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Mechanism of suppression in *Drosophila melanogaster**

VIII. Comparison of *su(s)* alleles for ability to suppress the mutants purple, vermilion, and speck

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SUMMARY

The suppressor of sable [$su(s)^2$] restores the function of vermilion (*v*), purple (*pr*) and speck (*sp*) as well as sable (*s*) in *Drosophila melanogaster*. In this report various alleles of *su(s)* are compared for their relative effectiveness on three target mutations, *v*, *pr* and *sp*. Three criteria for suppression of *pr* and *v* were employed: visible phenotype, eye pigment levels (drosopterins and xanthommatin) and enzyme levels (sepiapterin synthase and tryptophan oxygenase). For *sp* only the visible phenotype was examined. By all three criteria *pr* was found to be more easily suppressed than *v*; *v* and *sp* were comparable. By use of *pr* with various alleles of *su(s)* either homozygously or in heterozygous combination with $su(s)^+$, the extent of suppression of *pr* can be best demonstrated by observing the levels of sepiapterin synthase; normal levels of drosopterins were found in females when sepiapterin synthase was only 20 % of normal. On the other hand, the extent of suppression of *v* is best demonstrated by the amount of xanthommatin eye pigment, because even the suppressed vermilion fly has < 10 % of wild-type activity of tryptophan oxygenase when 1-day-old flies are examined; in older flies this enzyme can be as high as 50 % of wild type. From these results we also demonstrated that $su(s)^2$, and other alleles, are not recessive but, in heterozygous combination with $su(s)^+$, cause marked suppression of *pr* and slight, but reproducible,

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suppression of *v*. The purple mutation, therefore, is particularly useful for studying the mechanism of suppression as well as for obtaining new mutant alleles of *su(s)*.

1. INTRODUCTION

The mechanism by which suppressor of sable [$su(s)^2$, 1–0·0] restores the function of vermilion (*v*, 1–33·0), purple (pr, 2–54·5) and speck (sp, 2–107·0) in *Drosophila melanogaster* is being investigated. The evaluation of suppression is facilitated by examining the levels of enzymes and of eye pigments that are controlled by the suppressible mutants. There are drawbacks to using the visible phenotype and the pools of pigments to measure the extent of suppression, since a partial restoration of the enzyme activity may be sufficient to restore fully the amount of pigment. For example, these are conflicting interpretations of the mechanism of suppression of *v* by $su(s)^2$. With $su(s)^2$ and $su(s)^+$ in homozygous and heterozygous combinations the suppression of *v* was judged to be recessive on the basis of the visible phenotype (Bonnier, 1925; Schultz & Bridges, 1932; Marzluff, 1965; Tartof, 1969; Baillie & Chovnick, 1971). However, Baglioni (1960) observed that the eye colour of *v*; *bw* that was heterozygous for $su(s)^2$ had visually detectable amounts of brown eye pigment and therefore concluded that '$su(s)^2$ was not recessive'. Furthermore, when Shapard (1960) measured the amount of non-protein tryptophan in adult flies, she observed a significant decrease in $su(s)^2/+$ compared with homozygous $su(s)^2$ and concluded that '$su(s)^+$ was incompletely dominant'. Thus Baglioni and Shapard agree that $su(s)^2$ is not recessive.

In this study, we demonstrate that *pr* gives a clearer indication of weak and moderate suppression than either *v* or *sp*. In the cases of *pr* and *v*, we measured the amounts of the respective eye pigments and enzymes as well as the visible phenotype; in the case of *sp*, only the latter was observed. The response of *pr* to various alleles of *su(s)* was measured in terms of the drosopterin eye pigments that could be extracted and of the measurable activity of sepiapterin synthase, the enzyme for which *pr* is probably the structural locus (Yim, Grell & Jacobson, 1977). The response of *v* was measured in terms of the xanthommatin eye pigment that could be extracted and of the measurable activity of tryptophan oxygenase, the enzyme for which *v* is probably the structural locus (Baglioni, 1960; Baillie & Chovnick, 1971).

The *pr* mutant is more easily suppressed than either *v* or *sp*. Furthermore, *pr* is a leaky mutant, as evidenced: (1) by the orange colour of the eyes of *pr cn*; and (2) by the presence of sepiapterin synthase in *pr cn* and pr^{bw} *cn* at levels 20–30 % of that in the wild type (Yim *et al.* 1977; Krivi & Brown, 1979). More extreme alleles of *pr* have been isolated that are non-suppressible, homozygous lethal and lower in sepiapterin synthase (< 10 %) (Yim *et al.* 1977). By use of the suppressible *pr*, additional mutations at the *su(s)* locus may be obtained that either suppress or enhance the *pr* phenotype (Grell *et al.* in preparation).

2. MATERIALS AND METHODS

(i) *Drosophila*

The mutants *y* (1–0·0), *v* (1–33·0), *cn* (2–57·5), pr^{bw} (2–54·5), *sp* (2–107·0), and *bw* (2–104·5) are described in Lindsley & Grell (1967), as are $su(s)^2$ (1–0) and $su(s)^s$. A number of alleles of *su(s)* were isolated after X-ray treatment [$su(s)^{x1}$, $su(s)^{x4}$] or ethyl methanesulfonate treatment [*su(s)* e^1, *su(s)* e^6]. The following alleles were obtained from the source indicated: $su(s)^{ab}$, A. T. C. Carpenter and B. S. Baker; $su(s)^{e5\cdot6}$, A. S. Fox. Dp(1; f) 18 and Dp(1; f)164 are fragments of the *X* chromosome in which most of the euchromatin is deleted; they exist in the presence of the normal chromosomes. The former carries wild-type alleles y^+ and $su(s)^+$, whereas the latter carries the y^+ but not the $su(s)^+$ locus. All flies were reared in $\frac{1}{4}$-pint glass bottles on a cornmeal, sugar and yeast diet.

(ii) *Enzyme assays*

Sepiapterin synthase activity was measured by determining the amount of radioactive dihydroneopterin triphosphate that was converted to sepiapterin (unit activity = 1 pmole/30 min) (Yim *et al.* 1977). Specific activity is based on either protein or fresh weight of the fly, as indicated. All assays were performed in duplicate and agreed within 10–15%. An unexplained variability occurred from week to week such that wild-type enzyme activity sometimes varies 3- to 4-fold. The age of the flies (Tobler *et al.* 1979) is an important variable but did not seem to be the cause of the variability that we experienced. For comparison of genotypes, the assays were all performed in a group simultaneously. Within a set of flies, the relationship of sepiapterin synthase to the *su(s)* alleles is always the same but among different sets of flies the values can vary as much as 4-fold.

Tryptophan oxygenase activity was measured by determining the extent of oxidation of tryptophan (unit activity = 1 nmole/h) by converting the formylkynurenine produced to anthranilic acid with enzymes obtained from *Neurospora crassa* (Casciano & Gaertner, 1973), namely formylkynurenine formamidase and kynureninase. The reaction mixture contained 10 mM L-tryptophan, 3·6 mM β-mercaptoethanol, and 300 mM sodium phosphate (pH 7·4) (Pomato, 1974). Incubation was at 36 °C. The reaction was stopped by heating for 30 sec in boiling water. The *N. crassa* enzymes and pyridoxal phosphate (0·3 mg/ml) were added, and incubation occurred at 36 °C for 2–4 h. Glycine HCl was added to give 77 mM glycine-92 mM-HCl (pH 2), and then the anthranilic acid was extracted into ethyl acetate. The fluorescence was measured in an Aminoo-Bowman spectrofluorometer (340 nm excition, 400 nm emission) and compared with that of a standard solution of anthranilic acid. Tobler (1975) has used similar conditions for assaying Drosophila tryptophan oxygenase, and Jacobson (1978*a*) has shown that formylkynurenine is the reaction product of the Drosophila enzyme and that it is subsequently converted to anthranilic acid. Under these conditions the production

of formylkynurenine is linear with time [no time lag occurs, as observed by Sullivan & Kitos (1976)], and the rate is proportional to the concentration of the extract.

The extract for sepiapterin synthase was prepared as described previously (Yim *et al.* 1977). The extract for tryptophan oxygenase was made by homogenizing 150 mg of adult flies in 1·2 ml of 100 mM sodium phosphate (pH 7·4)–3·6 mM β-mercaptoethanol-10 % glycerol and 40 mg of 'Norite A' charcoal. After centrifugation at 45000 ***g*** for 30 min, the supernatant was filtered through a glass-wool plug and the filtrate was assayed for tryptophan oxygenase.

Protein was determined according to the procedure of Lowry *et al.* (1951).

(iii) *Determination of 'Drosopterin'*

Fly heads were homogenized in the dark in propanol:3·5 % NH_4OH (2:1) at a ratio of 200 μl of solvent to 20 heads as described by Wilson & Jacobson (1977). Following centrifugation, 20–40 μl of the resulting supernatant was subjected to thin-layer chromatography on cellulose (Eastman 24355) with *n*-butanol:ethanol:H_2O (10:3:7) as solvent. In this solvent system, all the red and orange pteridine eye pigments ('drosopterins') remain at the origin while other pteridines migrate away. The orange spot at the origin was measured directly on the thin-layer sheet by use of a Turner Model 110 fluorometer, primary filter 760, secondary filters 2A, NDI and 23A (filter numbers are according to the Turner manual).

(iv) *Determination of xanthommatin*

The method of Butenandt *et al.* (1960) was employed as described previously (Jacobson, 1978*b*). The mutants white and brown were employed to define the 0 and 100 % limits of xanthommatin, respectively.

3. RESULTS

(i) *Relative effectiveness of* su(s) *alleles on* pr

Three means of evaluating the effect of *su(s)* alleles on *pr* are available: (1) visually observe the phenotype; (2) determine the amount of sepiapterin synthase; and (3) chemically determine the amount of orange and red pteridine eye pigments ('drosopterins'). For these comparisons cinnabar (*cn*) was present in the genotypes to prevent synthesis of xanthommatin (brown eye pigment) so that the various levels of the pteridines could be observed more easily. In *pr cn* the orange eye colour of the females is less intense than that of the male.

By visual observation of the various homozygous and hemizygous genotypes in Table 1, we found that all alleles of *su(s)* caused readily detectable suppression. The flies with the alleles $su(s)^{e1}$, $su(s)^{2}$ and $su(s)^{x4}$ had eye colour that was uniformly a full red-orange colour that is typical of *cn*. Among the flies with $su(s)^{ab}$, $su(s)^{s}$ and $su(s)^{e5\cdot6}$ some individuals had somewhat less than a full complement of red-orange eye pigment, but the majority here, too, were fully pigmented. Thus,

the suppression of *pr* is readily caused by all alleles of *su(s)*, but some alleles are more effective than others.

Males were constructed to be $su(s)^2/su(s)^+$ by use of a fragment of the *X* chromosome, Duplication 18, that includes the y^+ and $su(s)^+$ loci. In combination with *pr cn* the eye colour remained the pale orange colour of unsuppressed males. In the presence of Duplication 164, which contains y^+ but not $su(s)^+$, the $su(s)^2$ allele suppressed *pr* and restored a full compliment of red-orange colour. Thus, a single $su(s)^+$ allele prevents suppression in these males.

The amounts of sepiapterin synthase in flies with eight different alleles of *su(s)* are shown in Table 1. The difference between males and females is evident in all

Table 1. *Sepiapterin synthase in* pr cn *with eight different alleles of* su(s)

	Sepiapterin synthase			
	Specific activity (pmoles/30 min/mg fresh wt)		% of Oregon-R	
Genotype	Expt 1	Expt 2	Expt 1	Expt 2
Males [hemizygous for *su(s)* allele]:				
Oregon-R	3·6	13·4	100	100
$su(s)^+$; *pr cn*	0·9	2·1	25	16
$su(s)^{e1}$; *pr cn*	3·6	7·5	100	56
$su(s)^2$; *pr cn*	2·25	8·2	62	61
$su(s)^{ab}$; *pr cn*	2·6	4·7	71	35
$su(s)^{e6}$; *pr cn*	2·4	nt*	67	nt
$su(s)^{x4}$; *pr cn*	nt	8·0	nt	60
$su(s)^s$; *pr cn*	1·8	3·6	50	27
$su(s)^{e5\cdot6}$; *pr cn*	1·7	2·8	46	21
Females [homozygous for *su(s)* allele]:				
Oregon-R	2·7	8·25	100	100
$su(s)^+$; *pr cn*	0·2	0·2	8	3
$su(s)^{e1}$; *pr cn*	2·15	3·5	80	42
$su(s)^2$; *pr cn*	1·3	6·15	49	75
$su(s)^{ab}$; *pr cn*	0·8	1·4	28	17
$su(s)^{e6}$; *pr cn*	nt	nt	nt	nt
$su(s)^{x4}$; *pr cn*	nt	3·9	nt	47
$su(s)^s$; *pr cn*	1·0	1·9	36	23
$su(s)^{e5\cdot6}$; *pr cn*	0·9	1·1	34	13
Females [heterozygous for *su(s)* allele]:				
$su(s)^{e1}/+$; *pr cn*	0·5	—	19	—
$su(s)^2/+$; *pr cn*	0·8	—	31	—
$su(s)^{ab}/+$; *pr cn*	0·4	—	14	—
$su(s)^{e6}/+$; *pr cn*	0·4	—	14	—
$su(s)^s/+$; *pr cn*	0·3	—	13	—
$su(s)^{e5\cdot6}+/$; *pr cn*	0·4	—	14	—

Age of flies was 0–2 days. * nt = not tested.

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the genotypes – males contain more sepiapterin synthase than females; these specific activities are based on fresh weight, but the same difference between sexes is observed when protein is used as a reference. Also the extent of depression of sepiapterin synthase in $su(s)^+$; *pr cn* females is usually greater relative to Oregon-R than the depression in $su(s)^+$; *pr cn* males; as noted above females also have less orange eye colour than males. The *su(s)* alleles have different abilities to restore sepiapterin synthase. Among the males, the relative effectiveness is as follows: $su(s)^{e1} > su(s)^{e6} = su(s)^2 > su(s)^{ab} > su(s)^s = su(s)^{e5\cdot6}$. Among the homozygous females the order is similar except that $su(s)^{ab}$ drops to the bottom of the list; $su(s)^{e6}$ females survive poorly and were not examined. Among the females that are heterozygous for the *su(s)* mutant alleles and $su(s)^+$, all show a partial restoration of sepiapterin synthase; $su(s)^2/+$ seems to be somewhat more active than the other heterozygotes, but there is no other indication of the order of effectiveness seen for the homozygous females and hemizygous males. In contrast to the males with Duplication 18, females that contained $su(s)^2/su(s)^+$ had the suppressed purple phenotype.

The amount of the 'drosopterins' was determined among the various suppressor alleles, and sepiapterin synthase was again determined in this group of flies. In Fig. 1 it is apparent that the level of sepiapterin synthase can decrease to less than one-fourth of normal before a decrease in the level of the 'drosopterins' becomes significant. Thus, the enzyme is a more sensitive indicator of the extent of suppression than the pigment level or the visible phenotype. It is possible that sepiapterin synthase may be a rate-limiting enzyme in the determination of 'drosopterin' levels, but only when its activity is less than 15% that of the wild type.

(ii) *Comparison of sepiapterin synthase in* pr$^+$ *with different alleles of* su(s)

Since one effect of *su(s)* alleles is to increase the level of activity of sepiapterin synthase produced by the *pr* mutant, we also asked whether certain *su(s)* alleles affected the sepiapterin synthase in pr^+. Table 2 shows that of the two strongest suppressors, $su(s)^2$ had a slight negative effect on the sepiapterin synthase and $su(s)^{e1}$ a small positive effect. The other alleles did not alter the enzyme. Among these genotypes the visual phenotypes were all identical. The presence of y^+ and y^2 was employed to identify unambiguously which allele of *su(s)* was present in each pair of genotypes. We conclude that the pr^+ allele is not affected appreciably by any of the *su(s)* alleles.

(iii) *Relative effectiveness of* su(s) *alleles on* v

The visible phenotype of the *v*; *bw* flies that contained different alleles of *su(s)* showed that the latter could be graded by their effectiveness in restoring brown eye colour: $su(s)^{x1}$, $su(s)^{e1}$ and $su(s)^2$ were the strongest suppressors in homozygous and hemizygous flies, and $su(s)^s$ and $su(s)^{ab}$ were weaker. Heterozygous females

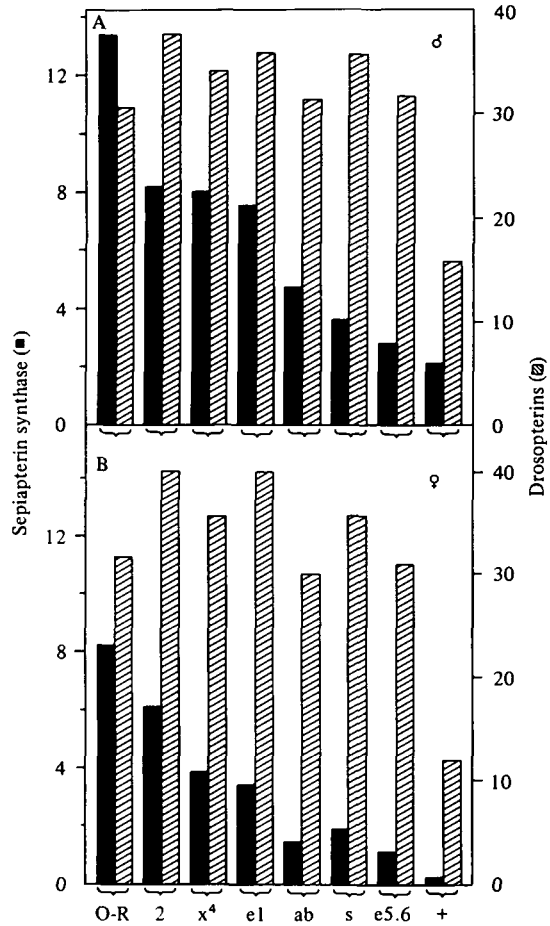

Fig. 1. Comparison of sepiapterin synthase and 'drosopterins' in hemizygous male (A) and homozygous female (B) alleles of *su(s)*. All except Oregon-R are of the genotype *pr cn* combined with different alleles of *su(s)*: O-R, Oregon-R; 2, $su(s)^2$ x4, $su(s)^{x4}$; e1, $su(s)^{e1}$; ab, $su(s)^{ab}$; s, $su(s)^s$; e5·6, $su(s)^{e5\cdot6}$; +, $su(s)^+$. The values for sepiapterin synthese are pmoles/30 min/mg fresh wt. and of 'drosopterin' are arbitrary fluorescent units/ 20 μl of extract, as described under (2) iii.

had a slightly increased amount of brown pigment in the cases of $su(s)^{x1}$, $su(s)^{e1}$ and $su(s)^2$, but had no change in $su(s)^s$ and $su(s)^{e5\cdot6}$.

The suppression of *v* by these five alleles of *su(s)*, as measured by the amount of tryptophan oxygenase, is shown in Table 3. In the hemizygous males, the strongest suppressors were $su(s)^{x1}$, $su(s)^{e1}$ and $su(s)^2$; in the homozygous females the same alleles were most effective in restoring tryptophan oxygenase. The weaker alleles $su(s)^s$ and $su(s)^{e5\cdot6}$ were also those that were least effective for restoration of sepiapterin synthase in *pr*. In contrast to the results with suppression of *pr*, the heterozygous *su(s)* alleles were much less effective in suppressing vermilion. When

the tryptophan oxygenase specific activity is around 0·05 or less, the limits of sensitivity of the assay are approached and the accuracy is low.

Fig. 2 shows the variation in tryptophan oxygenase with the age of Oregon-R, *su(s)*^{2}*v*; *bw* and *v*; *bw* flies. The levels of tryptophan oxygenase in Oregon-R and *v*; *bw* vary little, if at all, with the age of the fly, but in *su(s)*^{2}*v*; *bw* the activity increases 4- to 5-fold between 2 and 6 days of age. In the past, various laboratories have estimated that tryptophan oxygenase is restored to 10–30 % of wild type. The age-dependent change of this activity in *su(s)*^{2}*v*; *bw* is such that the activity relative to Oregon-R may be correctly said to be restored to 10–50 %. Thus, the discrepancy of values from various laboratories may be due to the variation in activity of tryptophan oxygenase of *su(s)*^{2}*v*; *bw* flies of different ages.

Table 2. *Sepiapterin synthase in male* pr$^+$ *with various alleles of* su(s)

Genotype	Sepiapterin synthase: Specific activity (pmoles/ 30 min/mg fresh wt)	Sepiapterin synthase: Ratio, *su(s)* allele/*su(s)*+
1. Oregon-R	13·5	—
2. y^+ *su(s)*^{s}; pr^+	17·95	0·97
y^2 *su(s)*$^+$; pr^+	18·6	—
3. y^+ *su(s)*^{2}; pr^+	11·3	0·85
y^2 *su(s)*$^+$; pr^+	13·3	—
4. y^+ *su(s)*^{ab}; pr^+	14·0	1·07
y^2 *su(s)*$^+$; pr^+	13·1	—
5. y^+ *su(s)*$^{e5\cdot6}$; pr^+	15·0	1·06
y^2 *su(s)*$^+$; pr^+	14·2	—
6. y^2 *su(s)*^{e1}; pr^+	13·6	1·24
y^+ *su(s)*$^+$; pr^+	10·95	—

Age of flies was 0–2 days.

The question then arises as to the relationship between the level of tryptophan oxygenase in the adult fly and the amount of xanthommatin that can be maintained in the eye at that level of enzyme. In contrast to *pr*, for which a graded series of sepiapterin synthase levels were found in the flies with the different alleles of *su(s)*, the level of tryptophan oxygenase in the two strongest suppressors, *su(s)*^{e1} and *su(s)*^{2}, is only a small fraction of the wild-type level (10 and 5 %, respectively, for males, and even less for females) (Fig. 3). The decrease in tryptophan oxygenase to values relative to the wild type of 10 % [*su(s)e*^{1} males] and ~ 3 % [*su(s)e*^{1} females] resulted in xanthommatin levels at 77 and 63 %. Thus, a 10- to 30-fold decrease in enzyme resulted in less than a 2-fold decrease in xanthommatin. Turning to the *su(s)*^{2} males and females in which the enzyme was only one-half the corresponding *su(s)*^{e1} values, the xanthommatin was also significantly lower. The trend continues

for $su(s)^s$, $su(s)^+$ and $su(s)^{e5\cdot6}$. These data lead to the suggestion that the concentration of tryptophan oxygenase is the main limitation to xanthommatin accumulation in the vermilion mutant in conjunction with various alleles of *su(s)*. Therefore, the wild type, Oregon-R, would appear to have an excess of enzyme over that required to maintain xanthommatin levels in the eyes. Since the xanthommatin occurs in the eyes and the enzyme determinations are from the whole body, it is appropriate to point out that kynurenine produced in another part of the body is available for xanthommatin synthesis in the eye of vermilion (Beadle & Ephrussi, 1936; Kikkawa, 1941).

Table 3. *Tryptophan oxygenase in* v; bw *with different alleles of* su(s)

	Tryptophan oxygenase	
Genotype	Specific activity (nmoles/h/mg protein)	% of Oregon-R
Males [hemizygous for *su(s)* allele]:		
Oregon-R	8·9	100
$su(s)^+v$; *bw*	0·02	< 1
$su(s)^{z1}v$; *bw*	1·0	11
$su(s)^{e1}v$; *bw*	0·90	10
$su(s)^2v$; *bw*	0·40	4
$su(s)^sv$; *bw*	0·05	< 1
$su(s)^{e5\cdot6}v$; *bw*	0·01	< 1
Females [homozygous for *su(s)* allele]:		
Oregon-R	8·1	100
$su(s)^+v$; *bw*	0·04	< 1
$su(s)^{z1}v$; *bw*	0·8	9
$su(s)^{e1}v$; *bw*	0·95	12
$su(s)^2v$; *bw*	0·55	7
$su(s)^sv$; *bw*	0·07	< 1
$su(s)^{e5\cdot6}v$; *bw*	0·04	< 1
Females [heterozygous for *su(s)* allele]:		
$su(s)^{z1}/+v$; *bw*	0·06	< 1
$su(s)^{e1}/+v$; *bw*	0·04	< 1
$su(s)^2/+v$; *bw*	0·07	< 1

Age of flies was 0–4 days.

Heterozygotes for $su(s)^{e1}$, $su(s)^2$ or $su(s)^{e5\cdot6}$ could not be distinguished from $su(s)^+$ on the basis of tryptophan oxygenase (Fig. 3). However, the xanthommatin levels of $su(s)^{e1}/+$ and $su(s)^2/+$ were higher than those of $su(s)^+$, as expected from the differences in visible phenotype.

These results may be compared with the relationship between 'drosopterins' and sepiapterin synthase (Fig. 1); there, too, the enzyme had to decrease by > 80 %

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before the pigment level was affected. In that respect, the relationship between pigment level and the enzyme concentration is similar for *pr* and *v*; on the other hand *pr* and *v* are different in that *pr* is more readily suppressed by heterozygous combinations of the *su(s)* alleles or by the weaker alleles, homozygously, e.g. $su(s)^{s}$ and $su(s)^{e5\cdot6}$.

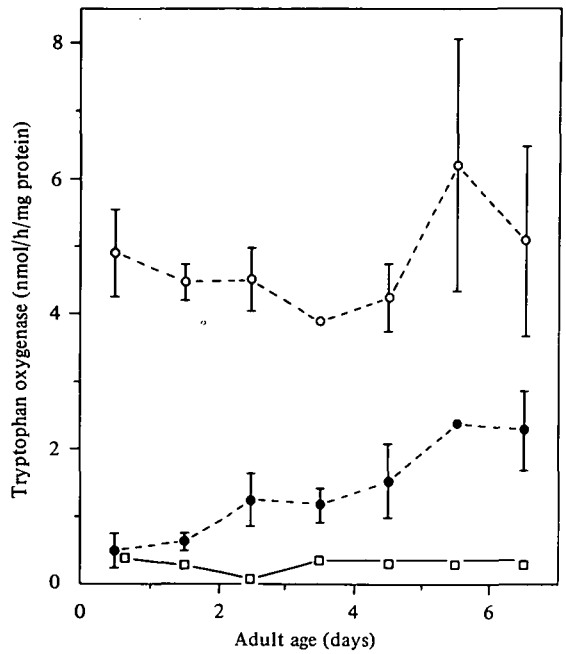

Fig. 2. Variation of tryptophan oxygenase with age of adult flies. ○, Oregon-R; ●, $su(s)^{2}$ *v*; *bw*; □, *v*; *bw*. Vertical bars are the standard deviation.

(iv) *Comparison of* su(s) *alleles for their ability to suppress* sp

The *su(s)* alleles in combination with *sp* were compared in the following genotypes: $su(s)^{2}$; *sp*, $su(s)^{x1}$; *sp*, $su(s)^{e1}$; *sp*, $su(s)^{ab}$; *sp*, $su(s)^{s}$; *sp*, and $su(s)^{e5\cdot6}$; *sp*. The first three caused the complete disappearance of the speck phenotype, whereas in the last three the speck trait was still visible.

A uniformity among the suppressor alleles seems to exist: $su(s)^{2}$ and $su(s)^{x1}$ are strong suppressors of *v*, *pr* and *sp*. The alleles $su(s)^{ab}$, $su(s)^{s}$ and $su(s)^{e5\cdot6}$ are weaker suppressors of these mutants. Other alleles that are probably uniformly strong suppressors are $su(s)^{e1}$, $su(s)^{e6}$ and $su(s)^{x4}$, but they were not tested with all three target genes.

Certain traits of $su(s)^{e1}$ and $su(s)^{e6}$ may be of peripheral interest; $su(s)^{e1}$ males are sterile at 17 °C; $su(s)^{e6}$ females have poor viability. In those females containing $su(s)^{e6}$, strong suppression was noted; limited viability cannot be a consequence of strong suppression solely, since $su(s)^{e1}$ is a strong suppressor and yet grows as a rather vigorous culture.

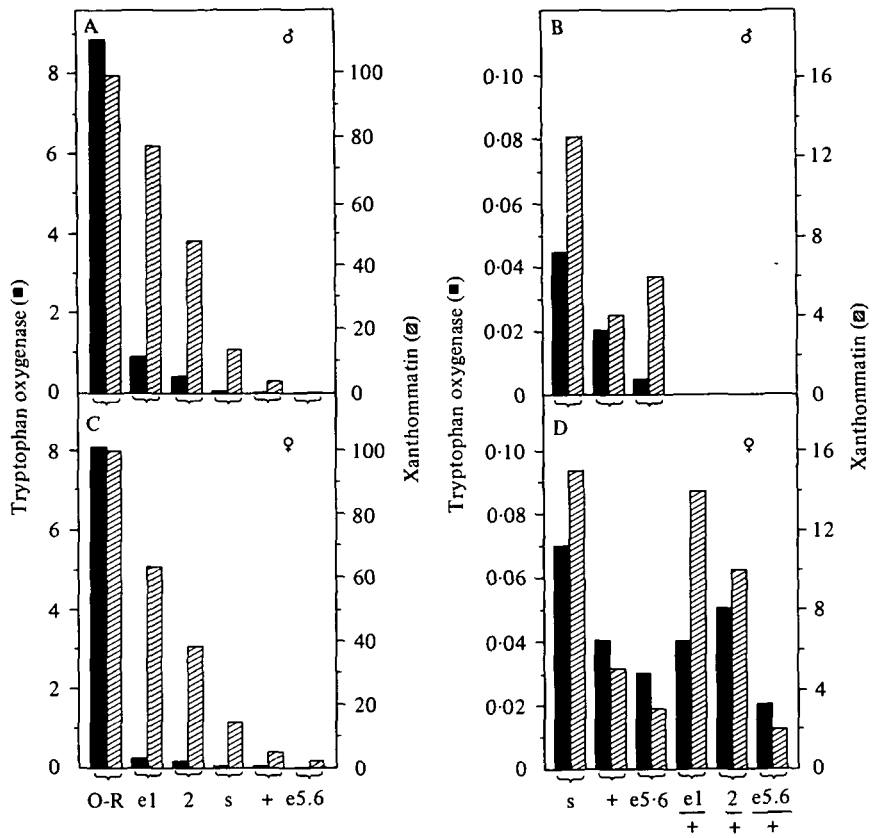

Fig. 3. Comparison of tryptophan oxygenase and xanthommatin in homozygous, heterozygous and hemizygous alleles of *su(s)*. All except Oregon-R are of the genotype *v*; *bw* combined with different alleles of *su(s)*. Panels A and B give values for male flies with different alleles of *su(s)*, the stronger suppressors are on the left in panel A, the weaker ones on the right of panel A and in panel B where the scale is expanded and the values replotted. Panels C and D give values for females organized in the same manner as the males in A and B except in panel D are given also values for three heterozygotes. The values for tryptophan oxygenase are nmoles/hr/mg protein and of xanthommatin are percent of the value obtained from the brown mutant. Allele designations are as in Fig. 1.

4. DISCUSSION

In the Introduction, several studies are mentioned that concluded that *su(s)* alleles are recessive; but two others concluded otherwise. All these studies used *v* as the target of *su(s)* action. In the case of *v*, the enzyme tryptophan oxygenase is abnormal (Baglioni, 1960; Marzluf, 1965; Tartof, 1969; Baillie & Chovnick, 1971). Several of these studies, as well as those of Jacobson (1971, 1978*b*), led to the suggestion that suppression was not the result of regulation or deregulation of transcription nor the alteration of a translational component but seemed to involve the restoration of the activity of the mutant enzyme. In the case of *pr*,

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Yim *et al.* (1977) found that sepiapterin synthase activity is decreased in *pr cn* and is restored to more than 50% of normal levels by $su(s)^2$. This afforded an opportunity to compare two mutant enzymes and the way they respond to the *su(s)* alleles. One goal of this study will be to determine whether or not the mechanism of suppression is the same for *v*, *pr*, and *sp*.

When changes in the eye pigments are the criteria for measuring the suppression of the mutant, xanthommatin in the case of *v* and 'drosopterins' in the case of *pr*, it is possible that their alteration may not reflect accurately the changes in activity of the mutant enzyme. If the enzyme is not the rate-limiting catalyst for the overall pathway, then the enzyme level could change and there would be little or no change in the pigment deposited in the granules of the eye cells. Indeed, in the case of *pr*, the suppressor alleles, $su(s)^{e1}$ and $su(s)^2$, were very effective in restoring sepiapterin synthase whereas $su(s)^s$ and $su(s)^{e5\cdot6}$ were much less effective. Yet the amount of the 'drosopterins' in all four suppressed flies was similar. It appears that sepiapterin synthase is not the rate-limiting step in 'drosopterin' biosynthesis until the activity is less than 15% of the wild-type level. Xanthommatin synthesis also seems to be insensitive to the level of tryptophan oxygenase until the activity of this enzyme is less than $\sim 15\%$ of normal.

Comparison of the response of *pr* and *v* to the various alleles of *su(s)* revealed that *pr* responds more readily than *v*. Heterozygous combinations of the *su(s)* alleles with $su(s)^+$ all caused a measurable increase in 'drosopterin' and sepiapterin synthase in *pr cn*, whereas the increase in xanthommatin and tryptophan oxygenase in *v*; *bw* was slight. Other evidence of the greater responsiveness of *pr* is found in the comparison of the *su(s)* alleles in homozygous or hemizygous genotypes. $su(s)^{e5\cdot6}$ and $su(s)^s$ are scarcely able to increase tryptophan oxygenase in *v*; *bw*, but they cause a marked restoration of sepiapterin synthase in *pr cn*. We found earlier that the amount of sepiapterin synthase varies with the dose of the pr^+ allele and suggested that this is the structural locus for the enzyme (Yim *et al.* 1977). Baillie & Chovnick (1971) observed that the amount of tryptophan oxygenase varies in proportion to the v^+ alleles and suggested that *v* is the structural gene for this enzyme. In agreement with Shapard (1960) and Baglioni (1960), we find that $su(s)^2$ is not recessive. The opportunity seems to be available to determine how the *su(s)* alleles exert their effect on *pr* and *v* and why to a greater extent on *pr* than on *v*.

In considering the mechanism of suppression, it is of interest to note that *v* and *pr* can become active by epigenetic manipulation. Flies of the *v*; *bw* genotype will produce xanthommatin when grown on a diet consisting exclusively of dried brewer's yeast (Beadle, Tatum & Cloney, 1939; Jacobson, 1978*b*). Flies of the *pr cn* genotype will produce normal amounts of 'drosopterins' when grown at 30° but not at 25° (Grell, in preparation). However, *pr cn* does not produce 'drosopterins' on the yeast diet, nor does *v*; *bw* produce xanthommatin at 30°. These observations demonstrate ways that the mutant alleles may be restored to essentially normal activity. It is clear that the inactivation of $su(s)^+$ is not the explanation, however, since *v* and *pr* respond to different treatments.

Earlier reports showed that one isoacceptor of $tRNA^{Tyr}$ was an inhibitor of the vermilion form of tryptophan oxygenase (Jacobson, 1971). This depended on the activation of the mutant form of this enzyme by ribonuclease; however, subsequently the ribonuclease activation has proven to be unreproducible (Jacobson *et al.* 1975; Mischke, Kloetzel & Schwochau, 1975). One might consider abandoning this tRNA inhibition hypothesis altogether if it were not for the fact that it derives support from an independent approach in which *v*; bw flies were raised on a diet, consisting exclusively of dead brewer's yeast, that caused xanthommatin appearance in the eyes and concomitant disappearance of the postulated inhibitory isoacceptor of $tRNA^{Tyr}$ (Jacobson, 1978*b*). When the diet was modified by the addition of glucose so that xanthommatin was no longer made, the inhibitory $tRNA^{Tyr}$ was present again. This correlation may be an important link to allow us to retain interest in the inhibition model. If the inhibition hypothesis is correct, is should be demonstrable by use of *pr* as well as *v*; experiments are in progress to investigate this possibility.

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