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The effects of nutritional deficiencies on the *maroon-like* maternal effect in Drosophila

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1. INTRODUCTION

Mutation at the *maroon-like* locus of *Drosophila melanogaster* (*ma-l*: 1–64·8) leads to a brownish eye colour, resulting from a reduction in the red (drosopterin) pigments, loss in activity of the enzyme xanthine dehydrogenase (Glassman & Mitchell, 1959), as well as loss in activity of pyridoxal oxidase (Forrest, Hanly & Lagowski, 1961) and aldehyde oxidase (Courtright, 1967). Chemical as well as genetic evidences argue that these enzyme activities are associated with distinct molecular species (Glassman, 1965; Courtright, 1967), and that the eye colour defect is associated with XDH (Keller & Glassman, 1965). If *ma-l* mutant flies are bred from non-mutant mothers, their eye colour is red, and although genetically incapable of making the enzyme they nevertheless contain detectable amounts of XDH (Glassman & Mitchell, 1959). Offspring of the reciprocal cross do not show this 'maternal effect', which is due to the transmission, through the egg, of a small amount of the *ma-l*$^+$ gene product present in the mother. This product is not XDH, which is scarcely detectable in the egg; but when maternally affected larvae hatch they contain about one tenth of the wild-type amount of XDH, and this is sufficient to cure their morphological abnormalities (Glassman & McLean, 1962).

The maternal effect is found only among flies which hatch during the first 4–5 days of emergence from ordinary Drosophila bottle cultures. By the seventh day or so, emerging adults are all mutant, and the maternal effect has disappeared. This is a consequence of the culture conditions under which the larvae develop, for offspring of old flies placed in new bottles again show the maternal effect, and vice versa (Glassman & Mitchell, 1959). There is therefore an interaction between some environmental factor(s) and a gene product involved in the formation of the functional enzyme. Glassman & Keller (1965) suggest that either (*a*) some substance in the food medium involved in this reaction is used up, or (*b*) an inhibitor accumulates, as cultures age. They have shown that when two larval excretory products (hypoxanthine and uric acid) are separately incorporated in the normal food, loss of the maternal effect happens earlier than in controls, in both cases. They therefore favour the second hypothesis. While the purpose of this paper is to examine the first hypothesis, since the two possibilities are not mutually exclusive, it is worth noting that, using the germ-free conditions described below,

Glassman & Keller's result for hypoxanthine has been confirmed, but not that for uric acid (Table 8).

Quantitative changes in the amount of the yeasts available to larvae reared in bottle cultures have been demonstrated, and these are accompanied by qualitative alterations in the nutrients available as the cultures age (Sang, McDonald & Gordon, 1949). It therefore seems possible that the disappearance of the maternal effect is a consequence of the malnutrition which overtakes larvae during the later phases of the life of a culture. The first issue to be settled, therefore, is whether any nutritional deficiencies modify the maternal effect. If they do not, accumulation of excretory products would probably be the sole agency involved.

2. MATERIALS AND METHODS

The experimental procedure in all but one experiment involved growing the progeny of *C(1)RA*, *yfma-l*$^+$ females by *ma-l*^{1} males. Eggs were collected from flies kept in normal culture bottles, and sterilized by the procedure described by Sang (1956). Larvae hatching from these eggs were then placed in batches of fifty in 5×1 in. tubes containing 5 ml of previously prepared and sterilized media. The synthetic medium (Table 1) was as defined by Sang (1956). Infected cultures were discarded. The eye-colour phenotype of males was used to measure the influence of nutrient deficiencies upon the maternal effect. Significance of difference from controls was assessed by the usual 2×2 χ^2 test.

Table 1. *The defined culture medium*

Constituent	g/100 ml	Constituent	g/100 ml
Agar	3·00	Nicotinic Acid	0·0012
Casein (Genatosan—low vitamin)	5·50	Ca pantothenate	0·0016
Sucrose	0·75	Pyridoxine	0·00025
Cholesterol	0·03	Biotin	0·000016
Lecithin	0·40	Folic acid	0·0003
RNA	0·40	Na HCO_3	0·140
Thiamine	0·0002	KH_2PO_4	0·183
Riboflavin	0·0010	Na_2HPO_4	0·189

In the experiment which studied the influence of lecithin upon enzyme activity, progeny of the cross of *C(1)RM*, *ypnma-l*$^+$ females by *ma-l*^{1}:*cu k-pn* males were grown on synthetic medium in the presence and absence of lecithin. Late third-instar males were collected and used as a source of enzyme. Oregon-R and *ma-l*^{1} adult males from standard stocks grown on cornmeal medium served as controls to the assay. Extraction and assay of XDH activity was accomplished by procedures described by Chovnick (1966).

3. RESULTS

At 25 °C the complete, defined medium (Table 1) permits the normal development of flies in $9\frac{1}{2}$ days, from hatching from the egg to eclosion of the adult. The

segregating population of *ma-l* behaved normally in this respect when reared on the medium, and there was no selective mortality among the phenotypes as judged by the sex ratio. The effects of limiting the nutrients one at a time is considered under two heads: major nutrients and vitamins. The influence of mineral deficiencies was not tested.

Table 2. *Effects of major nutrient deficiencies on expression of the maternal effect*

Medium	Maternally affected flies	*N*
Lecithin omitted	Lethal	—
Lecithin omitted, choline added	15**	20
Cholesterol reduced to 0·0004 g/100 ml	97·3	43
Sucrose omitted	91·0	45
Casein reduced to 2·5 g/100 ml	82·2	56
Control 1: Sang's medium C	90·9	44
Control 2: sterile cornmeal medium	100	37

**Significant at the 1 % level.

Table 3. *Effect of lecithin concentration on expression of the maternal effect, in the presence of 6·4 mg choline %*

Lecithin concentration (mg/100 ml)	Maternally affected flies (%)	*N*
0	11	9
25	34	44
50	39	61
100	69	29
200	85	54

Major nutrients. Table 2 summarizes the effects of lowering the provision of individual major nutrients. The procedure for making the larvae germ-free had no influence on the expression of the maternal effect when they were cultured on sterile, cornmeal media (control 2), but the standard, defined medium (control 1) is close to some threshold concentration of some nutrient(s) essential for 100 % expression of the maternal effect. Although all deficiencies slow development, only lecithin is directly, or indirectly, concerned in the maternal effect (Table 2). This is not compensated for by the small amount of choline provided, which is necessary for larval survival in the absence of lecithin. The lecithin-deficiency effect was confirmed by a dose-response test, which showed that the percentage of maternally affected flies was roughly proportional to the amount of lecithin in the diet (Table 3). Since choline was also provided in this test, it seemed improbable that the choline was the lecithin constituent involved, and a dose-response to choline showed that there was, indeed, no correlation with the dietary provision of choline, down to the lowest survival level. Larvae are synthesizing phospholipids under these condi-

tions, and utilizing the choline (which is an essential dietary requirement) to this end.

The other precursors and constituents of lecithin were tested, but only fatty acids were found to modify the maternal effect (Table 4). Both fatty acids listed

Table 4. *Effects of oleic and linoleic on the proportion of maternally affected flies*

Treatment	Maternally affected (%)	*N*	Development time
Control	38·6	62	0·716
Oleic acid			
0·1%	45·0	80	0·702
0·2%	86·8	68	0·644
0·4%	83·3	62	0·660
Linoleic acid			
0·1%	64·1	64	0·725
0·2%	72·6	64	0·749
0·4%	70·9	48	0·751

The medium was prepared without lecithin, but with 6·4 mg % choline, and the acids were neutralized with bicarbonate. Larval development time is measured in log. days.

Table 5. *Effects of vitamin deficiencies on expression of the maternal effect*

Medium deficiency	Maternally affected flies (%)	*N*
Ca pantothenate	69·5**	23
Nicotinic	0**	4
Pyridoxine	94·4	90
Thiamine	57·1**	14
RNA, omitted	87·5*	16
Biotin, omitted	65·0**	20
Folic, omitted	100·0	28
Riboflavin	94·6	56
Control: sterile cornmeal medium	100	64

Concentrations reduced to a hundredth of the level in Table 1, except where omitted.

* Significant at the 5% level. ** Significant at the 1% level.

affect development rate, but they do so in different ways: oleic speeding development and linoleic slowing it. This again shows that expression of the maternal effect is not just dependent on the rate of larval development, as was previously indicated by the failure of other deficiency treatments to change the proportions of maternally affected males developing in the cultures. Palmitic acid also increased the percentage of wild-type males in the same circumstances, but in all three instances the fatty acids are not as effective as lecithin. In part, this is a consequence of the difficulty of providing the fatty acids in the synthetic food medium. Use of the more readily soluble Tween 80 (polyoxyethylene sorbitan mono-oleate)

showed that about 2 % had to be incorporated in the food before all emerging flies were wild type.

It is concluded that only an adequate source of fatty acids has to be provided for the maternal effect to be expressed, and that deficiencies of the other major nutrients play no significant part in this phenomenon.

Vitamins. All the vitamin deficiencies slow larval development, but only four affect the *ma-l* phenotype. These are thiamine, nicotinic acid, pantothenic acid and biotin (Table 5). Dose responses were run which confirmed the effects of these vitamin deficiencies.

Table 6. *Interactions between lecithin supply and vitamin deficiencies*

Lecithin level	100 mg %		400 mg %		
	Maternally affected (%)	*N*	Maternally affected (%)	*N*	Difference
Control	65·8	79	90·3	41	24·5
Thiamine (10 μg %)	22·7	53	57·6	73	34·9
Pantothenate (100 μg %)	34·8	23	43·8	16	9·0
Nicotinic (160 μg %)	58·2	66	85·0	53	29·8
Biotin zero	25·3	27	70·6	34	45·3

Table 7. *Response to lecithin with low pantothenate in the diet*

Lecithin level (mg %)	100	200	400	800	1600
Maternally affected (%)	78·2	61·7	48·2	66·6	76·6
Number	55	34	56	51	60

Ca pantothenate was provided at 100 μg %

Vitamin deficiencies were examined on diets containing lecithin, so their effects are independent of the lecithin supply. However, it is also important to know if there are interactions between deficiencies of the effective vitamins and low lecithin. Table 6 shows that there are no such interactions between thiamine or nicotinic acid and lecithin level, as judged by the anticipated lowering of the proportion of maternally affected flies on the lesser provision of lecithin. On the other hand, there is evidence of such an interaction with pantothenate, where the lowering is less than would be anticipated, and with biotin, where it is greater (difference column: Table 6). Since lecithin is contaminated with biotin, the latter result is probably explained by the fact that the amount of biotin at the low lecithin level is one-quarter of that at the high level. The interaction with pantothenate is more complex. Unfortunately, a new sample of lecithin had to be used when looking at this interaction and it had different properties from the first. Nonetheless, the response to lecithin with low dietary pantothenate (Table 7) shows that there is an interaction between the two. As before, reducing the lecithin lowers the proportion of maternally affected flies, but with low pantothenate a limit is reached after which the proportion increases.

It is concluded that the consequences of dietary deficiencies of thiamine, nicotinic acid, and probably also of biotin, on the maternal effect are independent of any function they may have in the metabolism of fatty acids, normally provided as lecithin. Figure 1 shows, for example, that the level of thiamine provided has a constant effect on the response to lecithin. There is such an interrelation when dietary pantothenate is deficient, as Table 7 shows.

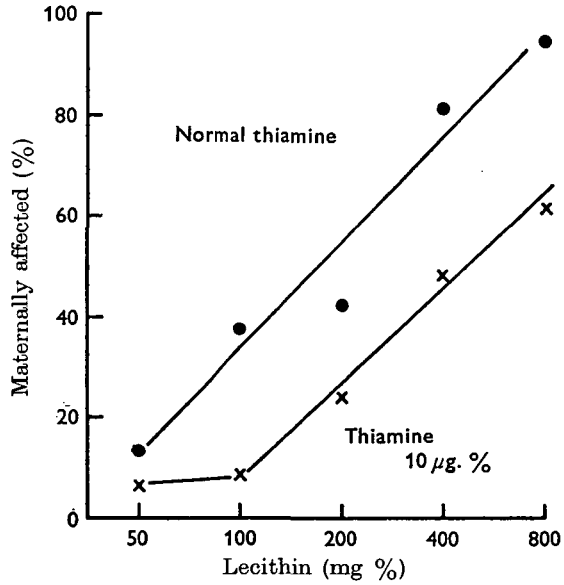

Fig. 1. Responses to lecithin with normal and reduced thiamine in the diet. The differences between equivalent points are significantly different from each other only at the lowest provision of lecithin.

Table 8. *Effects of XDH substrates on the percentage of maternally affected flies*

	Normal lecithin	No lecithin	Difference
Control	66·6 (39)	28·2 (45)	38·4
+ Hypoxanthine	15·3** (39)	5·4* (37)	10·1
+ Guanylic acid	65·6 (64)	38·2 (55)	27·4
+ Uric acid	55·5 (36)	23·8 (42)	31·7

All additions were at 0·1 % and all media contained choline. The number of males scored is shown in parentheses.

* Significant at the 5 % level. ** Significant at the 1 % level.

Other vitamin deficiencies, and low dietary RNA, have no important influence on the maternal effect. It is particularly interesting that a deficiency of riboflavine, which is required as a co-factor for XDH, comes in this category.

XDH substrates. Glassman & Keller (1965) have shown that addition of 0·1 % hypoxanthine, and of 0·1 % uric acid, to normal live yeast cultures, lowers the proportion of maternally affected flies hatching. Table 8 shows that hypoxanthine

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is also effective under germ-free conditions, but uric acid is not. Nor is guanylic acid, which was tested, since, like hypoxanthine, it is a precursor of xanthine. No certain effect was found with xanthine additions, even when 0·2% was used, and the hypoxanthine effect thus seems peculiar to this substance. It is less effective when lecithin is omitted from the diet than with lecithin (Table 8).

Guanylic acid is a precursor of insect pteridines (Brenner-Holzach & Leuthardt, 1961) and it is interesting to note that an extra supply of this substance has no effect on eye pigmentation (Table 8), even when the balance of eye colour phenotypes in the experimental populations is at a very sensitive level. Folic acid is also a possible pteridine precursor, and additional supplies (up to 16 times), had also no influence on the proportion of normal-eyed flies in the population. There seems therefore to be no limitation upon the supplies of pteridine precursors in *ma-l* larvae.

4. DISCUSSION

The above data show that dietary deficiencies of any one of four vitamins essential for *Drosophila melanogaster* (thiamine, pantothenic acid, nicotinic acid or biotin), or of available fatty acids, could lead to disappearance of the maternal effect, which is found when the *ma-l* genotype, bred from wild-type mothers, is reared in aged cultures. Accumulation of one possible excretory product, hypoxanthine, could also have the same consequences. Since no exact data exist concerning changes of the nutritional status of the ordinary live yeast culture with age, it is impossible to say which deficiency is usually effective, or if the normal progression from wild-type to *maroon-like* emergents is a consequence of the combined action of all the possible factors. It can be noted that certain mutant genes of variable penetrance and expressivity—for example, *eyeless* (Sang & Burnet, 1963) or *antennaless* (Gordon & Sang, 1941)—clearly indicate that nutritional changes do occur in such cultures, and that they are of sufficient magnitude to alter the penetrance of these genes. It is more difficult to be sure that accumulation of hypoxanthine in the food would also be effective in normal cultures. Two facts argue against this conclusion: the time course of the change from wild-type to *maroon-like* phenotypes can readily be modified by altering the medium composition, and the amount of hypoxanthine which has to be added to the medium to give significant effects (under germ-free conditions) is very much higher than the quantity which might be expected to accumulate as an excretory product. The nutritional changes are therefore the more likely cause of modification of the *maroon-like* maternal effect in ordinary cultures.

Although little or no active XDH is found in the eggs giving rise to maternally affected *ma-l* males, the largest amount is found in newly hatched larvae. It declines at a rate such that about half the initial activity is found in early pupae (Glassman & McLean, 1962). The hypoxanthine result implies that the greater part of the enzyme is combined with hypoxanthine, and is not available for pteridine oxidation when the wild-type eye pigments are being formed. It seems improbable that any of the nutritional deficiencies operate by a similar mechanism,

either directly or indirectly. That is, maternally affected *ma-l* larvae should have the expected complement of XDH even when reared on deficient diets. Figure 2 shows this is, in fact, the situation for larvae reared on low lecithin. It follows that the nutritional effects must influence the availability of the pteridines which form the red eye pigments (drosopterines), since it is only by the presence or absence of these that the phenotype is being assessed.

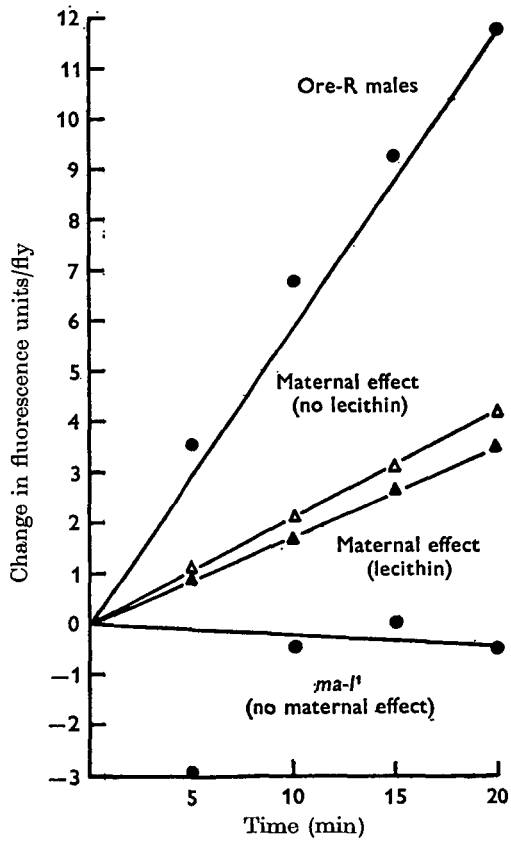

Fig. 2. The XDH content of male larvae. Maternally affected $ma\text{-}l^1$ (triangles) grown on diets containing normal lecithin or lacking lecithin are not distinguishable from one another, but distinctly different from wild-type (Ore-R), and from $ma\text{-}l^1$ male larvae derived from $ma\text{-}l^1$ homozygous mothers.

The dose-response to lecithin (Table 3), and the ability of fatty acids (Table 4) to substitute for lecithin, suggest that the *de novo* synthesis of fatty acids (which Keith (1967) has shown occurs in *Drosophila*) is important in its effects upon the formation of the $ma\text{-}l^+$ eye pigments. This fatty-acid synthesis must reduce the quantity of drosopterin formed, but so little is known about the biogenesis of this group of pteridines (Hubby & Forrest, 1960) that it is idle to speculate about detailed mechanisms. It is interesting, however, that the addition of phenylalanine to the diet (Fig. 3) results in a decline in the proportion of wild-type flies, since

phenylalanine might also be expected to divert some pteridine (the drosopterin precursor sepiapteridine?) to such a function, in this case, as a co-factor for phenylalanine hydroxylation (Kaufman, 1963). There is no evidence that the same co-factor is required for fatty-acid synthesis by Drosophila, but Kidder & Dewey (1963) have found that an unconjugated pteridine (biopterin or neopterin) is necessary for the desaturation of fatty acids by the trypanosome, *Crithidia fasciculata*.

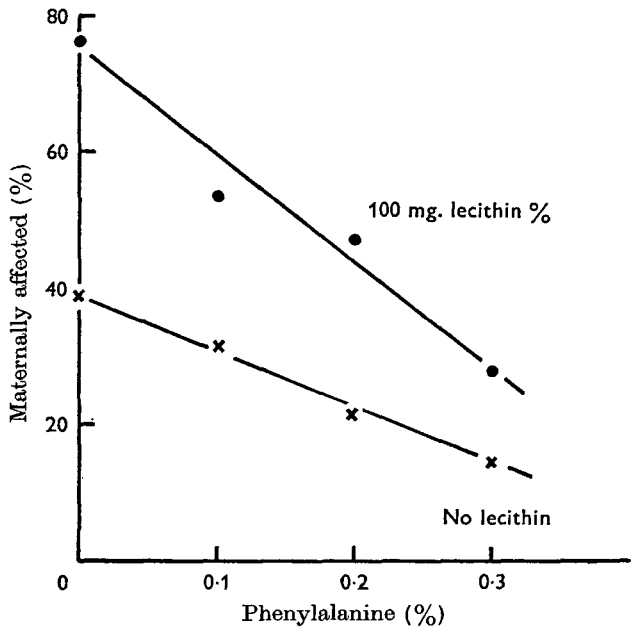

Fig. 3. The effects of adding l-phenylalanine to diets containing, or lacking, lecithin.

All the effective vitamin deficiencies could influence fatty-acid synthesis, since they are all involved in this pathway. But interpretation of their possible roles, and of how they might specifically affect eye pigment formation, is not possible in the present state of our knowledge of *Drosophila* metabolic biochemistry.

SUMMARY

1. The *maroon-like* maternal effect, present when *ma-l* flies are bred from non-mutant mothers, is not found among the late emergents from normal cultures. The hypothesis that this is due to a deficiency of some nutrient(s) in old cultures is tested by growing larvae on defined, germ-free media with the nutrients reduced one at a time.

2. Omission of lecithin (but not reduction of casein, sucrose, RNA or cholesterol) decreases the percentage of maternally affected emergents, in proportion to the lowering of the lecithin supply. This is not a consequence of choline deficiency but of the shortage of fatty acids. Oleic, palmitic and linoleic acids can substitute for lecithin, when adequate choline is provided.

3. Pantothenic acid, nicotinic acid, thiamine and biotin deficiencies (but not reduced pyridoxine, riboflavine or folic acid) also lower the proportion of maternally affected flies. There is an interaction between pantothenic acid and lecithin, but not between lecithin and the other effective vitamin deficiencies.

4. Addition of the excretory product hypoxanthine to the complete diet significantly lowers the proportion of maternally affected emergents, but equivalent amounts of uric acid, or of guanylic acid, do not. It is argued that the quantitative relationships make it improbable that accumulation of excretory products play any great part in the disappearance of the maternal effect, whereas it is more likely that nutritional deficiencies do.

5. *ma-l* larvae grown on low lecithin have the same complement of xanthine dehydrogenase as those reared on normal lecithin. The lecithin effect is therefore assumed to operate by affecting the pattern of pteridine synthesis. Phenylalanine, which would also be expected to alter this pattern, modifies the maternal effect similarly.

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