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A purine and pyrimidine requiring mutant of *Drosophila melanogaster*

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SUMMARY

The E.M.S.-induced auxotrophic mutant *1308* of *Drosophila* is located between 13·7 and 21·0 on chromosome 1. Survival of larvae is possible if whole RNA is provided in the diet, and this is shown to be a requirement for a source of both purines and pyrimidines. A proportion of larvae will grow on pyrimidine nucleosides alone. Tests for utilization of bases and of precursors suggest that *1308* is a double mutant, and not a single mutation preventing the synthesis of a common precursor of purines and pyrimidines.

1. INTRODUCTION

Three auxotrophic mutants of *Drosophila melanogaster* have been described. The first, an adenine-requiring mutant, proved to be genetically complex and its expression dependent on culture conditions, particularly the pH of the culture medium (Ellis, 1959). The second, again a nucleic acid auxotroph, also seemed to have a complex requirement (Vyse & Nash, 1969) because the requirement could not be met by provision of nucleotides in the diet, but could be met by RNA. This paper examines this problem in greater detail and shows that a source of both purines and of pyrimidines is necessary for larval survival. While the work was in progress, a third auxotroph was reported by Nørby (1969), who found that mutants at the *rudimentary* locus are lethal in the absence of pyrimidines, but can grow when provided with them. Unlike *rudimentary*, the mutant studied in this report is morphologically indistinguishable from wild-type.

2. MATERIALS AND METHODS

Isolation of the mutant strain *1308* has already been described (Vyse & Nash, 1969). For convenience of handling it has been marked with yellow (*y*) and forked (*f*). The marked strain so established has the same nutritional characteristics as the original, and has been used for some of the experiments. Location of the *1308* mutant has been further refined by crossing *1308* to *wsnm* (white eyes, singed bristles and minute wings) and backcrossing heterozygous females in the conventional way, except that their progeny were reared on the RNA-free media described below. About 100 larvae were inoculated into each sterile minimal culture, and the adults scored as they emerged. A more detailed description of the mutants and symbols used may be found in Lindsley & Grell (1968).

Stocks were maintained as mass cultures on Lewis's yeasted cornmeal medium and kept at 25 °C. Eggs were collected and surface-sterilized as described by Sang & Burnet (1963). Forty newly hatched larvae were transferred to the test media on sterile paper

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spoons using aseptic techniques. The culture medium was that described by Bryant & Sang (1969), but without RNA and supplemented as described in the Tables. This medium contained an all-potassium buffer in place of the usual sodium/potassium buffer (Sang, 1956) and there is some evidence that this improved survival (Sang, unpublished results). It was also supplemented with 0·062% magnesium sulphate. The RNA was provided in optimal amount (4·0 mg/ml), the nucleotides at 1·0 mg/ml, the nucleosides at 0·75 mg/ml and the bases and precursors at 0·5 mg/ml. Additives were all obtained from Sigma Chemical Company, except for orotic acid (Nutritional Biochemicals), RNA and 5-amino-4-imidozole carboximide ribotide (AICR) (Calbiochem). Effects of the treatments were measured in terms of survival to emergence.

3. RESULTS

In the mapping experiments the female classes are controls for the male classes, since heterozygous females are fully viable (Table 1). The expected lethal male class (+ + +) shows some survivors, for reasons which remain obscure. The large deficiency of + + *m* males indicates that the mutation lies to the left of *m*. The reduction of males in the *w* + + class, together with the near expected numbers in the reciprocal + *snw* class, indicates that the mutation lies between *w* and *sn*, but closer to the latter. Since the *1308* mutation is known to be to the right of crossveinless (*cv*) (Vyse & Nash, 1969), it must lie between *cv* and *sn* (13·7–21·0).

Table 1. *Results from testing the* w sn m/1308 ♀ × w sn m ♂ *progeny on defined media without RNA*

Phenotypic class	*wsnm*	+ + +*	*w* + +	+ *snm*	*wsn* +	+ + *m*
♀	225	251	42	35	46	44
♂	254	36	18	33	32	3

* *1308* but carrying the wild-type alleles of *w sn* and *m*. Only two double recombinants were found.

Table 2. *Tests for the survival of* y 1308 f *and of Oregon wild-type larvae in the presence or absence of nucleosides*

	y 1308 f		*Oregon*	
Additives	No. of larvae	Survival (%)	No. of larvae	Survival (%)
Nil	240	1·2	160	56·3
RNA	200	30·5	120	49·2
Adenosine	240	0·8	160	36·9
Uridine	200	6·0	160	50·0
Adenosine, uridine	240	23·4	160	62·0
Inosine, uridine	240	26·7	160	56·8
Adenosine, cytidine	240	24·2	160	51·3

In previous experiments (Vyse & Nash, 1969) individual nucleotides or combinations of nucleotides did not substitute for RNA in the diet. In contrast, the present experiments (Table 2) show that a source of both purine and of pyrimidine will permit survival of *1308*. Inosine will replace adenosine, and cytidine and uridine are interchangeable as pyrimidine sources, as they are in the nutrition of wild type (Burnet & Sang, 1963). Surprisingly, uridine permits some mutant survival under the improved nutritional

conditions of these cultures, but adenosine does not. This result is confirmed in Table 3, which also shows that guanylic acid cannot replace adenosine, as is again the case for wild-type nutrition. That is, *1308* requires a source both of purines and of pyrimidines in order to survive normally, but the requirement for purine is not absolute. Survival of a wild-type strain (Table 2) is but little affected by the absence of dietary purines or pyrimidines, or both.

In wild-type nutrition, purine bases can substitute for the nucleosides or nucleotides, but pyrimidine bases cannot (Sang, 1957). This is also the case for *1308* where adenine and

Table 3. *Survival of* 1308 *larvae in the presence or absence of nucleosides or nucleotides*

Additives	No. of larvae	Survival (%)
Nil	80	0
Adenosine	40	0
Uridine	80	12·5
Adenosine, uridine	80	32·5
Adenosine, cytidine	120	25·0
Adenosine, guanylic acid	40	0
Cytidine, uridine	80	15·0
Cytidine, guanylic acid	160	11·4
Uridine, guanylic acid	160	10·6

Table 4. *Effects of feeding purine or pyrimidine bases to* 1308 *larvae in the presence of adenosine or uridine*

Additives	No. of larvae	Survival (%)
Adenosine, uridine	240	20·8
Adenine, uridine	200	18·5
Hypoxanthine, uridine	240	25·4
Xanthine, uridine	320	6·9
Cytosine, adenosine	120	0·5

Table 5. *Effects of the addition of pyrimidine precursors on the survival of the* y1308f *strain in the presence of adenosine*

Additives	No. of larvae	Survival (%)
Nil	360	2·5
Uridine	360	26·6
Carbamyl aspartate	360	4·4
Orotic acid	120	0·8
Without adenosine	240	0

Table 6. *Effects of the addition of 5-amino-4-imidazole carboximide ribotide on the survival of* 1308 *larvae in the presence of uridine*

Additives	No. of larvae	Survival (%)
Adenosine	240	14·5
AICR	120	13·3
Without uridine	360	1·1

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hypoxanthine can replace adenosine (Table 4). Xanthine is apparently not used, although this conclusion is uncertain since the proportion of larvae surviving on uridine alone is variable (Tables 2, 3). Cytosine is not used by the mutant. So, purine bases are salvaged for incorporation into nucleic acids (by *1308* larvae), but pyrimidine bases are not.

The purine precursor AICR substitutes for adenosine, but neither carbamyl aspartate or orotic acid substitute for uridine (Tables 5, 6). Orotic acid is not used by wild-type (Sang, 1957).

4. DISCUSSION

Since differences in the nutritional adequacy of different batches of casein, of agar and of other constituents of the culture medium are known to affect larval survival (Sang, 1956), it is possible that the discrepancy between the results for uridine, reported here and in the previous publication (Vyse & Nash, 1969) are due to this cause. The change in the phosphate buffer and the addition of magnesium sulphate may also contribute to the differences found. Differences in the survival of wild-type (cf. Table 2 here, and table 3 of the previous paper) on RNA-deficient diets suggest that this explanation is correct. The present data show that the *1308* mutation has induced a double nutritional requirement: that for the pyrimidine nucleoside being more exacting than that for the purine nucleoside. There are two possible explanations of this double-requirement: either a single mutation has blocked the synthesis of a precursor common to both pathways, or two closely linked mutations have occurred, each affecting a separate pathway. The only precursor common to both pathways in other organisms is 5-phosphoribosyl-pyrophosphate (PRPP). Bases must combine with PRPP before they are incorporated into nucleic acids. Since purine bases are used by *1308* (Table 4), it follows that PRPP must be synthesized by the larvae and that the mutation does not block the synthesis of this common precursor. It is therefore more probable that *1308* is a double mutant. The fact that the mutation on the purine pathway is 'leaky', whereas the mutation on the pyrimidine pathway is not, would support this contention. However, too little is known about the synthesis of nucleic acids by *Drosophila* for us to be sure of this, and it will therefore be necessary to attempt to separate what are apparently closely linked mutations.

The data confirm previous findings concerning the utilization of dietary purines (Sang, 1957; Burnet & Sang, 1963) and extends them by showing that AICR is used for purine synthesis. The *1308* mutation must block purine synthesis at a step earlier than this.

Orotic acid is not utilized by wild-type *Drosophila* (Sang, 1957; Burnet & Sang, 1963) but it is utilized by rudimentary mutants (Nørby, 1970). A similar observation has been made with yeast in which wild-type strains do not utilize pyrimidines but pyrimidine requiring mutants do (Lacroute, 1968). The failure of *1308* to survive on media supplemented with orotic acid might be interpreted in either of two ways: (1) *1308*, a pyrimidine requiring mutant, is defective in the enzymic steps converting orotic acid to uridylic acid, or (2) *1308*, like wild-type, does not utilize orotic acid. Experiments to test these two alternatives are currently in progress.

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