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CHARACTERIZATION OF AN X-LINKED SEMI-DOMINANT SUPPRESSOR OF *BLACK Su(b)* (1-55.5) IN DROSOPHILA MELANOGASTER

by

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A newly described mutant gene in Drosophila melanogaster suppresses the phenotype of the *black* mutation *b* (2-48.5), which is known to cause melanization of the cuticle through a deficiency of β-alanine. The semi-dominant suppressor, *Su(b)* is X-linked and maps at 1-55.5 adjacent to or within the *rudimentary* locus *(r)* controlling the level of the first three enzymes in the biosynthesis of pyrimidines. The suppressor of *rudimentary, su(r),* known to block the degradation of uracil to β-alanine, enhances the melanization of the cuticle in the *black* mutant flies. This suppressor *su(r)* is epistatic over the suppressor of *black* resulting in an enhanced melanization in *su(r) Su(b); b* flies.

Feeding the pyrimidine analogue 6-azathymine to wild type larvae gives rise to a partial phenocopy of *black.* Resistance against melanization by 6-azathymine is provided by *Su(b).* The analogue 6-azauracil normalizes the mutant *black* but not the double mutant *su(r); b.* It is proposed that *Su(b)* is a mutation in the regulatory gene for the formation of the first three enzymes in pyrimidine biosynthesis leading to overproduction of uracil. The degradation of this extra uracil into β-alanine would then permit the normal tanning of the cuticle in flies homo- or hemizygous for *black.*

1. INTRODUCTION

The mutant *black* of Drosophila melanogaster has a black body colour and light pupal cases. This mutant phenotype is caused by a deficiency in β-alanine, which normally is involved in the tanning and melanization of the cuticle (5, 9, 10). Thus brown coloured pupal cases and wild type body colour are obtained by feeding the mutant larvae β-alanine (5). The dark cuticle colour in the *black* mutant as well as in the *ebony* mutant apparantly results from the polymerization of indole-5,6-quinones to melanin (23), whereas this condensation is prevented in the wild type by the presence of β-alanine (6, 10). In the wild type β-alanine also promotes the cross-linking of the quinones of N-acetyldopamine to protein in the tanning process (10).

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The pupae of the *black* mutant contain about half the wild type amount of β-alanine in the hemolymph and only 10% of wild type amount in the cuticle (26). A normal amount of β-alanine in the cuticle is restored when the recessive suppressor of *black su(b)* mapping at the tip of the X-chromosome (1- 0.0) is present in homozygous form. In the absence of the *black* gene the suppressor causes a doubling of the concentration of β-alanine in the hemolymph and a supranormal amount in the cuticle (26). The amino acid β-alanine seems metabolically derived from either uracil or aspartic acid (8) (cf. Figure 1). The only enzyme so far assayed in the *black* mutant is hydropyrimidine hydrase catalyzing a step in the catabolism of uracil and it was found to be present in wild type amounts (4). It therefore remains unknown which step in β-alanine formation is blocked by the black mutation.

In a search for additional mutants, which can suppress or enhance the effect of the *black* mutation a new semi-dominant suppressor was identified and mapped to the X-chromosome at position 1-55.5. The thymine analogue 6-azathymine has previously been shown to inhibit the dihydrouracil dehydrogenase of rat liver (15) and to prevent the degradation of uracil in mice resulting in a large excretion of this pyrimidine in the urine (19). It was therefore tested in feeding experiments on wild type Drosophila and found to give partial phenocopies of *black*. This indicates that a deficiency of β-alanine resulting in black cuticle colour can be effected by blocking the catabolism of pyrimidines.

An altered degradation of pyrimidines has previously been identified in the suppressor mutant of *rudimentary, su(r)* (1). This mutant is unable to convert uracil to dihydrouracil and β-alanine, indicating a defective dehydro-uracil dehydrogenase EC 1.3.1.2 (cf. Figure 1). The interaction of this suppressor mutation with the *black* mutation was studied and the suppressor found to have an enhancing effect on black. The suppressor of *rudimentary, su(r)* provides increased resistance to 6-azauracil; an analogue causing *rudimentary-like* phenocopies in the wild type (3, 20, 27). This compound was accordingly also tested on the *black* mutant and found to prevent melanization.

2. MATERIALS AND METHODS

2.1. Isolation of the suppressor of *black Su(b)*

The semi-dominant *Su(b)* fly was found in the progeny of a cross between an attached-X female of the constitution *y f/Y;b* and a *++/Y;b* male. The males had been treated with ethylmethane sulfonate by the method of LEWIS and BACHER (12). Among a total of 273 male progeny, a single wild type coloured male was found and crossed to attached-X *y f/Y;b* virgins. The male progeny consisted of wild type coloured flies. From this result it is concluded that the suppressor mutation is X-linked. The *Su(b)/Y;b* male progeny was crossed to M-5;*b* virgins and from the F_2 progeny a *Su(b);b* strain was established. The semi-dominant character of *Su(b)* is clearly expressed in the M-5/*Su(b);b* females (Figure 2).

2.2. Mapping of the *Su(b)* gene

The mutation was mapped with the aid of the following crosses of homozygous black flies:
a): *++Su(b)+/++Su(b)+* × *w m+f/Y*
b): M-5/*su(r)+f* × *+Su(b)+/Y*
c): *Su(b)* / *Su(b)* × *g r f/Y*
The distances between the loci were calculated from the crossover classes among the F_2 males.

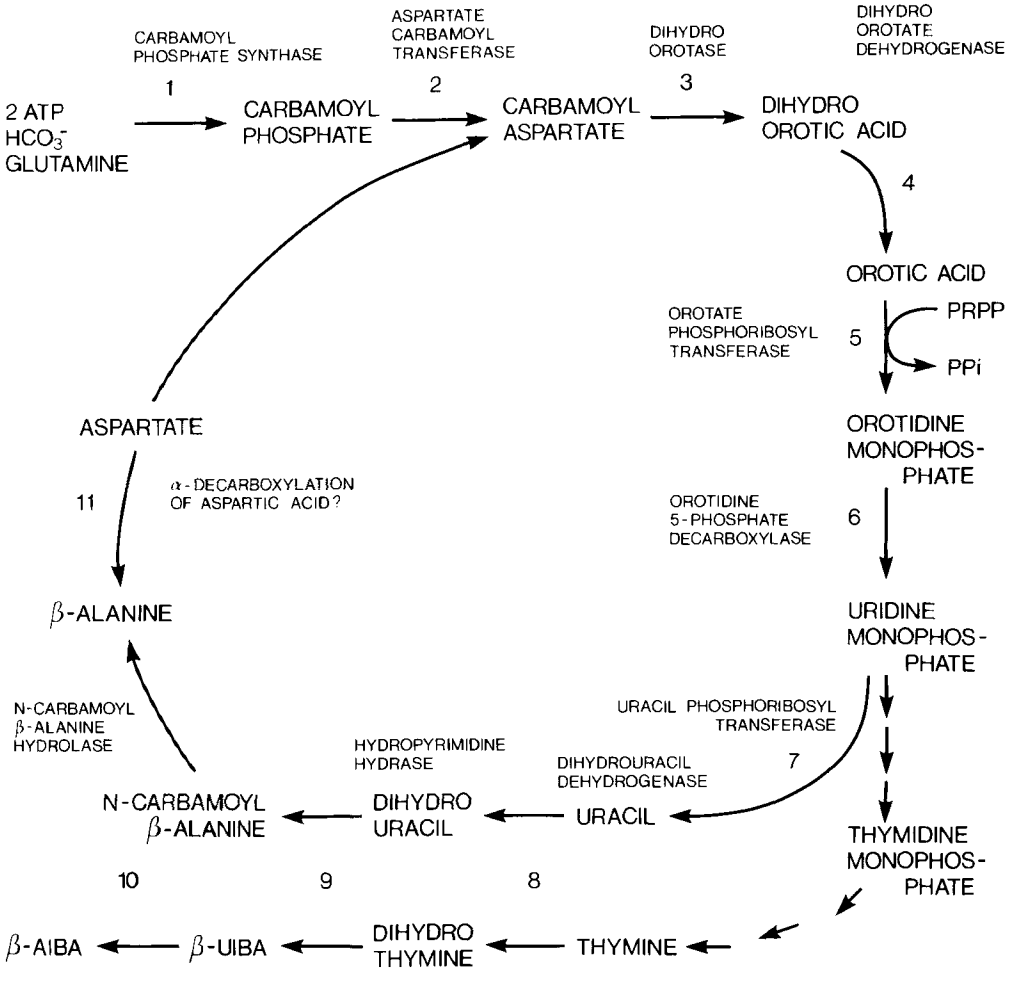
2.3. Construction of a *su(r);b* strain

The following crossing scheme was applied:
P: M-5/*su(r)f;Pm/b* × M-5/*Y;b*
F_1: M-5/*su(r)f;b* × *su(r)f/Y;b*
This balanced *su(r);b* strain was propagated with the M-5 chromosome as it proved difficult to keep the double recessive *black* flies alive on the standard yeast sucrose medium.

(M-5 = First chromosome balancer containing the genes *white* w^a, *scute sc* and *Bar B; Pm* = Second chromosome balancer containing the genetic marker *Plum Pm).*

2.4. Feeding with 6-azathymine and 6-azauracil

The strains were maintained on standard yeast sucrose medium, while the test medium, Eledon minimal medium, was made up according to NØRBY (17) and the inhibitor added to the medium during its preparation. The analogues 6-azathymine and 6-azauracil were obtained from Sigma, USA.



Mutants:		
r	(1-55.3)	defective in 1, 2 or 3
Dhod	(3-band 85A1-3)	defective in 4
r-l	(3-band 93)	defective in 5, 6
su(r)	(1-27.7)	defective in 8
b	(2-48.5)	β-alanine deficiency
su(b)	(1-0.0)	high β-alanine level
Su(b)	(1-55.5)	mutation in regulator gene for the pyrimidine biosynthesis ?

Inhibitors:	at:	Phenocopies of:
Phosphonacetyl-L-aspartate	2	rudimentary
6-azaUMP	6	rudimentary-like
6-azathymine	8	partial black
6-azauracil	?	normalizes black

Figure 1. Enzyme defects caused by mutants and analogues in the biosynthesis and degradation of pyrimidines in Drosophila. (β-AIBA = β-amino isobutyric acid; β-UIBA = β-ureidoisobutyric acid).

2.5. Determination of cuticle reflectance

For a quantitative analysis of the effect of the different mutant gene combinations the reflectance of the thorax cuticle was measured through a stereomicroscope with a photodiode according to the method of BARR and PEDERSEN (2). The shade of the cuticle is measured in mV by a voltmeter (Philips digital multimeter PM 2517E). In the diagrams reflectance is expressed in % of wild type, 100% corresponding to the average of the

reflectance exhibited by *Oregon R* wild type flies which have been raised on standard yeast sucrose medium. Usually 10 to 25 flies were analysed per treatment in the feeding experiments and care was taken to use for measurement only clean and undamaged flies.

3. RESULTS

3.1. Mapping of the suppressor of *black Su(b)*

Body colour was evaluated visually as well as objectively by measuring the reflectance of the cuticle with a photodiode (2). In Figure 2 the distribution of the reflectance values for a number of genotypes is presented. The average reflectance of wild type Oregon flies has been arbitrarily designated as 100. The black mutant flies then reveal a reflectance of about 40. In the presence of the suppressor of *black, Su(b),* the cuticles of flies homo- or hemizygous for this mutant gene give reflectance values close to wild type while *Su(b)* / + heterozygotes give intermediate values between wild type and black flies revealing the semi-dominant character of *Su(b).* The presence of the recessive suppressor of *rudimentary, su(r),* in homo- or hemizygous form gives an enhanced black body colour around 20 (cf. also Figure 5).

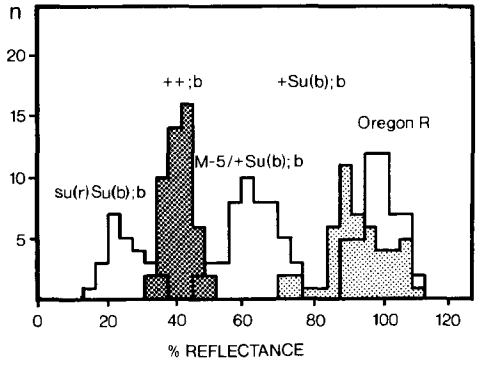


Figure 2. Histograms of reflectance values for wild type *Oregon R* flies, *black* flies and genotypes homozygous for *black* and hemi- or heterozygous for the *Su(b)* gene.

Also included is the distribution of reflectance values for *black* males containing both the suppressor for rudimentary, *su(r)* and the suppressor of *black, Su(b).* Each histogram is based on 50 individuals with the exception of the *su(b) Su(b); b* class which comprise 25 flies.

Table I.

Mapping of the suppressor of black *Su(b)* with the X-chromosome markers *white, miniature* and *forked.* The data show that *Su(b)* is closely linked to forked. All flies are homozygous black.

P generation	: *Su(b) / Su(b) × w m f / Y*		
F_1 generation	: *+ + Su(b) + / w m + f × M-5 / Y*		
F_2 males	:		
w m + f	: 335	*w + + f*	: 23
+ +Su(b)+	: 631	*+ m Su(b)+*	: 27
+ m + f	: 194	*w m Su(b)f*	: 0
w +Su(b)+	: 205	*+ + + +*	: 0
+ + + f	: 109	*w +Su(b)f*	: 1
w m Su(b)+	: 69	*+ m + +*	: 2
w m + +	: 4	*w + + +*	: 0
+ +Su(b)f	: 4	*+ m Su(b)f*	: 0

Mapping data for *Su(b)* are presented in Tables I to III. The first cross (Table I) showed that *Su(b)* is closely linked to forked (1-56.7) and not situated adjacent to *white* (1-1.5) like the recessive *su(b)* (1-0.0) described by SHERALD (26), since many flies among the F_2 males contained both *w* and *Su(b)* but only 8 out of 1604 males were the result of a cross over between *Su(b)* and *f.* The second cross (Table II) revealed that *su(r)* (1-27.7) is epistatic over *Su(b).* The *su(r) Su(b) / Y; b* males have an enhanced black body colour (Figure 2) and are phenotypically indistinguishable from *su(r); b.* The *su(r) Su(b) / Y; b* flies have dull red eyes and a low emergence rate compared to the *f/Y; b* males from the same cross over class (Table II). The third cross (Table III) involving *garnet* (1-44.4), *rudimentary* (1-55.3) (cf. 16) and *forked* (1-56.7) places *Su(b)* 1.2 cM to the left of *forked* giving a map position of 55.5 which is very close to the rudimentary gene known to control the three enzymes activities converting glutamine, ATP and bicarbonate via carbamoyl phosphate to dihydroorotic acid (cf. Figure 1). With a distance between *Su(b)* and *r* of 0.1 to 0.2 cM one would expect about 1 to 5 cross overs between *Su(b)* and *r* in a sample of 2655 F_2 males as analysed in Table III. Only one *f/Y ; b* male

Table II.

Mapping of the suppressor of black *Su(b)* with the X-chromosome markers *su(r)* and *f*. The double mutant males *su(r)Su(b)/ Y; b* have a low emergence rate compared to the + + *f*/ *Y; b* males from the same cross-over class. All flies are homozygous black.

P generation	: *M-5 / su(r) + f* × *+ Su(b) + / Y*
F_1 generation	: *su(r) + f / + Su(b) +* × *M-5 / Y*
F_2 males	:
su(r) + f	: 638
+ Su(b) +	: 777
+ + f	: 274
su(r)Su(b)+	: 49
su(r) + +	: 5
+ Su(b)f	: 18
su(r)Su(b)f	: 0
+ + +	: 1

Table III.

Mapping of the supressor of black *Su(b)* with the X-chromosome markers *garnet, rudimentary* and *forked*. It is not possible to determine on which side of *r Su(b)* maps. The one *forked* male optained may represent a cross-over between *r* and *Su(b)*, but it could not be test-crossed. All flies are also *b/b*.

P generation	: + + *Su(b)* + / + + *Su(b)* + × *g r f / Y*		
F_1 generation	: + + *Su(b)* + / *g r + f* × + + *Su(b)* + / *Y*		
F_2 males	:		
g r + f	: 1108	*+ r + +*	: 0
+ +Su(b)+	: 1303	*g +Su(b)f*	: 0
+ r + f	: 90	*+ + + +*	: 0
g +Su(b)+	: 121	*g r Su(b)f*	: 0
g r + +	: 11	*g + + +*	: 0
+ +Su(b)f	: 21	*+ r Su(b)f*	: 0
+ + + f	: 1	*g + + f*	: 0
g r Su(b)+	: 0	*+ r Su(b)+*	: 0

was found and non of the reciprocal cross over type *g r Su(b) / Y ; b*. As the former could not be test crossed it remains uncertain whether this single male represents a cross over between *r* and *Su(b)*. It is thus possible that *Su(b)* maps within the *r* locus.

3.2. Feeding experiments with 6-azathymine

A supplement of 6-azathymine in the food for wild type Drosophila larvae of strain *Oregon R* produces black body colour of the adults although the phenocopies are not quite as dark as the *black* mutant (Figure 3). On the other hand the phenocopies show a distribution of melanized areas which is identical to that of the *black* mutants. Flies homozygous or hemizygous for the black mutation do not react towards 6-azathymine with a changed cuticle colour (Figure 3).

When the suppressor of *black, Su(b),* is present in individuals either homo- or heterozygous for *black, b,* there is no response to feeding of 6-azathymine, i.e., the cuticle retains its wild type colouration (Figure 4). In this case flies of the attached-X/*Y; + /b* genotype served as control and responded readily with melanization upon feeding increasing amounts of 6-azathymine. It can be concluded that *Su(b)* provides resistance against melanization by 6-azathymine.

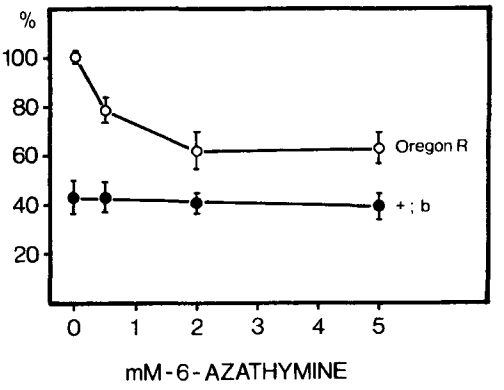


Figure 3. Effect of feeding increasing amounts of 6-azathymine on the relative reflectance of the thorax cuticle in the *Oregon R* and *+ ; b* strains.

While the wild type strain *Oregon R* becomes darker with increasing amounts og 6-azathymine in the medium, the black flies retain the same body colour. Bars indicate standard deviation.

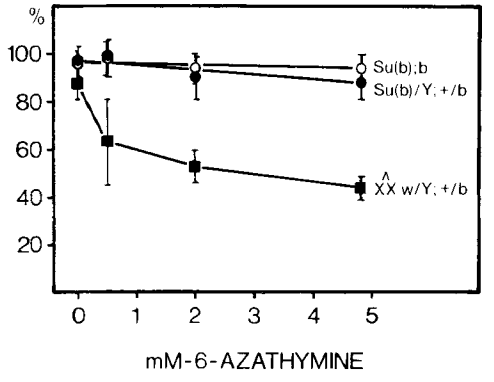


Figure 4. Relative reflectance of the thorax cuticle of three genotypes as a function of increasing amounts of 6-azathymine in the media.

(⊙-⊙) *Su(b) ; b*, (●-●) *Su(b) / Y ; b* males and (■-■) *XX w / Y + / b* females. Bars indicate standard deviation.

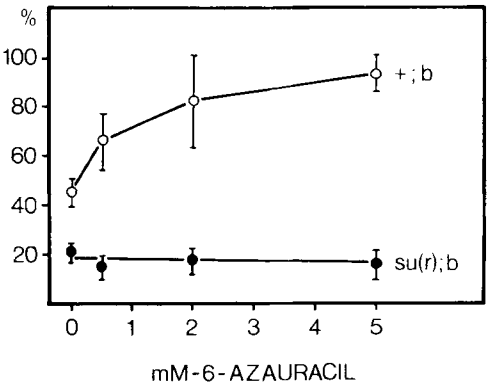


Figure 6. The difference in the reaction of + ; *b* and *su(r) ; b* flies to increasing amounts of 6-azauracil.

The *su(r) ; b* strain retained the enhanced black colour over all 6- azauracil concentrations tested, while the + ; *b* flies get lighter when the concentration of 6-azauracil increases. The bars give the standard deviations.

3.3. The interaction of the suppressor of rudimentary *su(r)* with *black b*

Flies homozygous for black and homozygous for the suppressor of *rudimentary* were obtained from the cross listed under 2.3. The cuticle of those flies had an enhanced black colour (Figure 5). Flies heterozygous for *su(r)* and homozygous for *b* are of the typical black phenotype. The mutant *su(r)* by itself causes a slight darkening of the cuticle relative to the wild type (Figure 5).

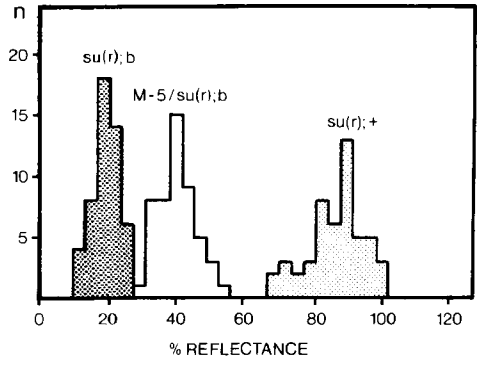


Figure 5. The effect of *su(r)* on *b* is a recessive trait, since the *M-5 / su(r) ; b* is of the same colour as + ; *b* (cf. Figure 2).

The *su(r) ;* + group seem to be slightly darker than *Oregon R* flies which have a value around 100. The *su(r) ; b* flies are distinctly darker than the two other genotypes with no overlap between the histograms of *su(r) ; b* and *M-5 / su(r) ; b*.

3.4. Feeding experiments with 6-azauracil

Amounts of 5 mM-6-azauracil in the food given to the larvae of the mutant *black* allows an almost complete normalization of its cuticle colour (Figure 6). This compound is, however, unable to prevent or decrease the extreme melanization caused by the combined action of the black mutation and the *su(r)* mutation. Wild type flies react slightly and homozygous or hemizygous *su(r)* flies (in the absence of *b)* do not react to 6-azauracil in the larval food with a changed cuti-

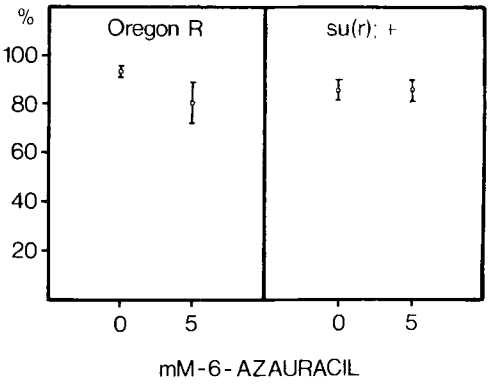


Figure 7. The two strains *Oregon R* and *su(r)* ; + retain their wild type appearance when fed with 5 mM-6-azauracil.

While the *su(r)* flies are quite resistant to the analogue only three *Oregon R* flies survived the treatment with 5 mM-6-azauracil.

Table IV.

Means and standard deviation of egg counts and emerged imagos in the strains raised on the minimal medium containing increasing amounts of 6-uracil. x_e is the mean of laid eggs in the vials *(n)* and x_i is the mean of the emerged imago flies.

Concentration of 6-azauracil	Genotypes: *Oregon R*			*sur(r)* ; +			+ ; *b*			*su(r)* ; *b*		
	x_e	x_i	*n*	x_e	x_i	*n*	x_e	x_i	*n*	x_e	x_i	*n*
0 mM	52.0 ± 9.0	22.8 ± 4.5	6	71.7 ± 35.4	25.0 ± 5.4	6	66.7 ± 11.2	22.8 ± 4.5	6	77.5 ± 20.9	53.8 ± 11.5	6
0.5 mM	37.8 ± 6.3	15.3 ± 9.1	4	n.d.			77.2 ± 15.3	19.4 ± 15.2	6	58.7 ± 18.7	21.8 ± 6.2	6
2 mM	81.2 ± 18.4	1.2 ± 1.8	6	n.d.			56.8 ± 18.4	1.7 ± 2.3	6	58.7 ± 17.4	16.0 ± 6.1	6
5 mM	65.7 ± 24.2	0.7 ± 1.0	6	79.3 ± 7.0	3.0 ± 2.8	3	68.0 ± 20.1	3.7 ± 2.8	6	45.3 ± 14.6	2.8 ± 4.5	6

Table V.

Ratios of emerged imago flies to laid eggs $\left(\frac{x_i}{x_e}\right)$ of the progeny raised on minimal medium containing increasing amounts of the pyrimidine analogue 6-azauracil.

Concentration of 6-azauracil	genotypes: *Oregon R*	*su(r)* ; +	+ ; *b*	*su(r)* ; *b*
0 mM	0.44	0.35	0.41	0.69
0.5 mM	0.41	n.d.	0.25	0.37
2 mM	0.01	n.d.	0.03	0.27
5 mM	0.01	0.04	0.05	0.06

n.d. not determined

cle colour (Figure 7). It should be pointed out that only few larvae can complete their development to an imago on food with the higher concentrations of 6-azauracil tested (Tables IV and V). Larvae homozygous for *su(r)* and *b* have a considerable higher survival rate on 6-azauracil containing media than the other genotypes studied.

4. DISCUSSION

The pathway of the biosynthesis of uracil and thymine as well as the pathway of the degradation of these two pyrimidines into β-alanine and into β-amino isobutyric acid is diagrammatised in Figure 1 which also lists the mutants in Drosophila melanogaster defective in specific steps of the pathway and the known effects of substrate analogues on wild type and mutant larvae.

The *rudimentary* locus controls the level of production of the first three enzymes in the biosynthesis of uracil (11, 18, 22): carbamoyl phosphate synthase EC 2.7.2.9, aspartate carbamoyl transferase EC 2.1.3.2 and dihydro-orotase EC 3.5.2.3. Dihydro-orotate dehydrogenase EC 1.3.3.1 is controlled by the *Dhod* chromosome segment of chromosome 3 (21), whereas the gene *rudimentary-like* on chromosome 3 controls the conversion of orotic acid to uridine monophosphate (3, 20). In the catabolic pathway from uracil to β-alanine the gene of the recessive suppressor of *rudimentary* has been shown to control the step converting uracil to dihydrouracil (1). The reduction in the degradation of uracil by the mutation *su(r)* normalizes the defect of the *rudimentary* mutations which cause a block in one or more of the first three steps in the biosynthesis of uracil. The *su(r)* mutation, however, does not lead to a black phenotype which indicates that β-alanine in this mutant is formed by the pathway from aspartate (8). The slightly darker cuticle colour observed in *su(r)* flies (Figure 5) indicates that a minor portion of the β-alanine preventing the melanization in the cuticle of wild type flies is derived through the degradation of uracil. The drastic enhancement of the black body colour in flies homozygous for the mutation *black* and the *su(r)* mutation (Figure 5) further supports the notion that the melanization in the *black* mutant is caused by a block in the formation of β-alanine from aspartate. The melanization can be intensified by adding to this block a reduction of β-alanine formation from uracil.

The mapping of the dominant suppressor of *black*, *Su(b)* revealed it to be located close to or within the rudimentary locus *r*. It should be em-

phasized here that homozygous *Su(b)/Su(b)* flies in the absence of *b* are wild type and not rudimentary. Furthermore, *r/r; b/b* flies are black with rudimentary wings. If *Su(b)* is a mutation inside the *r* locus, it must therefore be a mutation which does not decrease the activity of the three enzymes synthesizing dihydro-orotic acid. This is in agreement with the observation by L. SØNDERGAARD and E. BAHN (personal communication) that ethylmethane sulfonate induced *rudimentary* mutants in the *Su(b); b* strain are black. Thus the normalization of black flies by *Su(b)* requires active uracil synthesis and can be explained by an overproduction of uracil to provide for extra β-alanine via the catabolic pathway of uracil in order to compensate for the defect caused by the *b* mutation in the α-decarboxylation of aspartate. This readily explains the epistasis of *su(r)* over *Su(b)*: If uracil catabolism is blocked by *su(r)* through the elimination of the dihydrouracil dehydrogenase, an overproduction of uracil can no longer compensate for the absence of β-alanine formation from aspartate in the *black* mutant and the enhanced black cuticle colour (cf. Figure 2) characteristic for extreme β-alanine deficiency is observed. The *su(r) Su(b); b* flies have in addition to the enhanced black cuticle a dull red eye colour. This may be due to the accumulation of orotic acid and other intermediates in pyrimidine biosynthesis which could interfere with eye pigment synthesis. Eye mottling in the *rudimentary-like* flies has been interpreted along these lines (3, 20).

In the following it will be analyzed to what extent the effects of substrate analogues on cuticle melanization can be fitted into the metabolic model provided by the analysis of the mutants. In Drosophila, wild type and *black* flies decompose 1(^{14}C)-aspartic acid to the same extent (8). In Musca 56% of β-alanine is synthesized from uracil and 24% from aspartic acid (25), but these tracer experiments cannot decide whether the synthesis of β-alanine from aspartate occurs via uracil or directly by α-decarboxylation. Normal cuticular colouration is induced equally well by injection of β-alanine, uracil, dihydrouracil and N-carbamoyl-β-alanine into newly emerged adult Drosophila flies homozygous for the *black* gene (8). Thus endogenous regulation of the ana- and catabolic pathway of uracil should modify the availability of β-alanine and the cuticle tanning. 6-Azathymine inhibits dihydrouracil dehydrogenase (15, 19). This inhibitor causes in wild type flies partial phenocopies of the *black* mutant (Figures 3, 4) in agreement with the expectation that part of β-alanine for cuticle tanning is provided by degradation of uracil. The inhibitor has no effect on the normal cuticle colour in flies containing the *Su(b)* gene (Figure 4). As these flies are believed to overproduce uracil and thymine their resistance to 6-azathymine may be due to effective dilution of the inhibitor by the natural substrates. Unexpectedly, the thymine analogue has no enhancing effect on the melanization in the *black* mutant (Figure 3). The dihydrouracil dehydrogenase inhibitor thus cannot substitute for the *su(r)* mutation blocking the reaction catalyzed by this enzyme.

The uracil analogue 6-azauracil does not influence the degradation of uracil in mice (19) but may enhance its catabolic rate in the cocklebur plant (24). 6-Azauracil normalizes the *black* cuticle (Figure 6) which would be explainable by an increased catabolism of uracil to β-alanine. It has a small darkening effect on the cuticle colour of the wild type but no effect on the *su(r)* flies (Figure 7) nor on that of the *su(r); b* flies. The latter flies are however helped to a better survival by the compound. The normalization of *black* by 6-azauracil and the induction of melanization by 6-azathymine that is by compound effecting the same enzyme indicate complex effects of these analogues which cannot be interpreted without further detailed biochemical analyses. Finally it is known that 6-azauracil produces phenocopies of the *rudimentary-like* mutants (3, 27) and that this can be prevented by the presence of *su(r)*. It was mentioned above that the introduction of a *rudimentary* mutation in the *Su(b); b* strain results in a black cuticle. This can also be achieved by feeding the transition state analogue phosphonacetyl-L-aspartate (7) of aspartate carbamoyl transferase (L. SØNDERGAARD and E. BAHN, personal communication).

The interpretation that the semi-dominant suppressor of *black, Su(b)*, in Drosophila is a mutant of a regulator gene for the pyrimidine synthesis within or adjacent to the *rudimentary* locus points to similarities with the *PPR1* gene in the yeast Saccharomyces cerevisiae (13, 14). This

gene is not linked to the five genes coding for the enzymes involved in the synthesis of uridine monophosphate, but functions as a regulator in the de novo synthesis of pyrimidines. The semidominant *ppr1* mutation causes a constitutive overproduction of the dihydro-orotate dehydrogenase and the orotidine-5-phosphate decarboxylase coded for by the URA1 and URA3 genes, respectively (13, 14).

It will be of interest to determine if the *Su(b)* mutant gene in Drosophila causes an overproduction of carbamoyl phosphate synthase, aspartate carbamoyltransferase and dihydro-orotase and whether this suppressor gene is located within or adjacent to the *rudimentary* locus.

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