*Genet. Res., Camb.* (1979), **34**, *pp.* 143–161
*With 2 text-figures*
*Printed in Great Britain*



# Nutritional regulation of antennal/leg homoeotic mutants in *Drosophila melanogaster*

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(*Received* 3 *July* 1979)

SUMMARY

The expression of the antennal homoeotic mutant, *Nasobemia*, is shown to be strongly dependent on nutrition. Dietary deficiencies in any of the following: thiamine, calcium pantothenate, nicotinic acid or RNA, cause a *curing* of the homoeotic defect. It is demonstrated that the effects of thiamine and pantothenate are through limiting acetyl-CoA synthesis and that fatty acids are required for high penetrance of the *Ns* gene. Similar nutritional effects are described for other *Antennapedia* and *aristapedia* alleles. The period of sensitivity to nutritional changes is defined for *Ns* and compared with the period of temperature sensitivity. These results suggest the hypothesis that the homoeotic mutations *Antennapedia* and *aristapedia* share some common metabolic requirements which involve synthesis of acetyl-CoA.

## 1. INTRODUCTION

Homoeotic mutations in *Drosophila* are generally thought to occur in genes which are intimately involved in the determination of particular imaginal structures and as such are useful tools for examining the determination process (Garciá-Bellido, 1977; Kauffman, 1975; Lewis, 1978). Present theories of homoeotic gene action are based on observations involving clonal analysis and classical genetics, and although these theories have borrowed ideas from molecular genetics, no homoeotic transformation has yet been explained in molecular terms. Further understanding of homoeosis demands the biochemical definition of the processes involved, and the approach used here is the only one presently available which might permit this biochemical understanding.

The homoeotic mutations chosen for study cause antennal/leg transformations: *Antennapedia* (*Antp*) and *spineless-aristapedia* (*ss*$^{a}$). Several alleles are known at the *Antp* locus, corresponding to point mutations or to chromosomal rearrangements. All are dominant and homozygous lethal (Denell, 1972). Expression is variable among the alleles and is dependent on the culture conditions, but they all

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0016–6723/79/2828–0764 $01.00 © 1979 Cambridge University Press
https://doi.org/10.1017/S0016672300019388 Published online by Cambridge University Press

cause a transformation of more or less large regions of antenna into the homologous areas of the second thoracic leg. The mutation, *Nasobemia* (*Ns*, 3–48·0), used throughout the major part of this study, is considered a homozygous viable allele of *Antp* (Denell, 1973). Its phenotype differs from the other *Antp* alleles in that it additionally transforms the praefrons, arising from the eye disc, to sternopleural tissue. There are several important differences between *Ns* and *Antp* and the other major antennal homoeotic, *aristapedia* ($ss^a$, 3–58·5). Mutations at the $ss^a$ locus are recessive and give a partial transformation of distal segments of the antenna into tarsus. In the stronger $ss^a$ alleles, abnormalities on the tarsi of the thoracic legs also occur. The abnormal legs have fused segments with swellings and/or constrictions of the regions along their tarsi (Waddington & Clayton, 1953).

The antennal homoeotics are particularly sensitive to culture conditions. The expression of the mutations depends on genetic background, temperature, culture densities and nutrition. The thesis advanced in this study is that an investigation of the parameters which directly influence the expression of a mutation will allow conclusions to be reached about the nature of that gene action, and its products. The most potentially rewarding parameter for study is nutrition, as illustrated by the work on the *rudimentary* locus (Norby, 1973 and Falk & Nash, 1974). Here it was shown that dietary pyrimidines *cured* the *rudimentary* phenotype, and, conversely, that pyrimidine auxotrophs were mutant at the *rudimentary* locus. The system provides a model approach for the study of the effects of nutritional manipulation of a mutant phenotype. The antennal homoeotics *Antp* and $ss^a$ are known to respond to culture conditions. Indeed *Antp* has been demonstrated to respond to nutritional changes (Sang & Bradshaw unpublished). They are therefore potentially good candidates for intensive study, using axenically defined culture media.

## 2. MATERIALS AND METHODS

This study used a homozygous stock of *Nasobemia* (*Ns*) which had been maintained at Sussex for three years. Other stocks mentioned were obtained through the *Drosophila* Stock centres listed in Drosophila Information Service. All stocks were maintained on Lewis' yeast–cornmeal medium (Lewis, 1960).

The nutritional manipulations used Sang's (1956, 1978) culture medium. Egg collection and sterilization was performed as described by Sparrow (1971). Fifty newly hatched larvae were used to inoculate each $6 \times 1$ in. tube, containing 5 ml of defined medium, and five replicates were set up for each treatment. Tubes were incubated at 25 °C until the adults emerged. Infected tubes were discarded.

Flies were cleared for at least 24 h in a 150 % (w/v) fructose solution (Sang, 1966) before scoring transformations. Transformations of the antennae were scored using a dissecting microscope at $\times 60$ magnification. For the *Antennapedia* alleles penetrance and expressivity, as defined by Timoféef-Ressovsky (1931), were measured by dividing the possible transformations into three classes: (*a*) those flies with both antennae transformed; (*b*) those flies with only one antenna

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transformed; (*c*) those flies with no transformation. Two estimates of penetrance were made:

$$\text{penetrance per fly } (P_F) = \frac{a+b}{a+b+c},$$

$$\text{penetrance per antenna } (P_A) = \frac{2a+b}{2(a+b+c)}.$$

[The two values are clearly correlated but $P_A \leqslant P_F$, and the $P_A$ value statistically carries more weight since it is estimated from twice the number used for the estimation of $P_F$.] Significances of differences were tested by appropriate contingency chi-square tests. The relationship between penetrance and the dose of an effective agent that influences it is essentially non-linear. A linear dose response is obtained by converting the penetrance to its probit value and the dose to the log-dose (Sang & Burnet, 1963). This conversion is performed for all such studies described in the text. Expressivity was defined by the expression

$$\text{expressivity (Exp)} = \frac{a}{a+b}.$$

This measurement was less useful in the case of *Ns* and *Antp* alleles since the reduction in penetrance was not necessarily associated with reduced expression of the mutation. There were always several flies with extreme bilateral asymmetry: one antenna might be completely wild-type while the other showed an extreme transformation. Such flies fell into class *b* thereby reducing the value of Exp even though the expression was very high in one of the antennae. For this reason more emphasis was put on changes in penetrance in the experiments with *Ns* and $Antp^B$. In all tables in the text $P_F$, $P_A$ and Exp are expressed as percentages.

In the studies of $ss^a$, since these alleles were characterized by high penetrance and strong bilateral symmetry, Exp was the more useful measure of expression. The transformations of the aristae were classified into five groups:

| Class | *a* | *b* | *c* | *d* | *e* |
|---|---|---|---|---|---|
| Degree of transformation | 100% Complete tarsus | 75% | 50% | 25% | 0% Wild-type arista |

This classification incorporated the varied degrees of transformation of the different *spineless–aristapedia* mutations, and allowed comparisons of alleles and treatments using $p \times q$ contingency tests.

## 3. RESULTS

### (i) *Effects of nutrient deficiencies on* Ns *expression*

Table 1 shows the expression of the homozygous *Ns* stock cultured at the same densities on yeast–cornmeal medium, and on chemically defined medium. There was a significant reduction in penetrance on the defined medium ($P < 0{\cdot}001$) but

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Table 1. *Penetrance and expressivity of* Ns/Ns *on Lewis' and on Sang's media*

| | *n*† | Survival (%) | $P_F$ ♀♀ | $P_F$ ♂♂ | $P_A$ ♀♀ | $P_A$ ♂♂ | Exp ♀♀ | Exp ♂♂ | Dev.‡ time (days) |
|---|---|---|---|---|---|---|---|---|---|
| Lewis' yeast–cornmeal | 362 | 90·5 | 96·0 (175) | 95·7 (187) | 79·7 (350) | 80·2 (374) | 68·1 (166) | 67·6 (179) | 9·0 |
| Sang's defined | 1676 | 53·2 | 79·4 (838) | 76·8 (838) | 60·2 (1676) | 58·8 (1676) | 51·7 (665) | 53·0 (644) | 11·0 |

Numbers of antenna or flies scored are given in parentheses in this and subsequent tables.
† Number of adults eclosing. ‡ Time from egg hatch to eclosion.

Table 2. *Effects of nutrient deficiencies on the expression of* Ns *homozygotes*

| | *n* | Survival (%) | $P_F$ | $P_A$ | Exp |
|---|---|---|---|---|---|
| Control | 133 | 53·2 | 78·1 | 58·6 | 50·0 (104) |
| 2% casein | 66 | 26·4 | 68·7 | 53·8 | 54·3 (46) |
| Nil RNA | 43 | 17·2 | 51·2** | 32·6*** | 27·3 (22) |
| Nil sucrose | 128 | 51·2 | 84·4 | 62·9 | 49·1 (108) |
| Low cholesterol | 95 | 38·0 | 81·1 | 60·0 | 48·1 (77) |
| Low choline | 49 | 19·6 | 85·7 | 63·3 | 47·6 (42) |
| Low riboflavin | 104 | 41·6 | 88·5 | 64·9 | 46·7 (92) |
| Low pyridoxine | 26 | 10·4 | 84·6 | 65·4 | 54·5 (22) |
| Nil folate | 53 | 21·2 | 73·6 | 58·5 | 59·0 (39) |
| Nil biotin | 33 | 13·2 | 75·8 | 51·5 | 36·0 (25) |
| Low pantothenate | 62 | 24·8 | 48·4*** | 27·4*** | 13·3*** (30) |
| Low nicotinate | 41 | 16·4 | 46·3*** | 26·8*** | 15·8***(19] |
| Low thiamine | 63 | 25·2 | 65·1 | 46·0* | 41·5 (41) |

Numbers in parentheses are the individuals scored in estimating Expressivity, in this and all subsequent tables.

* Significant at 5% level; ** at 1% level; *** at 0·1% level in this and subsequent tables.

Table 3. *Effects on* Ns/Ns *expression of RNA and the interaction with different caseins*

| | | *n* | Survival (%) | $P_F$ | $P_A$ | Exp |
|---|---|---|---|---|---|---|
| Genatosan casein | Control | 107 | 53·5 | 82·2 | 60·3 | 46·3 (88) |
| | −RNA | 26 | 10·4 | 53·8** | 36·5** | 35·7 (14) |
| Wellcome Foundation | Control | 139 | 55·6 | 78·4 | 57·9 | 47·7 (109) |
| | −RNA | 39 | 19·5 | 56·4* | 35·9*** | 27·3 (22) |
| ICN casein | Control | 152 | 76·0 | 72·4 | 51·6 | 42·7 (110) |
| | −RNA | 22 | 8·8 | 31·8*** | 15·9*** | 0 (7) |

no difference in gene penetrance between males and females ($P > 0{\cdot}20$). Since the defined medium was adequate for homozygote growth and development, yet did not represent optimal conditions for expression of the mutant gene, it was feasible to make changes in the medium which would either shift the expression up to the level found on Lewis' medium, or reduce it further.

The level of each nutrient was altered in turn, to an amount causing a significant

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decrease in survival. Each manipulation was performed at least three times in a series of concentrations. Although all these dietary changes (except sucrose) reduced survival, not all affected penetrance (Table 2). Only changes in RNA, thiamine, nicotinic acid and calcium pantothenate reduced penetrance significantly from the control levels. The survival on RNA-free medium was dependent on the preparation of casein used (Table 3). Dose responses confirmed the effects of low

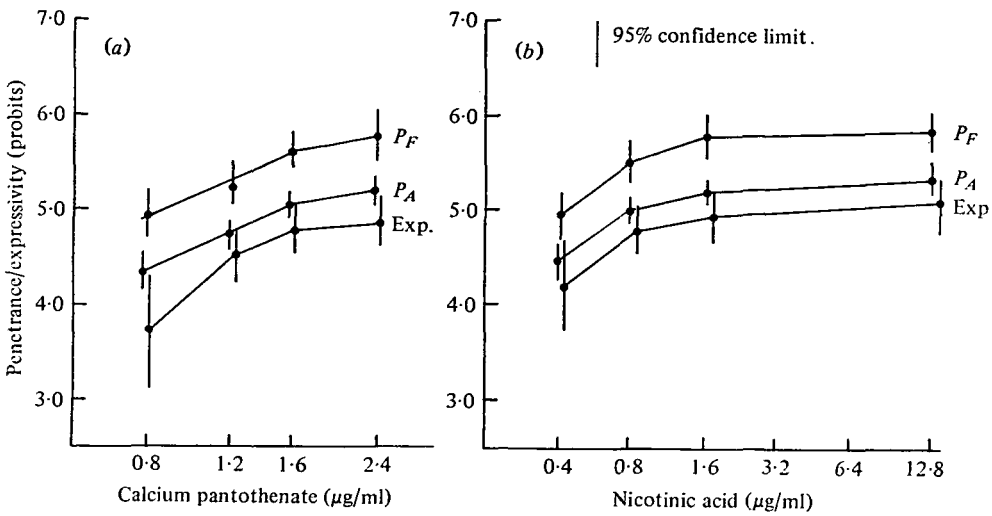


Fig. 1(*a*) Dose response of *Ns* homozygotes to calcium pantothenate. (*b*) Dose response of *Ns* homozygotes to nicotinic acid.

Table 4. *The pooled data of five experiments involving* Ns *homozygotes on low thiamine medium*

| Level of thiamine | *n* | Survival (%) | $P_F$ | $P_A$ | Exp |
|---|---|---|---|---|---|
| 2 μg/ml (control level) | 1676 | 53 | 78·1 | 59·5 | 52·3 (1309) |
| 0·08 μg/ml | 275 | 21 | 67·6*** | 48·2*** | 42·5* (186) |

pantothenate or nicotinate (Fig. 1*a*, *b*). The effects of low thiamine were more variable. A statistically significant reduction in penetrance was only given when the data from several experiments were pooled (Table 4). This may have been due to the low requirement for thiamine, and the contribution of maternal carryover of the vitamin in the egg.

Since each of the constituents of the defined medium is present in at least a tenfold excess over the levels limiting on survival, the higher penetrance on the yeast–cornmeal is likely to be due to the additional supply of preformed metabolic intermediates. However, the surprise is that deficiencies in the medium *cure* the homoeotic transformation. This contrasts to the findings in *rudimentary*, where deficiencies enhance the mutant phene. In order to characterize the area of

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metabolism important in the homoeotic expression of *Ns*, each of the nutrient deficiencies was explored in turn.

### (ii) *Effects of the omission of RNA nucleosides*

A balanced supply of its component nucleosides can be used to replace dietary RNA, but, as there is an interconversion of adenosine to guanosine and of cytidine to uridine, it is necessary to feed only adenosine and cytidine (Sang, 1957; Burnet & Sang, 1963). The results of such supplementations are given in Table 5.

Table 5. *Effect on* Ns/Ns *expression of adenosine addition in the presence of cytidine using RNA-free medium* (*Wellcome casein*)

| Type of medium | *n* | Survival (%) | $P_F$ | $P_A$ | Exp |
|---|---|---|---|---|---|
| No RNA (control) | 38 | 19·0 | 34·2 | 19·7 | 15·4 (13) |
| + 0·37 mM adenosine | 65 | 26·0 | 46·2 | 30·0 | 30·0 (30) |
| + 0·74 mM adenosine | 0† | 0 | | | |
| + 1·48 mM adenosine | 0† | 0 | | | |
| + 4·1 mM cytidine | 210 | 84·0 | 39·5 | 23·1 | 16·9 (83) |
| + 4·1 mM cytidine + 0·37 mM adenosine | 215 | 86·0 | 50·2 | 31·2 | 24·1 (108) |
| + 4·1 mM cytidine + 0·74 mM adenosine | 202 | 80·8 | 57·8* | 38·4** | 32·5 (117) |
| + 4·1 mM cytidine + 1·48 mM adenosine | 186 | 74·4 | 71·5*** | 50·0*** | 39·8 (133) |
| Complete medium | 210 | 84·0 | 63·3** | 41·0*** | 29·3 (133) |

All statistical comparisons made with no RNA control.
† No adults eclosed out of 250 larvae.

Survival and penetrance on RNA-free medium were both reduced. Addition of adenosine did not significantly change this response, although higher levels were lethal. Cytidine, however, caused normal survival but did not significantly change penetrance over the RNA-free control. Supplementation with both nucleosides together gave the same penetrance and survival rates as complete medium. These results suggest that the low survival on RNA-free medium is caused by a specific requirement for pyrimidines. Similar effects, in other *D. melanogaster* strains, have been reported by Kouni & Nash (1977) and earlier by Burnet & Sang (1963). High penetrance of the *Ns* gene requires adenosine, but, since the effect can only be shown in the presence of cytidine the possibility that transformation requires both purines and pyrimidines cannot be eliminated.

A requirement for nucleosides may be associated with a need for folic acid since folate is required in nucleic acid synthesis. However, omission of dietary folic acid failed to show any requirement for homoeotic transformation (Table 2). Venters (1971) demonstrated that *Drosophila* larvae are able to synthesize their general folate requirements, but require dietary folate to meet their needs at pupation. This larval synthesis of folate would therefore explain the absence of an effect on penetrance, if the determination event is early in larval development (see later).

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(iii) *Effects of low thiamine and low pantothenate*

Limiting levels of thiamine or of pantothenate reduce the penetrance of the *Ns* gene. Is there a common factor in these two effects? Thiamine, as the pyrophosphate, is a cofactor in the decarboxylation of the $\alpha$-keto acids, and also in the formation of $\alpha$-ketols. The most important of these reactions is the decarboxylation of pyruvate. Pantothenate is a precursor for coenzyme A (CoASH). A common feature of the roles of pantothenate and thiamine is therefore the production of acetyl-CoA, and we tested the hypothesis that acetyl-CoA was central in *Ns* expression. Limiting levels of thiamine or of pantothenate would both be expected to reduce acetyl-CoA production and this would imply that high acetyl-CoA is a requirement for high penetrance of the *Ns* gene: conversely, any treatment which lowers the endogenous level of acetyl-CoA should also lower penetrance.

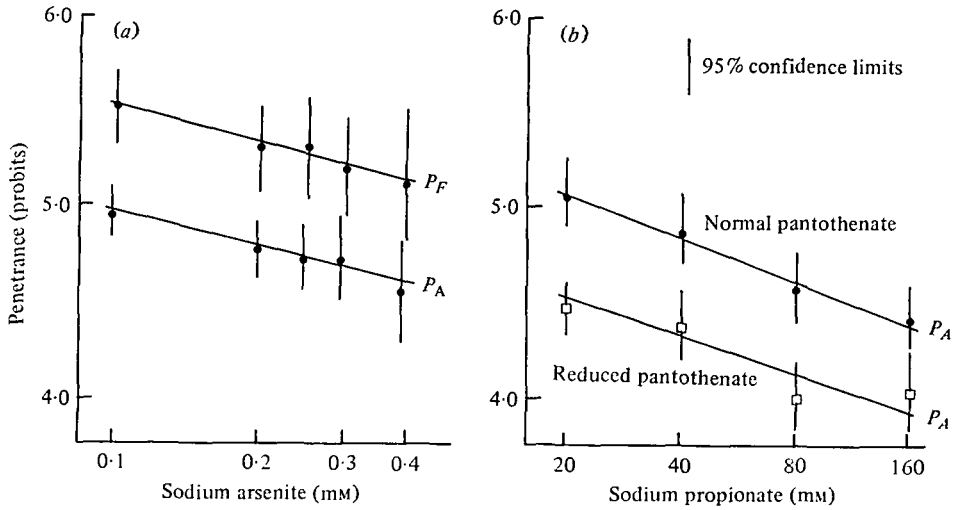


Fig. 2(*a*) Dose response of *Ns* homozygotes to sodium arsenite. (*b*) Dose responses of *Ns* homozygotes to sodium propionate on both normal and reduced levels of calcium pantothenate. ●, Normal calcium pantothenate (16 $\mu$g/ml); □, reduced (not limiting) calcium pantothenate (2·4 $\mu$g/ml).

In animals, acetyl-CoA is produced in two main ways: the most important is from pyruvate by the enzyme pyruvate dehydrogenase; and, secondly, from acetate by acetyl-CoA synthetase. Antagonists of these enzymes would be expected to reduce penetrance of the *Ns* gene. The degradation of fatty acids also gives rise to acetyl-CoA but since the defined medium contains no phospholipids this is unlikely to be an important acetyl-CoA source in these experiments.

Stoppani *et al.* (1953) examined the inhibition of yeast pyruvate dehydrogenase, and found that the purified enzyme is particularly sensitive to sodium arsenite. When arsenite was added to the defined medium there was a significant reduction in penetrance (Fig. 2*a*). However, the compound was very toxic, as was to be expected, and the effective concentration range was small. The inhibition of

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$\alpha$-keto acid oxidation by arsenicals has been demonstrated in many organisms and tissues, and such inhibition is characteristically associated with the accumulation of the keto acids (Webb, 1966). However, because of the large number of enzymes which have been demonstrated to be inhibited by arsenicals (Webb, 1966), it is difficult to be sure where the arsenite is exerting its effect in *Ns* larvae.

Table 6. Ns/Ns *expression and the antagonism of arsenite and acetate*

| Treatment | *n* | Survival (%) | $P_F$ | $P_A$ | Exp |
|---|---|---|---|---|---|
| Complete medium (control) | 210 | 84·0 | 70·0 | 48·3 | 38·1 (147) |
| 80 mM acetate | 209 | 83·6 | 72·7 | 51·4 | 41·2 (152) |
| 0·25 mM arsenite | 107 | 53·5 | 62·6 | 39·3* | 25·4 (67) |
| 0·25 mM arsenite + 80 mM acetate | 182 | 72·8 | 72·5 | 52·5† | 44·7† (132) |

† Significant at 1% level when compared with 0·25 mM arsenite values.

Table 7. Ns/Ns *expression and the antagonism of propionate and acetate*

| Treatment | *n* | Survival (%) | $P_F$ | $P_A$ | Exp |
|---|---|---|---|---|---|
| Complete medium | 141 | 56·4 | 78·7 | 58·2 | 47·7 (111) |
| Low pantothenate (control) | 115 | 57·5 | 80·9 | 60·9 | 50·5 (93) |
| Low pantothenate + 80 mM acetate | 172 | 68·8 | 76·2 | 54·4 | 42·7 (131) |
| Low pantothenate + 80 mM propionate | 125 | 50·0 | 34·4*** | 20·8*** | 20·9** (43) |
| Low pantothenate + 80 mM acetate + 80 mM propionate | 159 | 63·6 | 61·0† | 37·7† | 23·7 (97) |

† Significant at 0·1% level when compared with 80 mM propionate values.

Acetyl-CoA synthetase activates acetate, propionate and acrylate, but it does not affect the long-chain fatty acids. Its major role is in activating acetate for subsequent fatty acid synthesis. Propionate was particularly effective in reducing the penetrance of *Ns* without any significant effect on survival except at very high concentrations (Fig. 2*b*). The propionate effect was enhanced by reducing the level of (but not limiting) pantothenate. This suggests that propionate reduces endogenous pools of CoASH by its conversion to propionyl-CoA and thereby reduces acetyl-CoA synthesis.

The effects of arsenite and propionate are as expected, but do not confirm that it is the conversion to acetyl-CoA that is essential for homoeotic expression. However, these effects should be antagonized by supplementing with acetate. If arsenite is inhibiting pyruvate dehydrogenase, addition of excess acetate will allow synthesis of acetyl-CoA by acetyl-CoA synthetase, and thus by-pass the arsenite block. This is confirmed in Table 6. Arsenite caused reduced penetrance, but acetate itself had no effect. When acetate was added to the arsenite containing medium, the arsenite effect was abolished.

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Similarly, acetate should antagonize the effect of propionate according to the hypothesis that propionate competes for CoASH, thereby reducing acetyl-CoA levels. Indeed, in the presence of reduced (but not limiting) levels of pantothenate, it is found that the penetrance produced by propionate and acetate together is intermediate to their effects separately (Table 7). The effects of the antagonists, arsenite and propionate, are consistent with the original hypothesis that the low penetrance induced by limiting thiamine or pantothenate is through restrictions in acetyl-CoA synthesis, acetyl-CoA being necessary for the appearance of the homoeotic transformation. It should also be noted that under control conditions on the complete medium acetyl-CoA synthesis is not limiting since acetate supplementation does not increase penetrance above the control level.

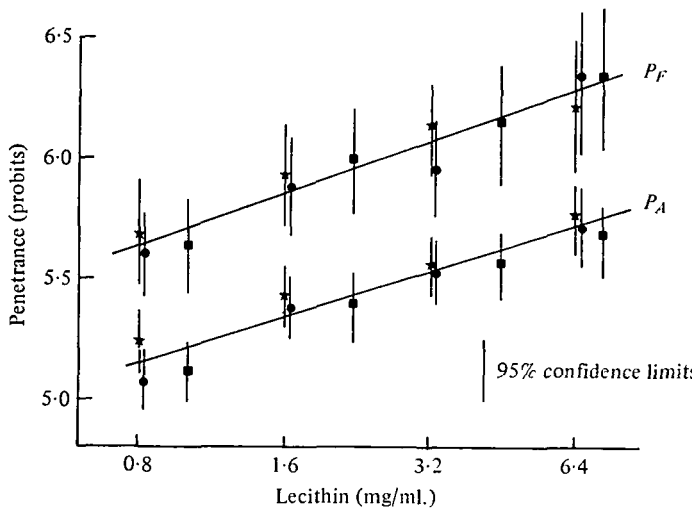


Fig. 3. Dose responses of *Ns* homozygotes to lecithin. ■, Egg-yolk lecithin; ★, soy-bean lecithin; ●, soy-bean lecithin with limiting calcium pantothenate (1·2 μg/ml).

(iv) *Effects of fatty-acid supplementation*

The comparison between the effects of yeast–cornmeal and defined media (Table 1) suggests that provision of extra metabolic products in the more complex medium favours higher penetrance. The defined medium used contains no fatty acids, and a number of the previous observations are explained by a specific requirement for fatty acid synthesis in *Ns* expression. First, fatty acid synthesis requires acetyl-CoA, which is consistent with the effects of pantothenate and thiamine, and the studies using arsenite and propionate. Second, nicotinic acid and adenine are precursors for NADPH which is required as a cofactor in fatty acid synthesis. A prediction of this hypothesis is that fatty acids added to the medium should increase penetrance. Indeed, when lecithin is added, just such an increase is found (Fig. 3).

There are three possible ways in which lecithin may be acting: (*a*) lecithin (fatty acids) is directly involved in *Ns* expression; (*b*) provision of lecithin in the

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diet reduces the requirement for acetyl-CoA in fatty acid synthesis and thereby allows endogenous acetyl-CoA to be redirected to another pathway involved in expression; (*c*) dietary fatty acids are broken down providing acetyl-CoA which causes an increase in endogenous levels and thereby allows increased expression by redirection into the homoeotic pathway. As previously stated the observation that dietary acetate does not cause an increase in mutant expression suggests that acetyl-CoA is not limiting under normal, control conditions. This would lead to the conclusion that (*b*) and (*c*) above are unlikely to be important, and that homoeosis involves fatty acid synthesis directly. However, are there other observations that support this conclusion?

Low pantothenate reduces endogenous CoASH which is required for activation of fatty acids before degradation. Under conditions of low pantothenate therefore, fatty acid activation should be minimal and endogenous acetyl-CoA levels should remain limiting. Fig. 3 shows the effect of adding lecithin in the presence of limiting levels of pantothenate. There is no significant difference from the dose response with normal pantothenate levels. This suggests that lecithin does not act by providing acetyl-CoA through the breakdown of fatty acids.

The majority of fatty acid synthesis from acetyl-CoA is through acetyl-CoA carboxylase (Lynen *et al.* 1963). This carboxylation step requires biotin as a cofactor (Knappe, 1970). Reducing dietary biotin should limit fatty acid synthesis and if this synthesis is directly involved expression should be lowered. As was seen in Table 1, omission of biotin causes no change in expression even though survival is reduced. However, this absence of response does not eliminate the possibility of fatty acid involvement. The requirement for biotin is very small and as a consequence maternal carryover may be important. Carryover is not sufficient to cause normal development throughout the life-cycle but it may be adequate to allow normal development for the early larval stages. If the process influenced by dietary changes occurs early in larval development, the endogenous biotin may be sufficient to support the larvae during this period.

Desthiobiotin (DTB) has been reported to be an effective biotin anti-metabolite (Lilly & Leonian, 1944). This anti-metabolite was added to the defined medium in the hope that it would compete with any biotin present through maternal carryover. However, it had a partial 'biotin-like' activity itself: increasing concentration of DTB improved survival but did not alter gene expression. It must be concluded that biotin and fatty acid involvement in homoeosis cannot be eliminated.

Since lecithin is a composite of several fatty acid derivatives, we wondered whether only certain fatty acids were important in bringing about high penetrance? Fig. 4 summarizes the results of several experiments in which the medium was supplemented by each fatty acid individually. Saturated fatty acids prove detrimental to survival; the effect is dependent on chain length. Myristate (14:0) and palmitate (16:0) both produce significant increases of penetrance. Of the mono-unsaturated acids, oleic (18:1) causes a significant increase in penetrance. The other unsaturated 18-carbon fatty acids (linoleic, 18:2; and linolenic, 18:3) are detrimental to survival at the higher levels although they both cause increased

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penetrance. In summary, the longer chain fatty acids increase the penetrance of *Ns* homozygotes.

Of more significance are the observations on medium with limiting pantothenate (Table 8). Saturated fatty acids do not alleviate the 'curing' effect of low pantothenate. Indeed laurate (12:0) and myristate (14:0) cause a further lowering of penetrance under these conditions. Survival is also reduced but less drastically than when the acids are added to complete medium. The only unsaturated fatty acid to be effective in completely alleviating the low penetrance effect was oleate (18:1). It may be that the other fatty acids are effective at higher concentrations.

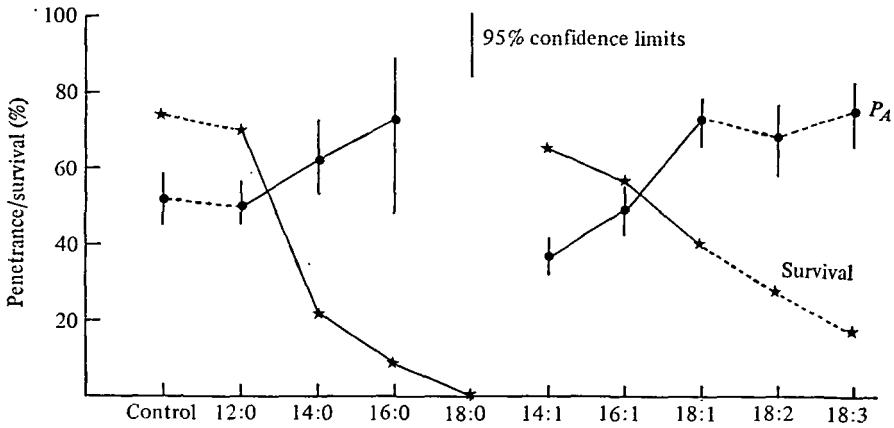


Fig. 4. The response of *Ns* homozygotes to fatty acids of control defined medium. All fatty acids were added at 4 mM level.

While lecithin increases the expression of *Nasobemia*, there is heterogeneity in the response to the individual fatty acids. The response depends on the acid and on the nature of the medium. Interpretation of these results is difficult since fatty acids may be interconverted, and as Keith (1967*a*, *b*) has shown, the degree of interconversion is dependent on the dietary fatty acids. Keith's work was performed on a medium containing some phospholipid while the medium used throughout this study contained none. Geer and Perille (1977) showed that the response to dietary fatty acids also depends on genotype. Therefore direct comparisons with Keith's and Geer's observations cannot be made.

The finding that oleic is the only acid that is effective on low pantothenate suggests that it may be directly involved in the appearance of the antennal leg. The increased penetrance observed with the other fatty acids on complete medium may reflect conversion to oleic (Keith, 1967). There is no other evidence that implicates oleate in the antennal/leg transformation and therefore this suggested role of oleic acid should be regarded as only tentative.

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Table 8. *Effects on* Ns/Ns *expression of fatty acid supplementation on medium containing limiting pantothenate*

| Treatment | *n* | Survival (%) | $P_F$ | $P_A$ | Exp |
|---|---|---|---|---|---|
| Complete medium | 185 | 74·0 | 70·8 | 51·9 | 46·6 (131) |
| Low pantothenate (control) | 130 | 52·0 | 58·5 | 37·3 | 27·6 (76) |
| +12:0 | 95 | 38·0 | 38·9** | 24·7** | 27·0 (37) |
| +14:0 | 93 | 37·2 | 40·9* | 25·8* | 26·3 (38) |
| +16:0 | 35 | 14·0 | 54·3 | 40·0 | 47·4 (19) |
| +18:0 | 135 | 54·0 | 59·3 | 37·4 | 26·3 (80) |
| +16:1 | 64 | 25·6 | 62·5 | 38·3 | 22·5 (40) |
| +18:1† | 130 | 52·0 | 83·8*** | 64·6*** | 54·1*** (109) |
| +18:2 | 15 | 6·0 | 66·7 | 43·3 | 30·0 (10) |
| +18:3 | 21 | 8·4 | 52·4 | 35·7 | 36·4 (11) |

All fatty acids were added to give a final concentration of 4 mM. All statistical comparisons are made with low pantothenate control.

† $P_F$ and $P_A$ also significantly higher than those of complete medium. ($P_F$, $P < 0{\cdot}02$; $P_A$, $P < 0{\cdot}01$)

(v) *Determination of the nutrition and temperature sensitive period of* Ns

The determination of the nutrition sensitive period (NSP) involved rearing larvae on two types of medium. One favoured high, and the other reduced, penetrance and expressivity. Larvae were transferred from one medium to the other at intervals during their development. The 'low' medium had a limiting level of pantothenate (6 $\mu$g/5 ml), 80 mM propionate and 0·15 mM arsenite. There were two forms of 'high' medium. The first was used for rearing larvae before transferring to 'low' medium. It contained reduced pantothenate, which was not limiting (12 $\mu$g/5 ml), to prevent pantothenate accumulation. The second, for larvae after they had been reared on 'low' medium, contained normal pantothenate (80 $\mu$g/5 ml) and soy bean lecithin (20 mg/5 ml). Fig. 5 shows the effects of this series of nutritional shifts. The 'low' and 'high' media gave different development times for the larvae. The developing larvae were examined at frequent intervals to determine the times of the larval moults, which were used to give appropriate development times for the larval instars on the two media.

The larvae are sensitive to transfers before the beginning of third instar. In the case of 'high' to 'low' the period of transition occurs during the second larval instar. However, the transfers 'low' to 'high' have a less well defined transition point. The greatest change occurs during the second instar but during the first instar the period of exposure to the 'low' medium has an accumulative effect on expression. This may be because the antagonists are not completely 'washed out' or countered by the 'high' medium.

The expression of *Ns* is dependent on temperature (Stepshin & Ginter, 1974), and this was confirmed for the strain used in the present study. The results of a series of temperature shifts at different developmental times are given in Fig. 6. The temperature sensitive period (TSP) occurs between the first quarter of the

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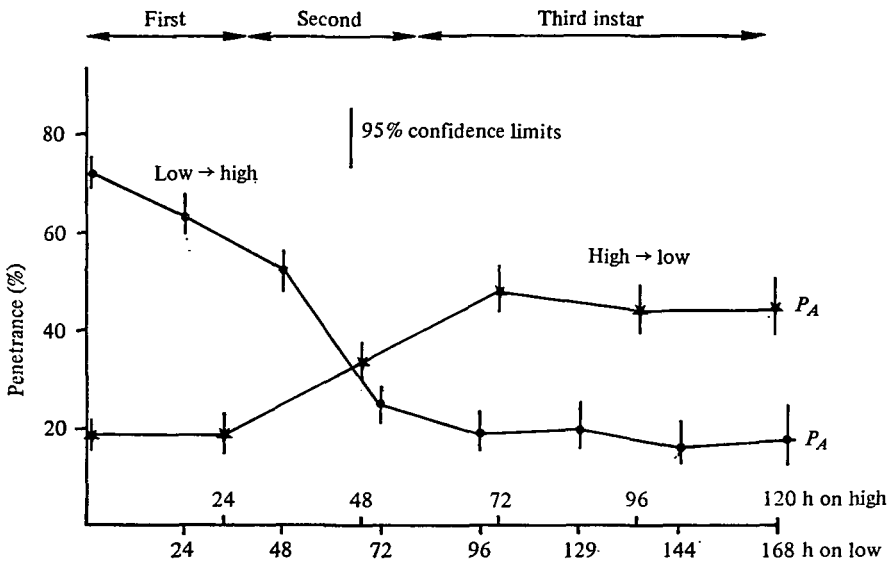


Fig. 5. Determination of the nutrition sensitive period (NSP) for *Ns* homozygotes. Larvae were transferred between media favouring high and low penetrance at intervals during their development. See text for the constituents of the media.

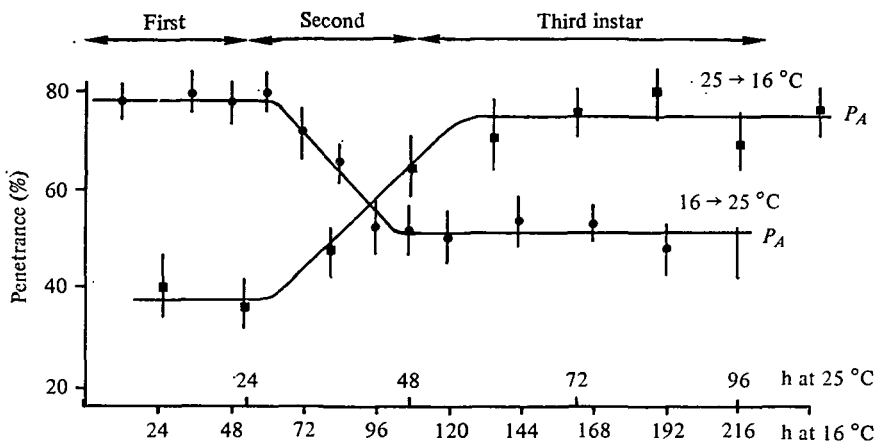


Fig. 6. Determination of the temperature sensitive period (TSP) for *Ns* homozygotes.

second larval instar and the beginning of the third. The TSP was confirmed by a temperature pulse experiment involving the shifting of developing larvae from 25 °C to 16·5 ± 2 °C for periods of 24 h (Table 9). The most effective pulse was that performed on 36 h larvae. The results confirm the TSP during the last three-quarters of the second larval instar and the beginning of the third instar.

Stepshin & Ginter (1972) demonstrated the TSP of *Apx* and *Ns*/+ to be between 24 and 72 h. (Based on a 96 h larval cycle). This is confirmed by the present study (30–54 h) and, furthermore, the NSP occurs at the same time.



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### (vi) *The responses of* Antp$^B$/TM1 *and* ss$^a$ *alleles*

Bradshaw (1976) showed that *Antp*$^{50}$ responded to low levels of nicotinic acid, calcium pantothenate, or thiamine: the same manipulations are effective with *Ns*. To confirm the similarity between *Ns* and *Antp*, a selection of nutritional manipulations were performed using another allele, *Antp*$^B$. The transformations in *Antp*$^B$ are restricted to the third antennal segment and the arista base. Pro-

Table 9. *Effect of low-temperature pulses of* 24 *h on* Ns/Ns *expression using yeast–cornmeal medium*

| | *n* | Survival (%) | $P_F$ | $P_A$ | Exp |
|---|---|---|---|---|---|
| 25 °C (control) | 274 | 68·5 | 88·7 | 76·8 | 73·3 (243) |
| 24 h larvae | 193 | 77·2 | 93·8 | 78·8 | 68·0 (181) |
| 36 h larvae | 284 | 71·0 | 70·1*** | 51·2*** | 46·2*** (199) |
| 48 h larvae | 310 | 77·5 | 82·6* | 62·9*** | 52·3*** (256) |
| 16·5 °C (control) | 280 | 70·0 | 70·4*** | 50·0*** | 42·1*** (197) |

All comparisons made with 25 °C control.

Table 10. *The response of* Antp$^B$/TM1 *to different nutritional treatments*

| | | | $P_F$ | | $P_A$ | | Exp | |
|---|---|---|---|---|---|---|---|---|
| | *n* | Survival (%) | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ |
| Control | 142 | 56·8 | 58·3 | 60·0 | 38·2 | 37·9 | 31·0 | 26·2 |
| 0·8 μg/ml pantothenate | 139 | 55·6 | 15·3*** | 35·8** | 8·3*** | 21·6** | 0 | 20·8 |
| Control | 120 | 48·0 | 63·0 | 74·2 | 42·6 | 59·1 | 35·3 | 59·2 |
| 160 mM propionate | 63 | 25·2 | 3·8*** | 35·1*** | 1·9*** | 24·3*** | 0 | 28·6 |
| Control | 158 | 63·2 | 57·1 | 63·0 | 36·4 | 41·4 | 27·3 | 31·4 |
| 8 mM oleate | 112 | 44·8 | 55·4 | 82·1* | 42·9 | 62·5** | 54·8* | 52·2 |
| Control | 182 | 72·8 | 75·0 | 68·3 | 52·5 | 53·0 | 40·0 | 55·4 |
| 8 mM linoleate | 131 | 52·4 | 61·5 | 53·0 | 36·9** | 34·8** | 20·0* | 31·4* |

The control in all cases was the normal complete medium with no additions.

trusions of leg tissue are commonly seen coming from the third segment. The similarity in expression between *Antp*$^B$ and *Ns* allows the use of the same criteria for estimation of penetrance and expressivity. However, unlike *Ns*, there is a significant difference between the responses of males and females. For this reason the $P_F/P_A$ and Exp values are given separately for the two sexes (Table 10).

Low levels of pantothenate or high levels of propionate are effective in reducing penetrance and expressivity. However, the responses of males and females to these nutritional manipulations are significantly different, whereas there is no significant difference between males and females on the complete defined medium. The responses to the two fatty acids are very different. Oleic acid causes an increase in expression. Again there is a significant difference between the responses of the two sexes. Males show a marked increase in penetrance and expressivity. Females show no significant change in penetrance although expressivity is significantly increased

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at the 8 mM level. Linoleic acid, in contrast, causes a significant fall in expression at the 8 mM level. The male and female responses are not significantly different in this case.

Similar nutritional studies were performed on three *spineless-aristapedia* alleles ($ss^{a40a}$, $ss^{aB}$, $ss^{a}$) to determine if these would respond to the specific treatments most effective with *Ns*. The alleles vary in their degree of transformation. Generally $ss^{a40a} > ss^{a} > ss^{aB}$ at 19 °C. These mutations show their maximum expression at lower temperatures. $ss^{a40a}$ shows the greatest temperature sensitivity such that at 25 °C the order of expression becomes $ss^{a} > ss^{a40a} > ss^{aB}$.

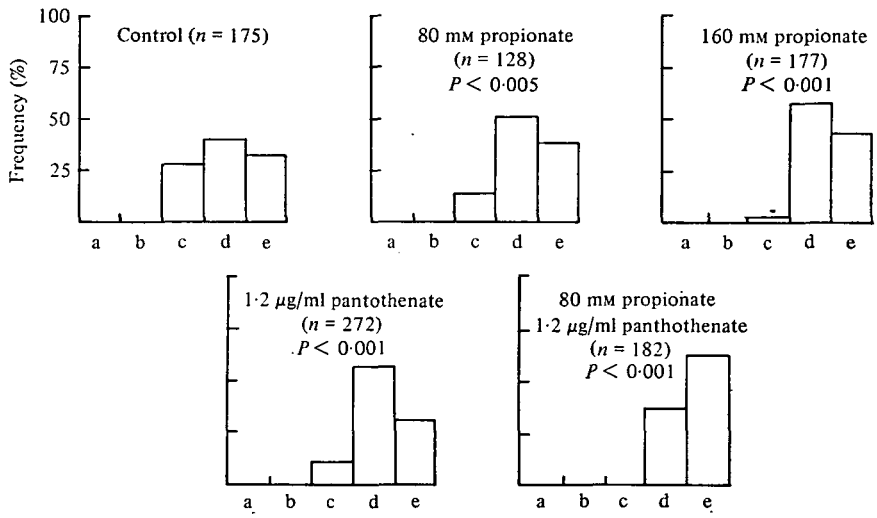


Fig. 7. The effect of limiting calcium pantothenate and high levels of sodium propionate on the expressivity of $ss^{aB}$ homozygotes. (All experiments were performed at 25 °C. The classes *a* to *e* on the abscissa represent decreasing transformation. Statistical comparisons are made with the control distribution).

When grown axenically on defined medium it is found that $ss^{a}$ does not respond to medium containing low pantothenate and high propionate. However, $ss^{a40a}$ homozygotes do respond to these manipulations. Both low pantothenate and high propionate shift expression towards the more weakly expressed classes. $ss^{aB}$, the weakest allele, proves to be the most responsive. At 25 °C low pantothenate and high propionate reduced expressivity (Fig. 7). These effects are also found at 19 °C but to a lesser extent.

In summary *Ns*, $Antp^{B}$, $ss^{a40a}$, and $ss^{aB}$ are all 'cured' by low pantothenate and high propionate. It appears that the processes involved in the antennal homoeosis of these mutations share some common requirements.

### (vii) *Effect of genetic background on* Ns *expression*

The homozygous *Ns* stock was maintained in the laboratory for over 40 generations during the course of this study. When outcrossed the expression of the



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heterozygote is significantly greater than the homozygote. This is the case both on yeast–cornmeal and on defined medium. Both genotypes respond to propionate (Fig. 8). During the course of the study the penetrance of the *Ns* stock progressively fell. However, outcrossing the homozygous stock and reselecting a new *Ns/Ns* stock brought about an increase in expression (Table 11).

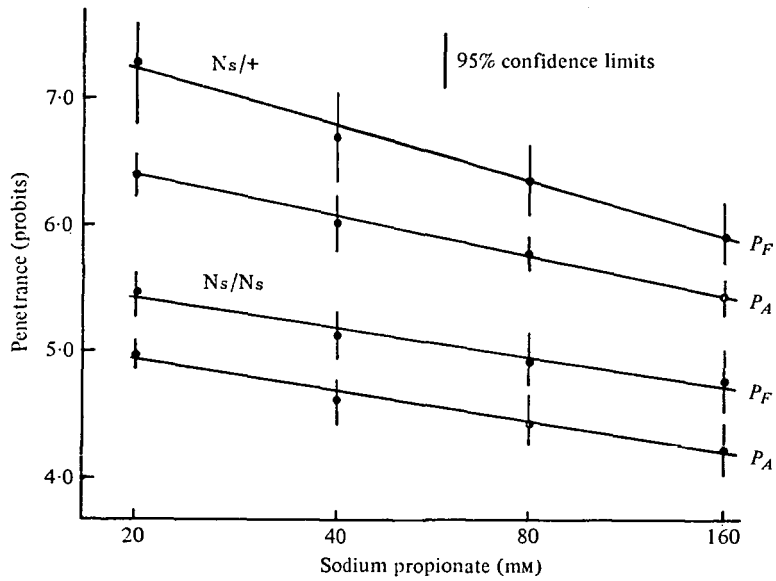


Fig. 8. Dose responses of *Ns* homozygotes and heterozygotes to sodium propionate.

Table 11. *Effect of outcrossing on* Ns/Ns *expression using defined medium*

| | *n* | Survival (%) | $P_F$ | $P_A$ | Exp |
|---|---|---|---|---|---|
| Ns/Ns (inbred) | 171 | 85·5 | 63·7 | 41·8 | 31·2 (109) |
| Ns/Ns (outcrossed) | 174 | 69·6 | 81·0*** | 66·4*** | 63·8*** (141) |

## 4. DISCUSSION

The expression of the antennal homoeotic *Nasobemia* is nutritionally dependent. Deficiencies of any one of four normal dietary constituents (RNA, pantothenic acid, nicotinic acid or thiamine) can lead to reduced penetrance of the *Ns* gene. The effects of these nutritional constraints are not through a non-specific effect on the developmental time of the homoeotic larvae, since several such treatments prolong larval life but not all affect expression. Nor can all the effects be explained by a reduced viability of the more strongly expressed progeny because certain treatments (lecithin and propionate) induce significant changes in penetrance without altering survival. Interestingly, applying specific nutritional constraints *cures* the mutational defect. This is contrary to the effect of dietary changes on *rudimentary*, but resembles phenylketonuria in humans where a deficiency in the diet (no phenylalanine) reduces the symptoms of the disease.

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The central involvement of acetyl-CoA is implicated by the specific requirement for thiamine and pantothenate. This is substantiated by the effects of the specific inhibitors propionate and arsenite, and their antagonism with acetate. However, the effects of low nicotinic acid or RNA are unlikely to be through limiting acetyl-CoA levels. A likely explanation is that these dietary restrictions limit NADPH levels. No direct proof of this hypothesis has been presented, but it is consistent with the observation that fatty acids bring about increased expression of *Ns* since synthesis of fatty acids requires acetyl-CoA and NADPH. However, not all fatty acids are equally effective, and the results are difficult to interpret because of the possible interconversions among the fatty acids. In the presence of low pantothenate, fatty-acid chain elongation and shortening are likely to be minimal, and the response to a fatty acid under such conditions is unlikely to be due to its contribution to endogenous acetyl-CoA or to its conversions to a longer chain fatty acid. Oleic acid is then the only fatty acid which causes increased penetrance on the low pantothenate medium. Laurate and myristate, on the other hand, cause a further reduction in expression. These findings may be interpreted in two ways. (*a*) Oleic acid is specifically required for antennal/leg homoeosis. The addition of myristate or laurate must therefore restrict oleate synthesis. Or (*b*) myristate and laurate are deficient in *Ns*. This deficiency brings about homoeosis, and provision of these acids in the diet *cures* the transformation. Supplementation with oleate must then similarly restrict myristate/laurate synthesis. Of these possibilities the latter is less likely since myristate and laurate do not *cure* the phenotype when added to the complete medium. As they stand these arguments are largely speculative, further work is necessary to confirm the direct involvement of fatty acids in antennal/leg homoeosis in *Ns*.

Acetyl-CoA has also been implicated in the other antennal homoeotics $Antp^{B}$, and $ss^{aB}$. In addition oleic acid causes elevated expression of $Antp^{B}$. However, the effect of linoleic acid conflicts with that in *Ns* since it reduces penetrance in $Antp^{B}$ and causes an increase in *Ns*. This may reflect the different genotypic backgrounds of, rather than a difference between, the two mutations. *Spineless-aristapedia* ($ss^{a}$) is generally less responsive to dietary changes than *Ns*, but the fact that it responds at all is significant and suggests some common metabolic feature between the two homoeotic loci.

There is a defined period in larval development during which *Ns* responds to both nutritional and temperature changes. *Ns* will respond to culture conditions until the beginning of the third instar. The nutritional and temperature shift experiments described suggest that it is during the second larval instar and the beginning of the third that the imaginal disc becomes determined for either antennal or leg development. These periods of sensitivity closely correspond to the phenocritical period and the onset of clonal inheritance found in $Antp^{R}$ by Postlethwait and Schneiderman (1971). The period of determination for *spineless-aristapedia* as shown by temperature shifts of $ss^{a40a}$ (Grigliatti & Suzuki, 1971) and in $ss^{a}$ by clonal analysis (Postlethwait & Girton, 1973; Ginter *et al.* 1975) occurs during mid-third instar and is therefore distinct from that in $Antp^{R}$ and *Ns*. This

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is significant in view of the fact that the preliminary nutritional study showed that $ss^{aB}$ also responded to the same nutritional changes as *Ns* and $Antp^{B}$, which argues that the nutrition effects described are acting generally on the homoeotic process. If this is so, do other homoeotic loci also respond to these changes?

The expression of *Ns*, *Antp* and $ss^{a}$ are strongly dependent on genetic background. During the course of this study several genetic combinations were made with the *Ns* mutation. Of particular note are recombinants with $ss^{a40a}$. One of several recombinants obtained was homozygous lethal, and the other non-lethal, chromosomes all had different homozygous viabilities. This makes interpretation of the effects of genetic compounds very difficult. However, there is increasing evidence to suggest that the *Antp* locus is part of a complex locus in the region of proximal 3R. Kaufman (1978) describes a deficiency, Df(3R)Scr, which exposes the *Antp* and *pb* loci and yet has a dominant phenotype expressed as a reduction in the number of sex comb teeth in the male. In addition Kaufman describes a new mutation, *Thickened arista* (*Ta*), which is similar to $ss^{a}$ in that only the arista is transformed to a tarsal-like structure. The region is also known to include several extra sex comb mutations (Denell, 1973). If these other loci are also sensitive to nutritional changes, the work described here could provide a useful new approach to looking at homoeotic transformations.

This work was supported by a Science Research Council studentship awarded to T. Jowett. Writing of the manuscript was supported by a National Institutes of Health research grant (GM24182) awarded to John H. Postlethwait.

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