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The physiological control of gene action in the *eyeless* and *eyegone* mutants of *Drosophila melanogaster*

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SUMMARY

The effect of dietary supplements of individual L-amino acids on the expression of the *eyegone* and *eyeless*^{K} mutants of *Drosophila melanogaster* are compared. In both mutants, eye size is reduced by excess levels of tryptophan, phenylalanine and methionine, and in each case the effects are independent of metabolic competition for pyridoxal phosphate. A dietary interaction involving methionine and RNA can be demonstrated in the *ey*^{K} strain, but the mechanism of action of this amino acid is obscure. Tryptophan metabolism is examined in detail. Although both tryptamine and serotonin have significant effects, the action of tryptophan on eye development is largely independent of its metabolic products. Conversely, the effect of dietary supplements of certain catecholamines is consistent with the action of phenylalanine. The action of certain metabolic inhibitors provides additional support for the suggestion that the catecholamines have an important effect on morphogenesis in the eye imaginal disks. Eye development is also affected by increasing concentrations of γ-amino-butyric acid, and this, taken together with the effect of the catecholamines and indolalkylamines, suggests that physiological control of the action of the mutant genes on eye development involves a group of compounds characteristically associated with nervous tissue. Eye development in the *ey*^{K} strain may be influenced by the availability of acetyl CoA, which would be expected to affect acetylcholine biosynthesis. Possible mechanisms of action of the effective dietary treatments are discussed, together with a tentative hypothesis regarding the mode of action of the mutant genes on eye development.

1. INTRODUCTION

Genes of variable penetrance and expressivity offer a unique opportunity to study the physiological control of gene action. Two such mutants, the non-allelic eye mutants *eyeless* (*ey*) and *eyegone* (*eyg*) of *Drosophila melanogaster*, produce identical phenotypic effects on eye development, and for both, expression is dependent on larval nutrition. A comparison of the dietary interactions of these mutants in a standardized genotype, the Pacific wild, has shown that expression is extremely sensitive to dietary cholesterol concentration, and that both mutants are closely concordant in the timing of their developmental sensitivity to deficiency

levels of this metabolite (Hunt & Burnet, 1969; Hunt, 1969), although sensitivity for *eyegone* is fractionally retarded compared to *eyeless*.

A number of differences between the two mutants are also apparent, especially with regard to the action of a deficient thiamine diet on eye development, and a detailed examination of the possible interactions of this metabolite with gene expression will be presented. Previous experimentation has tested the effect of excess and deficiency levels of dietary casein and hence of total available amino acids. However, since the metabolic effects of excess levels of individual amino acids are largely restricted to certain pathways of biosynthesis, a study of their interaction with gene expression is particularly suited to this type of investigation. Accordingly, an extensive analysis of their individual effects on eye development will be described.

2. MATERIALS AND METHODS

In order to facilitate direct comparisons between the dietary interactions of the two mutants without the possible complication of modification by differences in genetic background, the *eyg* and ey^K mutants were transferred into the Pacific wild genotype as described previously (Hunt & Burnet, 1969; Hunt, 1969). The derived Pacific strains were maintained in mass culture on a yeast maize-meal agar food medium at 25 °C. Nutritional studies reported in this publication are restricted to these two strains.

All nutritional tests were carried out with germ-free larvae in aseptic culture at 25 °C according to the procedure developed by Sang (1956). The components of the chemically defined diet follow Sang's medium C except that sucrose was substituted for fructose. For each dietary treatment, eight to ten replicate cultures, each containing 5 ml of medium, were inoculated with 50 early first-instar larvae. Individual amino acids were added after neutralization to the complete synthetic diet (with casein) to a final concentration of 40 mM where possible. However, L-threonine, L-cystine and L-lysine proved toxic at this concentration and lower concentrations were therefore used. For a number of dietary treatments, only the effects of concentrations sufficient to reduce significantly the larval development rate yet still be compatible with reasonable larval survival are reported, although a range of concentrations were tested. In this way, non-specific changes in gene expression associated with extreme dietary stress can be excluded from the results. Heat-labile compounds were added in solution directly after autoclaving the food medium. Sterility was ensured by filtering through sterile Millipore filters (0·22 μ pore size) carried in a Swinny hypodermic adapter.

Gauged estimates of mean facet number are presented for Pacific ey^K. For a discussion of the application of this analysis to the quantization of gene expression, see Hunt & Burnet (1969). The extreme expression of the Pacific *eyg* strain precludes reliable gauged estimates of mean eye size and, as previously (Hunt, 1969), expression is measured as the frequency (percentage) of eyes in a population or treatment group. Frequency differences are tested against the distribution of χ^2 by means of 2×2 contingency tables (Woolf, 1951).

3. RESULTS

(i) *Carbohydrate metabolism*

In a comprehensive dietary survey Hunt & Burnet (1969) showed that thiamine deficiency causes a decrease in mutant gene expression. The effect of the thiamine antagonist neopyrithiamine (Table 1) parallels the effect of thiamine deficiency in reducing gene expression in the Pacific ey^K strain. Jacobs (1968*a*, *b*) has shown that β-alanine inhibits glucose utilization in *Drosophila*, but the failure of this amino acid to influence gene expression (Table 1) tends to focus attention on the thiamine-dependent steps in pyruvate and α-ketoglutarate catabolism.

Table 1. *The effect of dietary supplements of some compounds important in carbohydrate metabolism*

Supplement	Pacific ey^K	Pacific *eyg*
0·01 % neopyrithiamine	+93·7**	.
40 mM β-alanine	+16·6	.
1·0 % acetoin	−30·4	.
0·5 % α-ketoglutarate	+20·9	.
0·5 % lactate	−131·9**†	−15·9*
0·75 % malonate	−259·5**	.

For Pacific ey^K the results are expressed in terms of facet number and for Pacific *eyg* in terms of % eyes. Deviations are from the within-experiment control value.

*, ** Significance at the 5 % and 1 % probability levels respectively.

† Tested on a 'limiting thiamine' concentration (1·6 μg/replicate) and compared with a limiting thiamine' control value.

The developmental effects of dietary supplements of acetoin, α-ketoglutarate and lactate are given in Table 1. Acetoin and α-ketoglutarate are without effect on eye development while excess lactate, tested on a limiting thiamine diet (1·6 μg/replicate) in an attempt to reduce the conversion of pyruvate to acetyl CoA and hence allow for an accumulation of pyruvate and lactate as predicted for a thiamine deficiency (Park & Gubler, 1969), produces a highly significant reduction in eye size. It is unlikely therefore that the observed effects of a thiamine deficiency depend on an accumulation of any of these compounds. Administration of malonate, a specific inhibitor of succinate dehydrogenase, also leads to a significant reduction in eye size, and in this case the effect can be attributed to a reduction in the turnover of the tricarboxylic acid (TCA) cycle. The common factor in the thiamine, lactate and malonate dietary interactions may be a change in the availability of acetyl CoA. The increase in eye size observed with a deficiency of thiamine and with neopyrithiamine may be associated with the expected reduction in acetyl CoA biosynthesis while the reduction in eye size obtained with lactate may depend on increased synthesis and with malonate on reduced utilization.

Although eye development in Pacific *eyg* is insensitive to a thiamine deficiency (Hunt, 1969), the relatively more extreme dietary treatment of excess lactate effectively reduces eye size (Table 1). Clearly, gene expression in this strain is not

entirely independent of acetyl CoA biosynthesis but is considerably less sensitive than in Pacific ey^K.

(ii) *Amino acid metabolism*

Individual L-amino acids were tested as supplements to the normal synthetic diet. For Pacific ey^K, excess levels of L-methionine, L-histidine, L-threonine, L-tryptophan and L-phenylalanine produced highly significant reductions in eye size while smaller reductions were obtained with L-cystine and glycine. Only L-aspartate gave a significant increase in eye size (Table 2). In view of the potential utilization of aspartate and glutamate by the TCA cycle, comprehensive dose

Table 2. *The effect of dietary supplements of individual* L-*amino acids on mean facet number in the Pacific* ey^K *strain*

Supplement	Mean facet no.
Control (none)	293·5
40 mM L-alanine	−3·8
40 mM glycine	−37·2**
40 mM L-serine	−31·3
40 mM L-leucine	−0·4
40 mM L-isoleucine	+24·5
40 mM L-arginine	−3·4
40 mM L-methionine	−111·0**
40 mM L-valine	−37·7**
40 mM L-histidine	−104·6**
40 mM L-tryptophan	−100·6**
40 mM L-phenylalanine	−160·7**
40 mM L-tyrosine	−3·2
40 mM L-glutamic acid	+4·4
40 mM L-aspartic acid	+50·4*
30 mM L-threonine	−71·4**
30 mM L-cystine	−36·5*
25 mM L-lysine	−6·3

An average control value is presented and results are otherwise expressed as the deviation from the within-experiment control values.

*, ** Significance at the 5% and 1% probability levels respectively.

responses to both amino acids on a limiting thiamine concentration (1·6 μg/replicate) were examined. Maximal eye size is obtained with 10 mM aspartate, while in contrast, this concentration of glutamate is least satisfactory for eye development (Fig. 1). On a control thiamine diet (10 μg/replicate), the optimal concentration of aspartate for eye development is shifted to 20 mM, but the form of the response profile is otherwise unaltered. It would appear unlikely therefore that the effects of these amino acids on eye development depend on their utilization by the TCA cycle. The γ-aminobutyric acid (GABA) 'shunt' pathway also utilizes glutamate (Bradford, 1968) and, although this amino acid is virtually restricted to nervous tissue (Neame, 1968), a similar response to aspartate is obtained with increasing concentrations of GABA (Fig. 1).

Previous observations (Sang & Burnet, 1963) have pointed to a role for RNA metabolism in the expression of the *eyeless* phenotype and the complex nature of this

dietary interaction is demonstrated by the opposed effects of cytidylic and adenylic acid on eye development in Pacific ey^K (Fig. 2). Aspartate and glutamate are important precursors for purine and pyrimidine biosynthesis and it is significant therefore that the aspartate dose response relations show considerable modification in the presence of a deficient RNA diet (Fig. 1). A methionine-RNA interaction is revealed in Pacific ey^K by the virtual elimination of the detrimental effects of excess methionine by a deficient RNA diet (Fig. 3). Eye development is also sensitive to dietary supplements of L-ethionine but, contrary to methionine, a deficient RNA concentration is unable to reduce the severity of this amino acid.

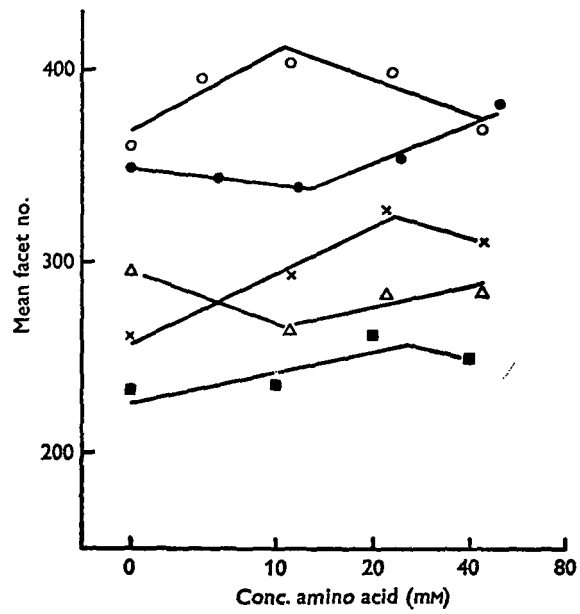

Fig. 1. Relation between gene expression and dietary L-amino acid concentration for the Pacific ey^K strain. ×, L-aspartate on control (10 μg/replicate) thiamine; ○, L-aspartate on 1·6 μg/replicate thiamine; △, L-aspartate on deficient (0·05 %) RNA; ●, L-glutamate on 1·6 μg/replicate thiamine; ■, GABA.

The effects of individual amino acid supplements on gene expression in the Pacific *eyg* strain are listed in Table 3. L-Methionine, L-tryptophan, L-phenylalanine, and L-lysine all reduce eye size, while only excess glycine produces a significant increase. In addition, a significant increase in the incidence of antennal duplications is obtained with tryptophan. Therefore, with the exception of lysine on Pacific *eyg* and histidine on Pacific ey^K, the extreme effects on eye development are restricted in both strains to excess levels of tryptophan, phenylalanine and methionine. For Pacific ey^K, a simple quantitative relationship exists between gene expression and increasing concentrations of the aromatic amino acids (Figs. 4, 5) and similar results are obtained for Pacific *eyg* (Fig. 6). Recent work by Sprince *et al.* (1969) in the rat has pointed to a competitive interaction between the pathways of tryptophan and methionine dissimilation for available pyridoxal phos-

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phate. This cofactor is also important for the decarboxylation of dopa and for tyrosine transamination. However, the failure of a twofold dietary excess of pyridoxine to modify the methionine, tryptophan and phenylalanine dose relations in Pacific ey^K (Figs. 3–5) argues against a role for this cofactor in these dietary interactions. Inhibition of gluconeogenesis by excess tryptophan has been demonstrated in the rat by Ray, Foster & Lardy (1966) and a similar interaction may

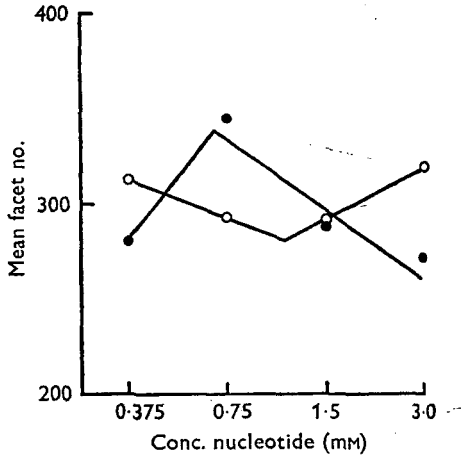

Fig. 2

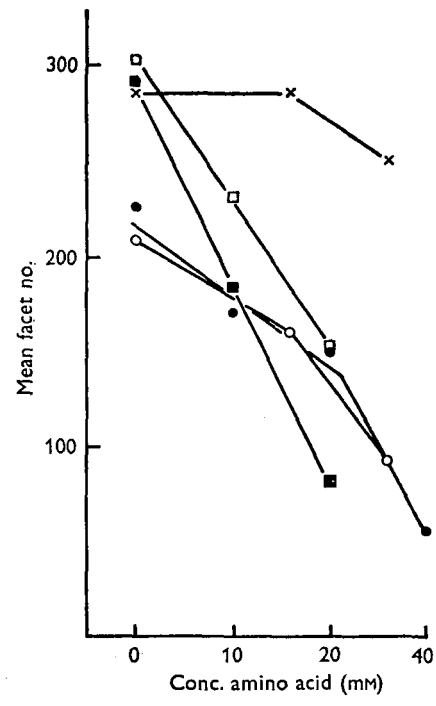

Fig. 3

Fig. 2. Relation between gene expression and dietary ribonucleotide concentration for the Pacific ey^K strain. ●, Adenylic acid on 1·5 mM cytidylic acid; ○, cytidylic acid on 3·0 mM adenylic acid.

Fig. 3. Relation between gene expression and dietary supplements of L-methionine on control (○), twofold excess (100 μg/replicate) pyridoxine (●), and deficient (0·05%) RNA (×), and supplements of L-ethionine on control (□) and deficient (0·05%) RNA (■) for the Pacific ey^K strain.

occur in *Drosophila*. In this case, the effect of excess tryptophan may depend on the accumulation of pyruvate, lactate and TCA cycle intermediates, with a correlated elevation in the synthesis of acetyl CoA. Repeat experiments testing tryptophan and phenylalanine on a limiting thiamine diet were carried out on Pacific ey^K (Figs. 4, 5) and although certain qualitative differences in the response profiles can be seen, the severity of the amino acid is certainly not reduced.

The synthesis of epinephrine requires the participation of 'active' methionine in the methylation of norepinephrine, and since both mutants are sensitive to excess dietary phenylalanine, the amino acid precursor to epinephrine (see Fig. 7), the primary effect of both amino acids may be an elevation in the synthesis of this phenylalkylamine. However, concentrations of 20 mM L-epinephrine are without effect on gene expression in Pacific ey^K (Table 4), although this result is open to the possible objection that oxidation of the supplement (darkening of the medium) occurs (Sang, 1969). In contrast, a highly significant reduction in eye size is obtained in both strains with 10 mM L-dopa, and a concentration of 20 mM L-norepinephrine likewise produces a reduction in eye size in Pacific ey^K, even though

Table 3. *The effect of dietary supplements of individual* L-*amino acids on eye size, measured as* % *eyes, and antennal duplication in the Pacific* eyg *strain*

Supplement	% eyes	Antennal duplications (%)
Control (none)	66·4	0·3
40 mM L-alanine	+9·4	0
40 mM glycine	+20·8**	+1·2
40 mM L-serine	+2·4	−0·5
40 mM L-leucine	+0·1	+1·0
40 mM L-arginine	−8·6	−0·5
40 mM L-methionine	−16·1*	0
40 mM L-valine	−3·3	+0·3
40 mM L-histidine	−6·0	0
40 mM L-tryptophan	−61·3**	+11·4**
40 mM L-phenylalanine	−22·8**	+2·5
40 mM L-glutamic acid	−2·2	−0·5
40 mM L-aspartic acid	−1·5	+2·4
30 mM L-threonine	+3·8	+0·4
30 mM L-cystine	+7·0	−0·5
25 mM L-lysine	−30·8**	+1·4

An average control value is presented and results are otherwise expressed as the deviation from the within-experiment control values.

*, ** Significance at the 5% and 1% probability levels respectively.

oxidation of the supplement was apparent in both cases. Considerable larval toxicity was encountered with dietary supplements of dopamine and the non-significant reduction obtained in Pacific ey^K with a concentration of 10 mM could not be confirmed with a higher concentration.

Additional support for the involvement of the catecholamine bio-synthetic pathway in eye development is provided by the action of certain metabolic inhibitors. α-Methyltryptophan, an inhibitor of dopa decarboxylase (Sourkes & D'Iorio, 1963), and sodium diethyldithiocarbamate, an inhibitor of tyrosine hydroxylase (Taylor, Stubbs & Ellenbogen, 1969) and dopamine-β-oxidase (Goldstein *et al.* 1964), both produce highly significant reductions in the expression of the mutant phenotype (Table 4). In contrast, administration of *p*-chlorophenylalanine, an inhibitor of phenylalanine and tryptophan hydroxylation (Koe & Weissman, 1966),

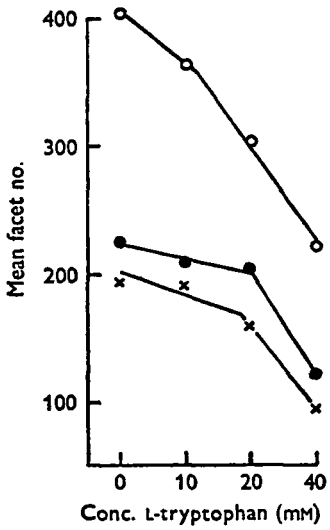

Fig. 4

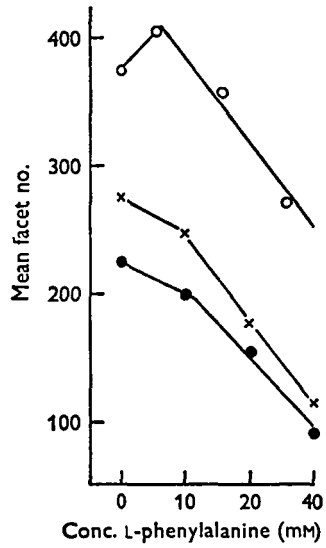

Fig. 5

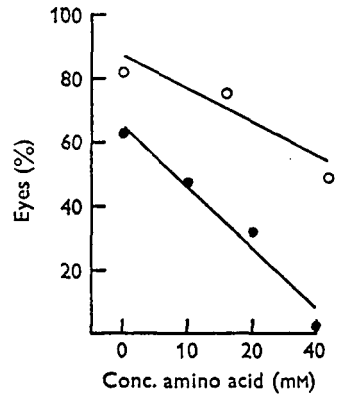

Fig. 6

Fig. 4. Relation between gene expression and dietary L-tryptophan concentration for the Pacific ey^K strain. ●, Control medium; ○, 1·6 μg/replicate thiamine; ×, twofold excess (100 μg/replicate) pyridoxine.

Fig. 5. Relation between gene expression and dietary L-phenylalanine concentration for the Pacific ey^K strain. ×, Control medium; ○, 1·6 μg/replicate thiamine; ●, twofold excess (100 μg/replicate) pyridoxine.

Fig. 6. Relation between gene expression and dietary L-tryptophan (●) and L-phenylalanine (○) concentration for the Pacific *eyg* strain.

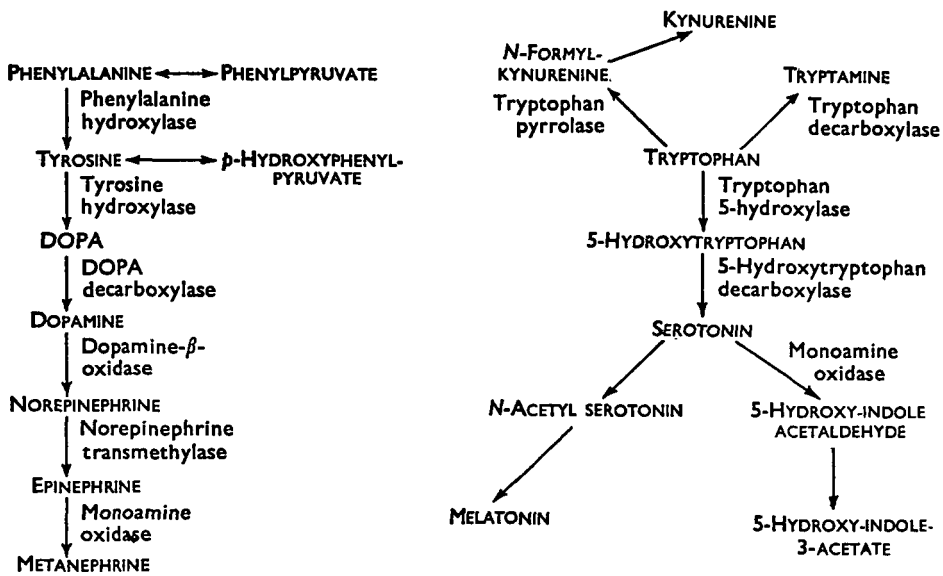

Fig. 7. Pathways of phenylalanine and tryptophan metabolism.

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leads to a significant reduction in eye size in both strains (Table 5). In the rat, this inhibitor produces a greater reduction in serotonin (see Fig. 7) than in dopamine or norepinephrine biosynthesis (De Schaepdryver, Preziosi & Scapagnini, 1969) and its effect on eye development may also depend on a reduction in tryptophan metabolism.

Table 4. *The effect of dietary supplements of* L-*dopa, the catecholamines and inhibitors of catecholamine biosynthesis on eye size in the Pacific* ey^K *and* eyg *strains*

Supplement	Pacific *ey*^{K}, mean facet no.	Pacific *eyg*, % eyes
20 mM L-epinephrine	−13·3	.
20 mM L-norepinephrine	−46·7*	.
10 mM dopamine	−42·0	.
10 mM L-dopa	−76·3**	−54·4**
40 mM DL-α-methyltryptophan	+101·2**	.
10 mM sodium diethyldithiocarbamate	+58·2**	.

Results are expressed as the deviation from the within-experiment control values.
*, ** Significance at the 5% and 1% probability levels respectively.

Table 5. *The effect of a number of metabolites and enzyme inhibitors on eye size in the Pacific* ey^K *and* eyg *strains*

Metabolite	Pacific *ey*^{K}, mean facet no.	Pacific *eyg*, % eyes
30 mM *p*-chloro-DL-phenylalanine	−56·4**	−49·0**
25 mM L-kynurenine	+5·7	.
0·05% allopurinol	+15·7	.
20 mM serotonin + 0·16% tranylcypromine	+66·3*	.
20 mM L-tryptamine	+65·4**	.
30 mM melatonin	+19·6	.
30 mM 5-hydroxyindole-3-acetate	+18·4	.
40 mM L-anthranilic acid	−103·9**	.
30 mM urocanic acid	−27·0	.
40 mM histamine	+52·3**	.

Results are expressed as the deviation from the within-experiment control value.
*, ** Significance at the 5% and 1% probability levels respectively.

As noted previously, both strains are extremely sensitive to excess levels of dietary tryptophan (Tables 2, 3). A dietary supplement of anthranilic acid, a non-essential amino acid closely related metabolically to tryptophan, also produces a highly significant reduction in eye size in Pacific *ey*^{K} (Table 5). Since *Drosophila* lacks the capability for *de novo* synthesis of niacinamide, the major intermediates in tryptophan metabolism are serotonin, kynurenine and tryptamine (see Fig. 7). The effect of kynurenine on eye development in Pacific *ey*^{K} was tested by the direct administration of the L-isomer and by feeding the tryptophan pyrrolase inhibitor allopurinol (Becking & Johnson, 1967). In neither test was a significant change in mean eye size obtained (Table 5). To examine the effect of serotonin on gene expression, use was made of the monoamine oxidase (MAO)

inhibitor tranylcypromine. In Pacific *eyg*, increasing concentrations of this inhibitor together with either 20 or 30 mM tryptophan resulted in a reduction in eye size below that achieved by the excess tryptophan alone (Fig. 8*a*), indicating that at least part of the tryptophan effect in this strain may depend on an elevated synthesis of this indolalkylamine. In direct contrast, similar experiments with Pacific ey^K led to an increase in eye size with increasing concentrations of inhibitor (Fig. 8*b*). This result is confirmed by dietary supplements of serotonin itself if immediate degradation by MAO is prevented by tranylcypromine (Table 5), whereas the products of serotonin metabolism, melatonin and 5-hydroxy-indole-3-acetate are without effect on eye development (Table 5). Finally, a reduction in the expression of the mutant phenotype is obtained with dietary supplements of tryptamine.

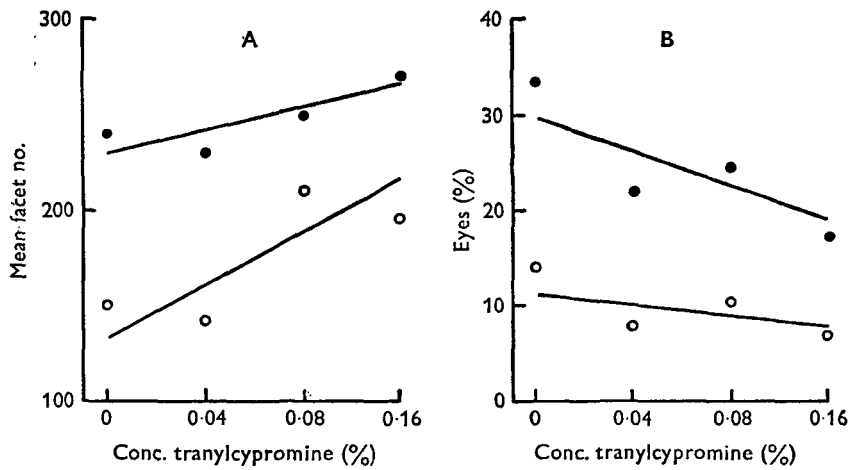

Fig. 8. Relation between gene expression and tranylcypromine concentration with 20 mM (●) and 30 mM (○) L-tryptophan. A, Pacific ey^K; B, Pacific *eyg*.

Histidine produces a comparable reduction in eye size in Pacific ey^K to tryptophan (Table 2). However, it is difficult to account for this dietary interaction since urocanic acid, the deamination product of histidine metabolism, fails to give a significant treatment effect, and histamine, the decarboxylation product, produces a highly significant increase in eye size (Table 5).

4. DISCUSSION

Although dietary interactions allow inferences to be made about the physiological control of gene expression, they are unlikely to yield definitive information about the underlying block in biosynthesis, and extreme caution is warranted in the interpretation of such data. For example, although the expression of the *eyeless* phenotype is extremely sensitive to dietary thiamine concentration, a primary gene effect of a metabolic block in the TCA cycle or in any of the other enzymic steps utilizing thiamine pyrophosphate as cofactor would be expected to have more

extensive effects than a reduction in eye size. Furthermore, although it is possible to relate the developmental effects of a number of the effective dietary treatments to certain pathways of biosynthesis, the mechanism of interaction is not entirely apparent. Clearly, changes in the basic composition of the synthetic diet must alter the general physiology of the organism and may thereby influence eye development. A more direct interaction with the processes of developmental control is also possible and the effects of certain of the non-essential metabolites amd metabolic inhibitors may depend on tissue specific changes in biosynthesis.

The results of dietary supplements of neopyrithiamine, lactate, and malonate on Pacific ey^K are consistent with an important role for acetyl CoA biosynthesis in eye development, a reduced availability leading to a reduction in the expression of the mutant gene, presumably by a limiting effect on another pathway of biosynthesis. Both aspartate and glutamate are potential precursors for the TCA cycle, but contrary to expectation, neither produces a significant reduction in eye size when fed in excess (Table 2), although mutant eye development is clearly sensitive to elevated levels of both amino acids over a wide dose range (Fig. 1). Aspartate and glutamate are also important precursors in the synthesis of the purine and pyrimidine ribonucleotides and it is significant therefore that a deficient RNA diet completely alters the response of Pacific ey^K to aspartate concentration.

The effect of valine, threonine and cystine on Pacific ey^K and of lysine on Pacific *eyg* is difficult to explain, although threonine and cystine are sources of lactate and pyruvate respectively, both of which would be expected to reduce eye size through an effect on the availability of acetyl CoA. Alternatively, the excess levels of these amino acids may influence eye development by altering the relative composition of the amino acid pool, and hence indirectly gene expression, by limiting the availability of other important metabolites. The effect of histidine on Pacific ey^K may involve a similar mechanism.

A highly significant reduction in eye size is obtained in both strains with excess concentrations of tryptophan, phenylalanine and methionine, and repeat experiments testing each amino acid on a twofold excess of pyridoxine, an important cofactor in the metabolism of all three amino acids, failed to reduce the severity of the dietary interactions. Tryptophan, phenylalanine and methionine have been implicated in the physiological control of melanotic tumour formation in *Drosophila* (Burnet & Sang, 1968), but in this case methionine acts to reduce the tumorigenic effects of the aromatic amino acids and is without effect when administered by itself. *De novo* synthesis of thymine ribonucleotides requires the participation of 'active' methionine for the methylation of deoxyuridylic acid to thymidylic acid and it is significant therefore that a RNA-methionine interaction can be demonstrated for Pacific ey^K. Earlier observations that a deficient RNA diet leads to an increase in eye size (Sang & Burnet, 1963; Hunt & Burnet, 1969) may be explained in terms of an increased demand for methionine in *de novo* ribonucleotide biosynthesis. With a normal concentration of dietary RNA, less methionine is utilized for this purpose and it is the final concentration of this amino acid that is the important component in the RNA dietary interaction.

Ethionine, a potent inhibitor of protein synthesis, produces a reduction in eye size in Pacific ey^K considerably in excess of equivalent concentrations of methionine, but in contrast to methionine the action of this amino acid is independent of dietary RNA concentration. Marzluf (1969) reports a similar reduction in eye size with supplements of ethionine to normal yeasted culture although no differences could be detected in the methionine metabolism of wild type and ey^2 larvae. Likewise, the present investigation offers no clue to the mechanism of action of these amino acids.

The reductions in eye size obtained in both strains with dietary supplements of phenylalanine and dopa suggest an important role for catecholamine biosynthesis in eye development. This is supported in Pacific ey^K by the action of norepinephrine and the inhibitors of catecholamine biosynthesis, sodium diethyldithiocarbamate (Goldstein *et al.* 1964) and α-methyltryptophan (Sourkes & D'Iorio, 1963). The absence of a significant tyrosine interaction is clearly at variance with this interpretation, but it is doubtful, in view of the insolubility of this amino acid in the synthetic medium, whether the developing larvae can receive an effective concentration.

Comprehensive tests with the products of tryptophan metabolism fail to provide a satisfactory explanation for the observed dietary effects of this amino acid on gene expression, although the opposed responses of Pacific *eyg* and Pacific ey^K to tranylcypromine imply that the action of serotonin differs in the two mutants. The extreme reduction in eye size obtained with excess tryptophan may depend on a direct interaction of this amino acid with the biosynthetic pathways important in the physiological control of eye development.

The amine derivatives of tryptophan metabolism, serotonin and tryptamine are consistent in producing a significant increase in eye size in Pacific ey^K, and a similar result was obtained with dietary supplements of histamine. These amines are effective *in vitro* inhibitors of dopamine-β-oxidase (Goldstein & Contrera, 1962) and may also reduce norepinephrine synthesis *in vivo*. In view of the opposed effects of tranylcypromine on gene expression in the two strains, such an interaction is unlikely for serotonin, but it may account for the developmental effects of tryptamine and histamine.

A recent developmental study of the ey^D mutant by Arking (1969) has pointed to a defective release of brain hormone and resulting hormonal imbalance in mutant individuals. The pleiotropic effects of this mutant include a high mortality at pupation and just prior to adult eclosion, abnormal development of the head and gonads, and the failure of the larval salivary glands to histolyse during the pupal instar. These effects may be accounted for in terms of a reduced ecdysone titre. Where examined, the mutant effects of *eyeless* and *eyegone* closely follow ey^D. Both mutants act as conditional semi-lethal factors with a lethal crisis towards the end of the pupal period (D. M. Hunt, unpublished data); both have manifest effects on cephalogenesis which are considerably exaggerated in the double mutant homozygote, also a synthetic lethal (Hunt, 1970); and both mutants are extremely sensitive to deficiency concentrations of dietary cholesterol (Hunt & Burnet, 1969;

Hunt, 1969), the sterol precursor to ecdysone. Gene expression in both strains is dependent on catecholamine biosynthesis, and eye development in Pacific ey^K is also sensitive to changes in the level of acetyl CoA. In addition, dietary supplements of GABA and serotonin effectively alter gene expression, and these results, together with those for the catecholamines, indicate that the regulation of eye development involves metabolites characteristic of nervous tissue. Furthermore, the effect of acetyl CoA may depend on changes in the synthetic capacity for acetylcholine – another compound with important nervous function.

In addition to the above considerations, the striking bilateral asymmetry in the development of the eye disks in these mutants must be taken into account and this indicates an extremely localized breakdown in developmental regulation. Such a mechanism could involve the direct passage of a neurosecretion to each eye disk, and evidence in support of this interpretation is provided by observations on the *in vitro* culture of eye disks (Fugio, 1962; Schneider, 1964), where normal growth and differentiation may depend on the continued attachment of the eye disks to the brain hemispheres. The release of this neurohumor, together with other neurosecretions including brain hormone, may be regulated by changes in the relative concentrations of the biogenic amines, and especially of norepinephrine. Whether mutation at the *eyeless* or the *eyegone* loci results in deficient synthesis or release of brain secretions is open to conjecture, but such an interpretation would certainly account for the semi-lethality of these mutants in terms of a reduced ecdysone titre. In effect therefore, the two mutants would involve essentially identical mechanisms, with the differences in dietary sensitivity to a number of amino acids and especially to thiamine concentration reflecting relatively minor discrepancies either in the time course of mutant gene action or in the step in biosynthesis primarily affected.

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