ENVIRONMENTAL MODIFICATION OF THE EYELESS PHENOTYPE IN *DROSOPHILA MELANOGASTER*

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IN his studies of the eyeless mutation of *Drosophila melanogaster*, MORGAN (1929) showed that penetrance and expressivity of this gene was dependent upon the environment, since the frequency and extent of the defect was different for flies hatching early or late during the life of a culture. He also demonstrated that both aspects of gene manifestation could be altered by selection. These consequences of selection have been confirmed and extended (SPOFFORD 1954), but no detailed work has been done to find which components of environment alter the exhibition of the gene. This is the first concern of this communication.

Underlying some of the work on the biochemical genetics of multicellular organisms, particularly man (see HARRIS 1959), is the expectation that a genetic defect may be rectified by environmental manipulation, either by preventing the accumulation of a substrate not properly utilized as a consequence of an enzymic block, as in phenylketonuria, or by providing the missing product of the gene-controlled reaction, as in diabetes. This expectation has received powerful support from work with microorganisms, because mutation screening techniques depend on just the latter corrective procedure. However, it does not follow that genes not so isolated will respond in the same way, or that the situation is as simple in higher organisms as the microbial model might imply. Examination of the reactions of eyeless may therefore provide a better indication of the limitations on, and possibilities for, amelioration of genetic defects in multicellular organisms.

Two classes of reaction might be anticipated: (1) a specific response when the missing gene product is supplied, resulting in 100 percent normal development of the eyes or (2) generalised responses when nonspecific agencies encourage (or discourage) the manifestation of the gene by interfering with competing or related processes. The kind of metabolite involved in (1) cannot be anticipated since we lack the necessary biochemical information concerning eye development, but a knowledge of (2) might suggest lines of approach to this question. For this and other reasons, particular attention has been paid to nutritional manipulations in the experiments described below.

MATERIALS AND METHODS

An Oregon-K strain of *Drosophila melanogaster*, which had been brother-sister mated for over 300 generations, was used in the experiments which follow. The strain carried an allele of eyeless, and is here designated as *ey^K* (SANG and

McDONALD 1954), although it also has a second chromosome recessive of low penetrance which results in the appearance of melanotic tumors in some treatment conditions (SANG and BURNET 1963). All the nutrition tests were carried out germ-free on defined media, using the techniques described by SANG (1956). Five ml of the food medium was used in each 6×1 inch culture tube. Six to eight replicates, each containing 40 larvae, were examined at each nutrient concentration. Infected cultures were discarded.

Changes of eye size were measured by grading, the grades being similar to those illustrated by SANG and McDONALD (1954) and SANG (1961). The two sides of the head were classed separately, since there is a low correlation between sides, and the sexes were also separately scored because there is a regular difference between them (Figure 11). The grading was done by one of us (B.B.) to ensure consistency of classification. Eye areas without facets were graded as zero, and apparently normal eyes as 5, with four intermediate grades. The grading system therefore set upper and lower limits, or thresholds, to each class.

On the assumption of an underlying normal distribution of eye size in the population, it is possible to determine the thresholds using the inverse probability transformation (see GREEN 1954 and SANG 1964 for details). Since the majority of eye sizes fall in Grade 2 or below, the difference between t_1 , the threshold separating completely eyeless and Grade 1, and t_3 , the threshold separating Grade 2 from Grade 3, was taken as the unit of scale. Consequently, negative mean values are found when the majority of eyes in a population are in Grade 0. The mean (μ) and standard deviation (σ) are calculated from the formulae

$$\mu = (-\text{probit } p_1)/(\text{probit } p_3 - \text{probit } p_1), \text{ and}$$

$$\sigma = 1/(\text{probit } p_3 - \text{probit } p_1),$$

where probit p_1 and probit p_3 are the probit values (FISHER and YATES 1953), minus the 5 added in the tabulated values, for the percentage of the population falling below t_1 and below t_3 , respectively. These values, and all tests, have been calculated separately for the two sexes, but since the difference between them is constant only the average is shown in the tables.

Larval development time was measured in log days, and dosages are plotted on a logarithmic scale, for reasons already given (SANG 1956).

RESULTS

The phenotypic effects of ey^K . Adult individuals from the ey^K strain show varying degrees of reduction of the faceted area of the compound eye, which may be asymmetrical with respect to the two sides of the head. In live yeast cultures, variation in eye size extends from complete absence of the eye to one which is similar to the wild type in size. Associated with eye reduction are irregularities in the orbital hypodermis and abnormalities of the number and distribution of chaetae in the frontal and ocellar regions. Absence of the eye on one side frequently distorts the position of the ocelli and of the antenna of that side; in addition the number of ocelli may also be affected.

FIGURE 1.—(A) Wild-type compound eye and associated optic glomeruli. (a) ommatidia, (b) outer optic glomerulus or perioptic, (c) external optic chiasma, (d) middle glomerulus or epipticon, (e) internal chiasma, (f) anterior and posterior inner glomerulus or opticon, (g) antennal glomerulus. (B) *ey^K* showing reduction in eye size, and distortion of the perioptic. (C) *ey^K* showing low grade eye size, and severe abnormalities of the perioptic. The eye disc has failed to make nerve fibre connections with the optic glomeruli of the brain which are hypoplastic. The epipticon and opticon are fused together. (D) Total absence of the eye and perioptic, with extreme reduction of the epipticon. (Chlorazol black)

The decrease in number of ommatidia in the eye is associated with hypoplasia of the adjacent optic glomeruli due to reduction in the number of centripetal axons which normally form the greater proportion of their volume (POWER 1943). Figure 1 shows in frontal section the brain of a wild-type individual compared with the eyeless condition. Absence of the eye results in absence of the external optic glomerulus and reduction in size of the middle and inner optic glomeruli which tend to fuse together. POWER (1946) has shown that these effects on the optic glomeruli are also associated with hypoplasia of the antennal glomerulus of the same side.

FIGURE 2.—Head of an eyeless fly with a duplicated antenna on the right side and small supernumerary antenna above the left orbit. Note the distortion of the vertex and occiput. Left, ventral aspect and right, dorsal aspect.

In addition to reduction in eye size, supernumerary antennae occur in a proportion of flies. These are usually mirror image duplications of one or both antennae and may be separate or united by a common base (Figure 2). In rare instances, however, miniature antennae or palpi may also occur which have no fixed orientation with respect to the normal antenna of that side, and are never united with it. Such a structure is shown in Figure 2, in addition to the antennal duplication on the opposite side. Unlike the duplicate antennae these miniature antennae are often structurally incomplete and appear to have a separate origin from the duplicate type, possibly by abnormal development from part of the eye disc.

The occurrence of antennal duplication is very highly correlated with absence of the eye (Grade 0). Table 1 shows that of 561 cases of antennal duplication 96.8 percent were associated with absence of the eye on the same side of the head. These observations suggest that the *ey^K* gene affects the organisation of the eye and the antenna at an early stage before the separation of individual eye and antenna discs, and are in agreement with the conclusion of VOGT (1947) that reduction of the eye is a favourable condition for antenna duplication, though not in itself the necessary direct cause.

The effect of temperature and larval density: MORGAN (1929) and CHEN (1929) observed that adults of an inbred eyeless strain hatching from live-yeast cultures on successive days had different mean eye sizes. Exhibition of the eyeless phenotype became less extreme with ageing of the culture. This may be due to

TABLE 1

Association between duplicate antennae and eye grade of the same side

	Eye grade						Total
	0	1	2	3	4	5	
Number of flies with duplicate antennae	543	10	2	4	2	0	561

differences in the amount of food available to the larvae, to qualitative changes in the yeast population, or to interactions between larvae due to crowding (cf. SANG 1950), or, of course, to a multiplicity of these causes. The situation was examined by culturing larvae under varying degrees of crowding on aseptic media at two temperatures, 25°C which is the optimum for larval growth, and 18°C which more than doubles the time spent in the larval stadia (Figures 3 and 4).

In the 25°C series, mean eye size and antennal duplication remain at a constant level over the range of crowding tested, but there is a well defined growth rate optimum at 40 larvae per culture, indicating that at this temperature penetrance and expressivity of the eyeless gene are likely to be influenced only by the qualitative composition of the food medium. At 18°C, larval development time remains constant below 160 larvae per culture. Eye size is much lower than at 25°C and there is a significant increase in the frequency of duplicate antennae. There is also a progressive rise of both mean eye size and frequency of duplicate antennae with larval density, which suggests that larval interactions influence both of these phenes at the lower temperature. All further experiments were carried out at 25°C at 40 larvae per culture in order to exclude this source of variability.

The effect of temperature on the *ey^K* strain is the reverse of that observed by BARON (1935) for *ey^s*, for which eye size at 18°C is greater than at 25°C. However, if SCHARLOO's (1961) observations have general validity, little can be

FIGURE 3.—Effect of temperature and larval density on eye size and on the frequency of duplicated antennae. Small solid circles, mean eye size and large solid circles, duplicate antennae at 25°C. Small hollow circles, mean eye size, and large hollow circles, duplicate antennae at 18°C. Control cultures contain 40 larvae each.

FIGURE 4.—Effect of temperature and larval density on the rate of larval development. Solid circles 25°C, and hollow circles 18°C.

deduced from this contradiction since mutant strains may be selected for the direction of the phenotypic response to temperature, which presumably reflects differences in the effects of the residual genotype rather than of the mutant locus concerned. Consequently, different eyeless strains may be expected to have characteristic temperature responses, according to differences in their respective genetic backgrounds.

Specific nutritional manipulations: In the control medium each vitamin is supplied at a concentration about ten times greater than the minimum level required for optimum larval growth rate, since vitamin excesses have no adverse effect on growth (SANG 1956), so that the possibility of an increased vitamin requirement in the *ey^K* strain is already covered by the control supply. The effect of individual vitamin deficiencies was tested by maintaining all vitamins at the control level with the exception of the one to be tested, which was reduced to the level shown in Table 2. Each deficiency causes a lengthening of the larval period compared with control. Folic acid deficiency also causes failure of eclosion of the adult from the pupal case necessitating an assessment of eye size from such unhatched pupae as were fully differentiated. The major nutrients in the synthetic medium have adverse effects on growth rate both as deficiencies and when supplied in excess (SANG 1956), and the tests of these were carried out at both deficient and excess levels (Table 2). The results for cholesterol require special mention. In media used to test the effect of cholesterol deficiency, lecithin is

replaced by choline to exclude extraneous cholesterol which is a contaminant of commercial preparations of lecithin. Consequently the results must be compared with a separate control using a choline containing medium (figures in brackets).

Of the vitamins, folic acid and biotin deficiencies bring about a significant reduction in eye size, but the effect is greatest in the case of biotin deficiency, which also causes a dramatic increase in the frequency of antennal duplication. This result might imply that the concentration of these vitamins in the control, although high, is not high enough, and that higher concentrations might be attended by an increase of eye size. However, full dose-response curves (unpublished data) show that even very high levels (up to 100 μg per tube) do not raise mean eye size above the control level. Excess dietary levels of lecithin and sucrose cause significant changes in mean eye size compared with the control value, but casein and RNA alone show convincing evidence of a systematic change in eye size with increasing concentration, and they are examined in greater detail below.

We have shown above that the mean eye size of flies cultured at 18°C is much lower than at 25°C and that the rate of development is greatly increased at the lower temperature, suggesting that there may be some direct relationship between the rate of development and the final eye size attained. No systematic correlation between larval development time and mean eye size is evident from the data in Table 2, however, which excludes the possibility of any simple relationship of this kind. We shall see further confirmation of this below.

TABLE 2

The effect of vitamin deficiencies, and of deficiency and excess of the major nutrients of the synthetic medium

	<i>t</i>	μ	σ	P
Control	0.72	0.240	0.302	4.1
Thiamine 0.4 μg .	0.80	0.259	0.280	3.15
Biotin nil	1.22	—0.08	0.333	32.6
Folic nil	no adults	0.190	0.352	4.55
Niacin 2.5 μg	0.95	0.250	0.405	10.2
Pantothenate 3.0 μg	0.89	0.242	0.325	4.12
Pyridoxine 0.25 μg	0.81	0.212	0.310	4.12
Riboflavin 1.5 μg	0.83	0.237	0.340	11.4
RNA nil	0.93	0.313	0.310	6.0
3.2 percent	0.79	0.072	0.386	3.87
Casein 2.5 percent	0.90	0.144	0.418	7.4
10 percent	0.74	0.327	0.473	3.8
Sucrose nil	0.80	0.294	0.271	3.78
8.0 percent	0.80	0.312	0.250	6.25
Lecithin 0.0125 percent	0.88	0.245	0.287	11.9
0.6 percent	0.78	0.175	0.317	4.2
Cholesterol 0.00156 percent	0.88	0.186	0.343	2.06
(0.05 percent)	(0.86)	(0.207)	(0.318)	(6.90)
0.20 percent	0.87	0.182	0.396	12.7

t, mean larval development time in log days; μ and σ , mean and standard deviation of eye size on the assumed scale (see MATERIALS and METHODS); P, percentage of individuals with antenna duplication.

Biotin deficiency causes a large increase in the frequency of duplicate antennae in addition to reducing the mean eye size. However, both niacin and riboflavin deficiencies, lecithin deficiency and excess cholesterol also cause a significant rise in the frequency of antennal duplications, although these treatments have no effect on eye size, suggesting that the processes which lead to the destabilisation of antenna development differ from those which affect development of the eye. Nevertheless, the association between antenna duplication and the most extreme expression of *ey^K* (eye Grade 0) shows that the two are not completely unconnected (Table 1), and the result for biotin deficiency points to a key role of some biotin mediated process during the differentiation of the eye-antenna complex.

Casein: Figure 5 shows the relationship between mean eye size and the concentration of dietary protein. There is a steady increase of eye size with casein concentration that is not correlated with development rate, which is at its optimum when 5.5 percent casein is provided. This suggests that development of the mutant eye disc to normal size may involve a higher requirement for a particular amino acid than is necessary for larval growth. Supplementation of the control 5.5 percent casein diet with individual amino acids may help to clarify this possibility.

Nucleic acids: *Drosophila* larvae are able to synthesize ribonucleic acid (RNA) to a limited extent from simple precursors, but a dietary supplement of 0.4 percent is required for the maximum rate of growth to be achieved. Figure 6 shows

FIGURE 5.—Relation between casein concentration and mean eye size. Control cultures used in Table 2 contain 5.5 percent casein.

FIGURE 6.—Relation between dietary nucleic acid concentration and mean eye size. Solid circles, ribonucleic acid in the absence of DNA, and hollow circles, deoxyribonucleic acid in the absence of RNA. Control cultures contain 0.4 percent RNA but no DNA.

that excess dietary RNA intensifies the expressivity of the ey^K gene, but lower concentrations lead to a decrease in exhibition with eye size reaching a maximum at 0.1 percent, a quarter of the amount required for the optimal rate of larval growth. Below this level, however, there is a further decrease in mean eye size. Larval development time at 0.1 percent RNA is 0.82 log days compared with 0.74 log days at the control 0.4 percent RNA level. Thus conditions which are most favourable for development of the mutant eye discs are different from those which permit the optimal rate of development of the organism as a whole, and we shall examine this distinction in greater detail below.

We have shown elsewhere (BURNET and SANG 1963) that *Drosophila* larvae may utilize deoxyribonucleic acid (DNA) to some extent, possibly by a sparing effect on the requirement for RNA. Figure 6 shows that the response to DNA is similar in form to that for RNA, but maximum eye size is obtained at a concentration four to eight times greater than that for RNA. We found (*loc. cit.*) that approximately the same proportional relationship between the two nucleic acids applies with respect to penetrance of the melanotic tumor gene tu^K , also present

in this strain. The agreement between the two mutants in this respect is striking, but its significance is still obscure.

Pentose nucleotide interactions: *Drosophila* larvae do not utilize the whole RNA molecule provided in the diet directly but as a source of pentose nucleotides. SANG (1957) has shown that the dietary growth requirement for RNA is completely satisfied by providing the nucleosides or nucleotides of adenine and cytosine in the diet, and SANG and BURNET (1963) have also shown that there is an interaction between adenylic and cytidylic acid with respect to the penetrance of the *tu^K* gene in this strain. The correlation between the concentration of dietary RNA and the expressivity of *ey^K* (Figure 6) may therefore in reality involve only part of the whole RNA molecule.

The effect of adenylic acid was examined as a dose response (Figure 7) on media from which RNA is omitted. When the medium is supplemented with 0.186 percent cytidylic acid, the response in mean eye size is similar in form to that for RNA (Figure 6) giving an inflexion at 0.025 percent adenylic acid. It can be seen from Figure 8 that, as in the case of the RNA response described above, there is a discrepancy between the conditions most favourable for growth of the mutant eye disc (0.025 percent) and for the optimal rate of larval development (0.10 percent). Comparing these two optima with those obtained from the

FIGURE 7.—Relation between purine ribonucleotide concentration and mean eye size. Small solid circles, adenylic acid with 0.186 percent cytidylic acid in the medium; hollow circles, adenylic acid in the absence of cytidylic acid. Large solid circles, guanylic acid with 0.023 percent cytidylic acid. The guanylic acid was supplied at equimolar concentrations to those on the adenylic acid scale.

FIGURE 8.—Relation between purine ribonucleotide concentration and the rate of larval development. Explanation of points in Figure 7.

RNA response (0.1 percent for mean eye size, 0.4 percent for larval development time), and assuming that adenylic acid forms a quarter of the RNA molecule, we obtain a close numerical correspondence to what would be expected if the RNA response in Figure 6 were due to the effect of adenylic acid alone. When cytidylic acid is omitted from the medium, however, (Figure 7) maximum eye size is given at 0.025 percent adenylic acid and there is no longer a marked over-dose effect. Eye size remains high throughout the range tested, suggesting that cytidylic acid increases the expressivity of the *ey^K* gene.

The effect of cytidylic acid is shown in greater detail in Figure 9. On media supplemented with 0.1 percent adenylic acid, increasing concentration of cytidylic acid causes a decline in eye size which levels off beyond 0.046 percent, the optimum concentration for larval growth rate (Figure 10). On 0.025 percent adenylic acid the adverse effect of cytidylic acid on eye size is no longer so apparent (Figure 9) and there is a well defined maximum at approximately 0.023 percent, but under these conditions the optimum for larval growth rate is 0.0116 percent (Figure 10).

Guanylic acid, tested on media containing 0.023 percent cytidylic acid (Figure 7) causes a steady increase in the expressivity of *ey^K* with increasing concentration, whereas uridylic acid tested on 0.025 percent adenylic acid is essentially similar in effect to cytidylic acid under the same conditions (Figure 9). These observations are in accord with those of SANG (1957), namely, that uridylic and

FIGURE 9.—Relation between pyrimidine ribonucleotide concentration and mean eye size. Large solid circles, cytidylic acid with 0.1 percent adenylic acid in the medium. Small solid circles, cytidylic acid with 0.025 percent adenylic acid in the medium. Hollow circles, uridylic acid with 0.025 percent adenylic acid in the medium. The uridylic acid was supplied at equimolar concentrations to those on the cytidylic acid scale.

FIGURE 10.—Relation between pyrimidine ribonucleotide concentration and the rate of larval development. Explanation of points in Figure 9.

cytidylic acids are, by transamination, freely interconvertible, whereas larvae cannot utilize guanylic in place of adenylic acid.

Acid amides: KAJI (1954, 1960) has found that inclusion of certain acid amides in the larval diet (live yeast) causes an increase in the number of facets in the compound eye of the Bar mutant. Recently FUJIO (1960) claimed that acetamide improves the growth of eye-antenna discs of both Bar and ey^2 mutants in tissue culture. Dose-response curves over a wide range of concentrations of acetamide and succinamide in our larval food medium have failed so far to disclose any comparable effect of these substances on eye size in the ey^K strain. In FUJIO's (1960) account it is not clear that growth of the eye disc refers to an increase in facet number in ey^2 discs. This author removed eye discs from late third instar larvae (95 hours after eclosion from the egg at 25°C) which is well beyond the termination of the phenocritical period determined here for ey^K , an allele of ey^2 . It would seem therefore that, in spite of certain morphological similarities between the Bar and ey mutants, the underlying biochemical defect is different in the two cases.

The phenocritical period of ey^K : The relationship between the concentration of dietary RNA and expressivity of ey^K permits us to examine the effective period for the gene by adding RNA to nucleic acid deficient media at timed intervals during the larval period by means of bulb pipettes (SANG 1962). Figure 11 shows the effect of changing the RNA concentration from 0 to 3.2 percent.

Addition of RNA at successively later intervals is attended by a steady rise in the mean eye size (reduced exhibition of ey^K) which levels off after approxi-

FIGURE 11.—The effect of adding RNA to a nucleic acid-less medium at different times during development to give a final concentration of 3.2 percent in the medium. The time is measured in hours after eclosion from the egg and proportionally adjusted to the standard 96-hour larval period (BODENSTEIN 1950). Hollow circles, males, solid circles, females.

mately 42 hours of larval life, at the same values found when no RNA addition is made at all. In fact, there are two points of inflexion in the ascending portion of the distributions. In addition to that at 42 hours there is a definite change in slope at about 24 hours, suggesting that RNA influences two separate developmental processes, one taking place prior to 24 hours of larval life, and another between 24 and 42 hours, approximately. The second period agrees fairly closely with the period of boron sensitivity of *ey^K* (27–47 hours) found by SANG and McDONALD (1954), allowing for differences in the culture media (aseptic killed yeast media) from those employed in the present study. Both the RNA and boron sensitive periods cover the phase of growth of the eye-antenna complex after formation of the optic stalk, and terminate at the point where differentiation of the complex into separate eye and antenna buds begins (BODENSTEIN 1950), and this presumably marks the end of the period of activity of the *ey^K* gene. There is no evidence that boron treatment has any influence on the expressivity of *ey^K* prior to 27 hours, which limits the action of boron to the second phase of the phenocritical period. The first phase of the period extends to the earliest time of treatment (3 hours of larval life), which strongly suggests that the phenocritical period begins during the embryonic stage when it is no longer accessible to dietary environmental manipulations of the kind described above.

DISCUSSION

MEDVEDEV (1935) has found that a difference in size between the eye-antennal complex of wild-type and *ey²* larvae can be recognized at 24 hours of larval life, showing that at the commencement of the second phase of the eyeless phenocritical period some loss in disc size has already taken place. By 48 hours, when the complex has differentiated into separate eye and antenna buds, the characteristic asymmetry on the two sides of the head can be recognized (CHEN 1929; MEDVEDEV 1935). These observations, together with the effects of RNA treatment (Figure 11), suggest that the activity of the eyeless gene probably commences at some point in the embryonic period and lasts until about the 42nd hour of larval life, before the separation of the eye discs. This view is supported by the observations of DE MARINIS (1959), who found that temperature treatment of *ey²* eggs during the first 10 hours of embryonic development produced changes in eye size, and by SHIFRIN's (1947) data which show that progeny from old females are more eyeless than those from young females.

It was suggested above, and elsewhere (SANG 1961), that eyeless may be regarded as a hypomorph, and that modification in expressivity of the gene is due to directional shifts of the population mean of an underlying distribution of gene product, relative to fixed thresholds separating the different eye grades. It seems that different environmental influences can bring this about. If the primary genetic lesion is a defective enzyme we cannot necessarily repair it by nutritional means, but it does seem that the pressure along the enzyme mediated pathway can be relieved or intensified by altering the pattern of metabolic utilization at other points. For example, shifting the emphasis in the pattern of nucleotide

metabolism to *de novo* synthesis, which in turn must have repercussions on the pattern of amino acid and vitamin utilization, causes an increase of eye size.

It is sometimes possible to bypass mutant enzyme blocks entirely by providing the missing substrate for the succeeding reaction, and thus bringing about restoration of metabolic activity indistinguishable from the wild type. This is a commonplace for workers with microorganisms, but it has been achieved only in rare instances (KÜHN and ENGELHAAF 1955; KAJI 1960) for complex organisms with a developmental history.

We have shown that alteration of the pattern of protein and nucleic acid utilization can shift the population mean in the direction of higher or lower expressivity, and that new conditions can be defined which maximize the buffering of development in the direction of normal eye size. But the system only becomes accessible to dietary environmental manipulation after the phenocritical period of the *ey^K* gene has begun, and it seems that we cannot abolish effects on the development of the eye which accrue from influences already at work in the embryo. These considerations lead us to the conclusion that further progress in environmental correction of the eyeless phenotype may be made only by making the embryonic period the primary point of attack. Recently SANG and KING (1961) have defined the quantitative nutritional requirements of *D. melanogaster* adults, and this gives a starting point for influencing embryonic development by subjecting mature females to different dietary treatments during oogenesis.

The original observations of MORGAN (1929) and of CHEN (1929) on the increase of eye size with age of culture, are not to be explained by larval crowding (Figure 3) and are in the opposite direction to that which would be expected from maternal age effects (SHIFRIN 1947). It therefore seems most probable that they result from the relative depletion of the yeast population which SANG, McDONALD and GORDON (1949) have shown to occur during the later stages of the life of a culture. The data presented above suggest that the increase of mean eye size then found is not likely to be a consequence of protein shortage or of vitamin deficiencies since these would lower mean eye size. It could result from a reduction of the RNA supply.

SUMMARY

The effects of dietary manipulations on the exhibition of *eyeless^K* are examined using germ-free, chemically defined diets. Nutritional deficiencies which prolong larval development do not generally affect eye size. Folic acid and biotin shortages result in significant reductions of eye size and, with biotin, an associated increase of antennal duplication. Excess sucrose and lecithin also alter eye size, but of the major nutrients only casein and RNA seem to cause systematic changes with nutrient concentration.

Eye size increases with rising amounts of casein in the diet, but full, normal eye growth is not achieved at the highest level tested. On the other hand, there is an optimal provision of RNA (or DNA) for eye development, which does not correspond to the growth optimum. The response to RNA is shown to be equiva-

lent to the response to adenylic acid in the presence of an optimal supply of cytidylic acid, but the amount of cytidylic acid provided affects reactions to adenylic acid and *vice versa*.

Acid amides which are known to alter the exhibition of Bar do not affect eyeless when fed in the synthetic medium.

The phenocritical period of *ey^k* appears to have two phases, one ending with the first instar and the other at about 42 hours, when the separation of eye and antenna buds begins. There is evidence that the phenocritical period commences during embryogenesis.

These results are discussed in terms of the general consequences of altering competing reaction systems on a defective gene-controlled process.

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