). These results suggest that *su-s* and v^+ may have similar, but not identical, effects on the product of a *v* gene. It is proposed, then, that the function of *su-s* is to produce a cellular environment (CE_{su-s}) in which $P_v{}^s$ subunits can be refolded, facilitating dimerization and, therefore, producing active tryptophan pyrrolase. Figure 5 is a diagrammatic form of the model put forth for the mechanism of *su-s* action.

If *su-s* now allows P_v subunits to dimerize, then flies of the genotype su^2-*s* v^+/su^2-*s* *v* will yield dimers $P_v{}^+P_v{}^+$, $P_v{}^+P_v$ and P_vP_v in the ratio of 1:2:1, respectively. The instances of reduced tryptophan pyrrolase activities among certain suppressed v^+/v flies (Table 6) are particularly striking among v^u alleles where less than additive TP activities are observed. These cases may be interpreted as due to tertiary distortion of such P_v subunits (as influenced by *su-s*) which then interact negatively with the active site of the $P_v{}^+$ subunit and reduce its catalytic efficiency when in the $P_v{}^+P_v$ dimer. In Neurospora a similar case of negative interaction for the oligomeric enzyme, glutamate dehydrogenase, has been observed (SUNDARAM and FINCHAM 1967). Here, the activity of wild-

type monomers is considerably reduced as a result of hybridization with mutant ones.

The molecular weights of purified tryptophan pyrrolase from rat liver and Pseudomonas have been determined to be between 103,000 and 180,000 (SCHIMKE, SWEENEY and BERLIN 1965; CHO-CHUNG and PITOT 1967; TOKUYAMA 1968). On the basis of studies with Sephadex gel filtration, Drosophila TP has a molecular weight from 100,000 to 200,000 (MARZLUF 1964). These physical data are consistent with the subunit structure of Drosophila tryptophan pyrrolase proposed here.

The fact that the mutant suppressor gene appears completely recessive to *su-s*$^+$ indicates that *su-s* exerts a negative control towards the aggregation of mutant tryptophan pyrrolase subunits. That is, it removes something from the cellular environment allowing change and interaction of the mutant enzyme monomers. The function of *su-s*$^+$ may be viewed, then, as an "organizing influence" which prevents the interaction of only mutant tryptophan pyrrolase subunits.

Note added in proof: Since this manuscript was submitted for publication POILLON, MAENO, KOIKE and FEIGELSON (J. Biol. Chem. 1969 **244:** 3447–3456) have demonstrated by direct physical techniques that the tryptophan pyrrolase of *Pseudomonas acidovorans* is composed of four polypeptide chains of equivalent mass. These subunits are devoid of enzymic activity and further evidence indicates that only the tetrameric form, stabilized exclusively by non covalent interactions, is the enzymatically active structure.

It is a pleasure to thank Dr. TAHIR M. RIZKI for his counsel and suggestion of this area of research to me, Dr. GEORGE W. NACE for the hospitality of his laboratory, and Mr. JOHN C. HAGENAUER who has taught me much biochemistry. I also wish to express my deep gratitude to Dr. ROWLAND H. DAVIS for his hours of inspective, incisive and thoroughly stimulating discussion.

SUMMARY

Tryptophan pyrrolase (TP) is absent in vermilion (*v*) mutants of *Drosophila melanogaster*. The non allelic suppressor of vermilion (*su-s*) partially restores TP activity in flies carrying suppressible vermilion (v^s) alleles. Of the various *su-s* v^s strains examined, aberrant TP characteristics are found for one v^s allele. The results suggest that v^+ is the structural gene for tryptophan pyrrolase.—Suppression of various suppressible vermilion alleles restores tryptophan pyrrolase activity to different levels. These differences depend on the particular v^s allele, since three independent suppressors equally conserve the degree of suppressibility of a given v^s allele. TP activity in v^+/v^s heterozygotes is super-additive, being greater than half the TP activity in the v^+/v^+ flies. The degree of super-additivity is strictly related to the suppressibility of the particular vermilion allele. These *in vivo* observations on heterozygotes are paralleled *in vitro* by mixing extracts of v^+ and v^s homozygotes. Other evidence indicates that the v^s material responsible for super-additivity is a protein. Certain vermilion alleles heterozygous with v^+ interact to yield reduced TP activities in the suppressed, as compared to the

unsuppressed, condition. These data suggest the following model. v^+ defines a polypeptide which must dimerize to form active, homomultimeric TP. The suppressible vermilion alleles produce locally misfolded subunits which are unable to form the dimer. The effect of *su-s* is to provide a particular cellular environment in which these misshaped subunits can be more easily refolded. Hence, dimers are formed capable of tryptophan pyrrolase activity.

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