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THE UNIVERSITY OF ALBERTA

# SECOND CHROMOSOME PURINE AUXOTROPHS OF *DROSOPHILA MELANOGASTER*

by

MARLENE EVELYN JOHNSTONE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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Supervisor

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Date November 9, 1984

## ABSTRACT

Mutations at three second chromosomal loci of *Drosophila melanogaster* have been characterized. All are auxotrophic for purines. Pteridine complements in adult flies were visualized chromatographically. Assays were carried out to assess enzyme activities which might, potentially, account for the auxotrophic phenotypes.

Mutants at the *adenosine2* locus, which is located at map position 18.4, require supplementation with adenosine, inosine or adenine during the second larval instar. Adult homozygotes have a dilute dull-red eye-colour which probably results from their severely reduced drosopterin content. *In vitro* assays show that the *ade2-1* mutant has six-fold lower guanine deaminase activity than wild-type.

The second locus, *adenosine3*, also produces mutants which require adenosine, inosine or adenine. The nutritional requirement persists throughout the larval stage and has a more consistent effect on development time than on mutant viability. The *ade3* locus is at map position 20.0 in the cytological interval, 26A7 to 27E1. The adult eye-colour and pteridine content, and larval guanine deaminase activity are all wild-type. The location and phenotype of *ade3-1* suggest the possibility that it affects the region shown by Henikoff and his co-workers (1983) to encode GAR transformylase.

Guanosine supplements mutants at the *burgundy* locus (55.7); this locus was described previously through a pteridine eye-colour defect, but identified as an auxotrophic locus after the isolation of a new allele, *bur-gua2-1*. Homozygotes of *bur-gua2-1* require supplementation until after the second larval moult. In adults, the pteridine content is less obviously disturbed than expected. Guanine deaminase activity is wild-type.

In the original isolate of *bur-gua2-1*, IMPdehydrogenase activity and female fertility were low. Although reduced synthesis of GMP would account for the auxotrophic phenotype, it transpired that IMP dehydrogenase activity returned to wild-type levels apparently as a concommitant of natural selection for increased fertility.

without altering the nutritional requirement. The relationship of IMPdehydrogenase and the *bur* locus is therefore unclear.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| A | Adenine |
| AMP | Adenosine-5'-monophosphate |
| APRT | Adenine phosphoribosyltransferase |
| AR | Adenosine |
| C | Cytosine |
| CR | Cytidine |
| G | Guanine |
| GMP | Guanosine-5'-monophosphate |
| GR | Guanosine |
| H | Hypoxanthine |
| HGPRT | Hypoxanthine-guanine phosphoribosyltransferase |
| HR | Inosine |
| IMP | Inosine-5'-monophosphate |
| NAD | Nicotinamide adenine dinucleotide |
| U | Uracil |
| UA | Uric acid |
| UR | Uridine |
| X | Xanthine |
| XDH | Xanthine dehydrogenase |
| XMP | Xanthosine-5'-monophosphate |
| XO | Xanthine oxidase |
| XR | Xanthosine |

# I. INTRODUCTION

## 1. Auxotrophy

The class of mutants known as auxotrophs is characterized by an abnormal requirement for compound(s) normally synthesized endogenously. The discovery of these mutations (Beadle and Tatum, 1941) led to significant advances in understanding fundamental genetic mechanisms in prokaryotes. When the nutritional requirements of Drosophila were defined (Sang, 1956) and an exceedingly effective technique for mutagenesis became available (Lewis and Bacher, 1968) the stage was set for the isolation of auxotrophs in a multicellular animal.

From the outset, the search for auxotrophic mutants in Drosophila was restricted mainly to those deficient in nucleotide metabolism. The nucleotide pathways are the only extensive pathways in the organism which are well-defined biochemically, known to function, and plausibly subject to replacement by dietary supplement. A number of mutants which are unable to survive without exogenous purines (Vyse and Nash, 1969; Naguib and Nash, 1976; Johnson, *et al.*, 1977) or pyrimidines (Norby, 1970; Falk and Nash, 1974; Lastowski and Falk, 1980; Porter and Rawls, 1984) have been recovered. Mutations affecting *de novo* pyrimidine biosynthesis (the *rudimentary*,*rudimentary-like* and *dihydroorotate dehydrogenase* loci) have been characterized enzymologically. However, none of the purine auxotrophs has yet been unambiguously assigned an enzymatic defect. (See the review by Nash and Henderson, 1982).

Largely because it is so easy to work with, most of the purine auxotrophs known in Drosophila are located on the X-chromosome. Mutations at three of these loci require an adenine source. Two are newly isolated, and beyond their observed mutant brown eye-colour, and their location between *vermilion* (1-33.0) and *forked* (1-57.6), little is known of them (Nash, unpublished). The third locus, *adenosine1* (1-57.0) (Falk and Nash, 1974b), is also poorly defined, primarily because the

auxotrophic phenotype is not very strong. Homozygous *ade1-1* individuals survive, but they develop very slowly in the absence of an adenine source. It is known from radioactive tracer studies (Johnson, *et al.*, 1977) that purine metabolism is not grossly perturbed in this mutant.

The best characterized X-linked purine auxotrophs in Drosophila are mutant at the *guanosine1-purine1-raspberry* complex (1-32.8) (Nash, *et al.*, 1980). The complex includes 3 non-allelic, but interacting, loci: *pur1* mutants respond to either an A or a G source, and *gua1* mutants require guanosine but *ras* homozygotes do not require supplementation (Falk and Nash, 1974b). In addition, *pur1* mutants exhibit a slight morphological wing defect which differs from that of pyrimidine auxotrophs (Nash, *et al.*, 1980), the *gua1-1* auxotrophy is temperature-sensitive (Falk and Nash, 1974b) and *ras* homozygotes have mutant-coloured brown eyes. Lethal alleles of *ras* are also allelic with *pur1* and *gua1*. In fact, the heterozygous combinations of *gua1-1* and most *ras-l* alleles are auxotrophic at all temperatures, in contrast to the temperature-sensitive phenotypes of *gua1-1/gua1-1* and *gua1-1*/deficiency (Johnson, *et al.*, 1979). Of the mutant *pur1* alleles, one is lethal and the other temperature-sensitive lethal against a deficiency on all culture media (Johnson, *et al.*, 1979). Heterozygous *pur1-1/gua1-1* individuals develop slowly in the absence of guanosine. Although the biochemical nature of these defects is unclear, purine synthesis appears normal in *pur1* mutants (Johnson, *et al.*, 1977). Because the same work showed that catabolism is enhanced, the suggestion has been made that the wild-type allele of *pur1* has a role in regulating the rate of catabolism.

Four auxotrophic loci are known on the second chromosome. Three are the subject of this work and will be discussed presently. The fourth, *nucleoside1* (approx. 2-104), does not, strictly speaking, yield purine auxotrophs. Mutants of *nuc1* require purine or pyrimidine ribonucleosides, but do not respond to deoxyribonucleosides (Naguib and Nash, 1976; Nash, unpublished). These mutants appear to suffer from a deficiency

of PRPP.

Neither of the third or fourth chromosomes have yet been screened for auxotrophs.

The work presented here addresses the characterization of three autosomal recessive purine auxotrophs. Some observations were already available regarding the mutants under study. Two of the mutants, *ade2-1* (Naguib, 1976) and *ade3-1* (Nash, unpublished), were known to respond to supplementation with AR or HR. The third, *bur-gua2-1* (Nash, unpublished) had been shown to require GR. (This mutant was originally identified as *gua2-1*; the designation was changed in response to results to be presented later.) All of the auxotrophic mutations were known to be located on the second chromosome, two near *Sternopleural* (2-22.0) and the third near *purple* (2-54.5). The initial portion of this work confirms and extends the nutritional characterization and provides more precise map locations.

*In vivo* radioactive tracer studies (Johnson, *et al.*, 1977; Johnson, 1978) provided information about the metabolism of purines in homozygous larvae. This suggested biochemical bases for two of the auxotrophic mutations. These hypotheses were tested directly by *in vitro* enzyme assays.

One other feature of the auxotrophs attracted attention: in two of them, the eye-colour of the homozygous adults is visibly mutant. Pteridines, one class of pigment molecules found in Drosophila eyes, are derived from purines. This knowledge prompted an examination of the pteridine content of the mutants.

The eye-colour phenotypes of these mutants can be used to illustrate one of the inherent difficulties of studying auxotrophy in a multicellular system. Based on nutritional studies alone, two of the mutants, *ade2-1* and *ade3-1*, appear to be nearly identical. However, only one, *ade2-1*, has a visibly mutant eye-colour. On the other hand, the two mutants which have in common visibly mutant pigmentation, *ade2-1* and *bur-gua2-1*, are dissimilar with respect to supplementation patterns. The simple

correlation between the biochemical defect and the resulting phenotype which often typifies auxotrophic mutations in prokaryotes is somewhat obscured in Drosophila.

## 2. *De novo* Purine Biosynthesis

The pathway of *de novo* biosynthesis of IMP is presented in Figure 1. The supposition that this is the universal pathway (Henderson, 1972) is certainly supported in Drosophila; radioactivity from labelled formate (Johnson, *et al.*, 1980b) or glycine (Johnson, 1978) fed to growing larvae can be found in IMP. Use of "*de novo* synthesis" in this text will be understood to refer to the pathway in Figure 1.

The reaction catalyzed by phosphoribosylpyrophosphate (PRPP) amidotransferase is the first step committed solely to the biosynthesis of purines. As such, it is a probable site of regulation of the pathway. This issue is discussed in detail in Henderson (1972), where extensive references are to be found, and will be touched upon only briefly here. In preparations from a variety of microbial, avian and mammalian sources, PRPP amidotransferase activity has been found to be regulated by the concentration of purine nucleotides. However, although subject to endproduct inhibition *in vitro*, it is not clear that this is an important control mechanism *in vivo*. To the contrary, Gordon *et al.* (1979) showed, in intact human fibroblasts, that PRPP amidotransferase is not endproduct inhibited.

The rate of *de novo* synthesis in mammalian cell lines appears, instead, to be regulated by the availability of PRPP, the substrate of the PRPP amidotransferase reaction (de Batisse and Buttin, 1977; Bashkin and Sperling, 1978; Holland, *et al.*, 1978; Taylor *et al.*, 1982). The synthesis of PRPP from ribose-5'-phosphate and ATP, catalyzed by PRPP synthetase, is, itself, feedback inhibed by A and G nucleotides *in vivo* (Bagnara, *et al.*, 1974). The amount of PRPP available for *de novo* synthesis is further affected in human fibroblasts (Gordon *et al.*, 1979) and in lymphocytes of five mammalian species (Peters and Veerkamp, 1979) by its preferential utilization in

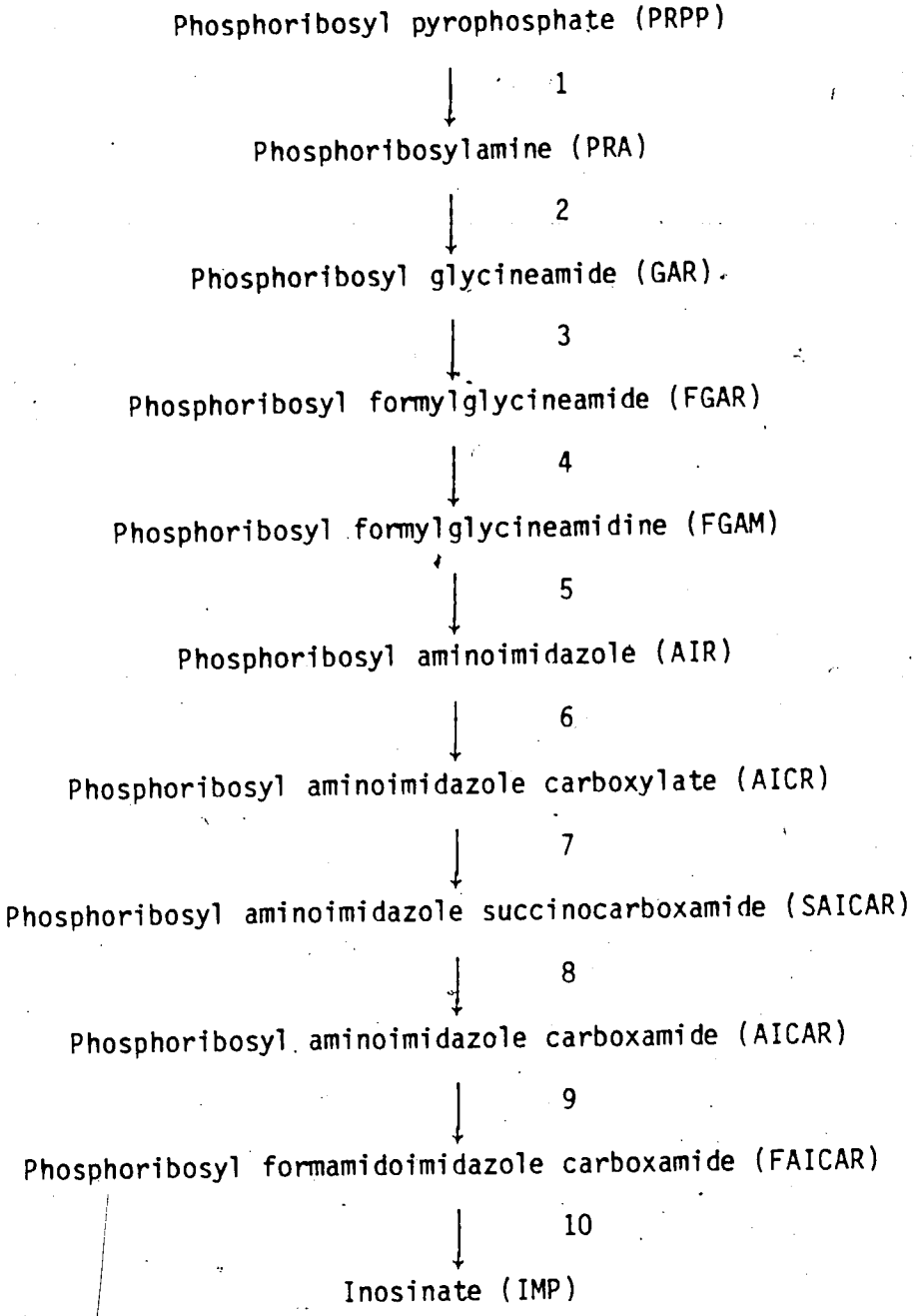



FIGURE 1: The Pathway of Inosinate Biosynthesis *de novo*

The pathway of the *de novo* biosynthesis of IMP is shown. The numbers refer to the names of the enzymes responsible for each step and are defined in Table 1.

The information is taken from Henderson (1972).

phosphoribosyltransferase reactions. It is known that in humans, mutations which increase the available pool of PRPP by either increasing PRPP synthetase activity or reducing HGPRT activity (Lesch-Nyhan syndrome) (reviewed by Boss and Seegmiller, 1982) result in overproduction of purines.

In at least some prokaryotes, *de novo* synthesis is regulated by repression as well as feedback inhibition (Koduri and Gots, 1980; Levine and Taylor, 1981). Repression acts at the level of the nucleic acids to block the production of specific enzymes. Whether this mechanism is involved in the regulation of purine biosynthesis in eukaryotes is not clear. However, Taylor *et al.* (1982) found no evidence for a repressor molecule involved in regulating the early steps of *de novo* synthesis in Chinese hamster cells.

The only biosynthetic enzyme which has been studied in Drosophila is phosphoribosyl-glycineamide formyltransferase (GAR transformylase) which catalyzes the third step in the pathway. Using recombinant DNA techniques, Henikoff *et al.* (1981) identified a segment of DNA from the left arm of Drosophila chromosome 2 which complements *ade8* (GAR transformylase$^-$) mutants in *S. cerevisiae*. *In vitro* assays have shown that the activity encoded is indeed GAR transformylase (Kelly and Henikoff, unpublished).

The recent work of Henikoff *et al.* (1983) has shown that the locus encoding GAR transformylase in Drosophila is unexpectedly complex; sequence analysis suggests that the protein product is a large, possibly multifunctional, polypeptide with GAR transformylase as the COOH-terminal "domain". In addition, a polyadenylation signal in an intron allows alternative processing of the RNA transcript. Thus, the locus can encode two polypeptides which differ in the presence or absence of the carboxy-terminal region including GAR transformylase.

A multi-enzyme complex including GAR transformylase and five enzyme activities involved in the synthesis and use of one-carbon tetrahydrofolate cofactors is known in

chickens (Caperelli, *et al.*, 1980; Smith, *et al.*, 1980). It is thought that proximity to GAR transformylase during synthesis may result in preferential utilization of folate intermediates in *de novo* synthesis, although tetrahydrofolate is also required in other metabolic pathways. A multi-enzyme complex of this type has not been demonstrated in Drosophila although the work described above (Henikoff *et al.* 1983) is suggestive.

In contrast to the paucity of such information for Drosophila, fifteen years of work has resulted in the identification, in Chinese hamster cells, of the genes encoding all but one of the *de novo* synthetic enzymes. The mutations listed in Table 1 are all recessive auxotrophies which produce a nutritional requirement for A, H or their ribonucleosides or ribonucleotides (See Kao and Puck (1982) for references.). Characterization of the mutational blocks has proceeded by identification of accumulated intermediates, presumed to be the substrates of the blocked reactions (for instance, Feldman and Taylor, 1974; Patterson, 1975), by comparison with phenocopies produced by specifically inhibiting known reactions (Patterson, 1976a) and by *in vitro* enzyme assays (Irwin, *et al.*, 1979; Patterson, 1976b). As shown, mutations are now known which affect every step except the seventh, phosphoribosyl-aminoimidazole succino-carboxamide synthetase.

It is possible to create hybrid cells containing both Chinese hamster and human chromosomes. Although the human chromosomes are usually lost fairly quickly, individual chromosomes will be retained if they carry the only active copy of a gene essential for the survival of the hybrid. Since human chromosomes can be identified in the hybrid cells, it is possible, under the appropriate selective conditions, to determine which human chromosome encodes the activity under selection. Using this technique, it has been shown that the human gene which complements *adeA* mutants, is on chromosome 4 (Stanley and Chu, 1978), *adeB* (Kao, 1980) and *adeE* (Jones, *et al.*, 1981) are on chromosome 14 and *adeC* (Moore, *et al.*, 1977) and *adeG* (Patterson, *et al.*, 1981) are on chromosome 21. Although not necessarily significant, the synteny

TABLE 1: Mutants of Chinese Hamster Cells Defective in *de novo* Purine Biosynthesis

| Step | Enzyme | Mutant | References |
|---|---|---|---|
| 1 | Phosphoribosyl pyrophosphate amidotransferase EC. 2.4.2.14 | ade A | a b c d |
| 2 | Phosphoribosyl glycineamide synthetase EC. 6.3.1.3 | ade C | b c e f |
| 3 | Phosphoribosyl glycineamide formyltransferase EC. 2.1.2.2 | ade E | b c g |
| 4 | Phosphoribosyl formylglycineamidine synthetase EC. 6.3.5.3 | ade B | a b c h i |
| 5 | Phosphoribosyl aminoimidazole synthetase EC. 6.3.3.1 | ade G | f j |
| 6 | Phosphoribosyl aminoimidazole carboxylase EC. 4.1.1.21 | ade D | b c |
| 7 | Phosphoribosyl aminoimidazole succino-carboxamide synthetase EC. 6.3.2.6 | | |
| 8 | Adenylosuccinate lyase EC. 4.3.2.2 | ade I | k |
| 9 | Phosphoribosyl aminoimidazole carboxamide formyltransferase EC. 2.1.2.3 | ade F | b c |
| 10 | Inosinate cyclohydrolase EC. 3.5.4.10 | ade F | b c j |

The step numbers in this table refer to the steps in the pathway of the *de novo* biosynthesis of IMP presented in Figure 1. The enzyme names are taken from Henderson (1972). The mutants named have been isolated in Chinese hamster cells in culture. All are auxotrophic and respond to appropriate dietary supplementation. The lower case letters indicate which of the following list of references contain further descriptions of each mutant.

References:
a. Kao and Puck, 1972.
b. Patterson, Kao and Puck, 1974.
c. Patterson, 1975.
d. Feldman and Taylor, 1975.
e. Moore *et al.*, 1977.
f. Irwin, Oates and Patterson, 1979.
g. Jones, Patterson and Kao, 1981.
h. Feldman and Taylor, 1974.
i. Kao, 1980.
j. Patterson, Graw and Jones, 1981.
k. Patterson, 1976b.

of the *adeB* and *adeE* genes is interesting since it is the first time that genes encoding sequential metabolic steps have been located on a single chromosome in humans.

In Chinese hamster cells two cases of apparent coordinate regulation of steps in the *de novo* pathway are known, *adeA* and *B* (Oates, *et al.*, 1980) and *adeC* and *G* (Patterson, *et al.*, 1981). In both cases, single mutations, *adeP AB* and *adeP CG* respectively, simultaneously eliminate the two enzyme activities and single reversion mutations restore the activities.

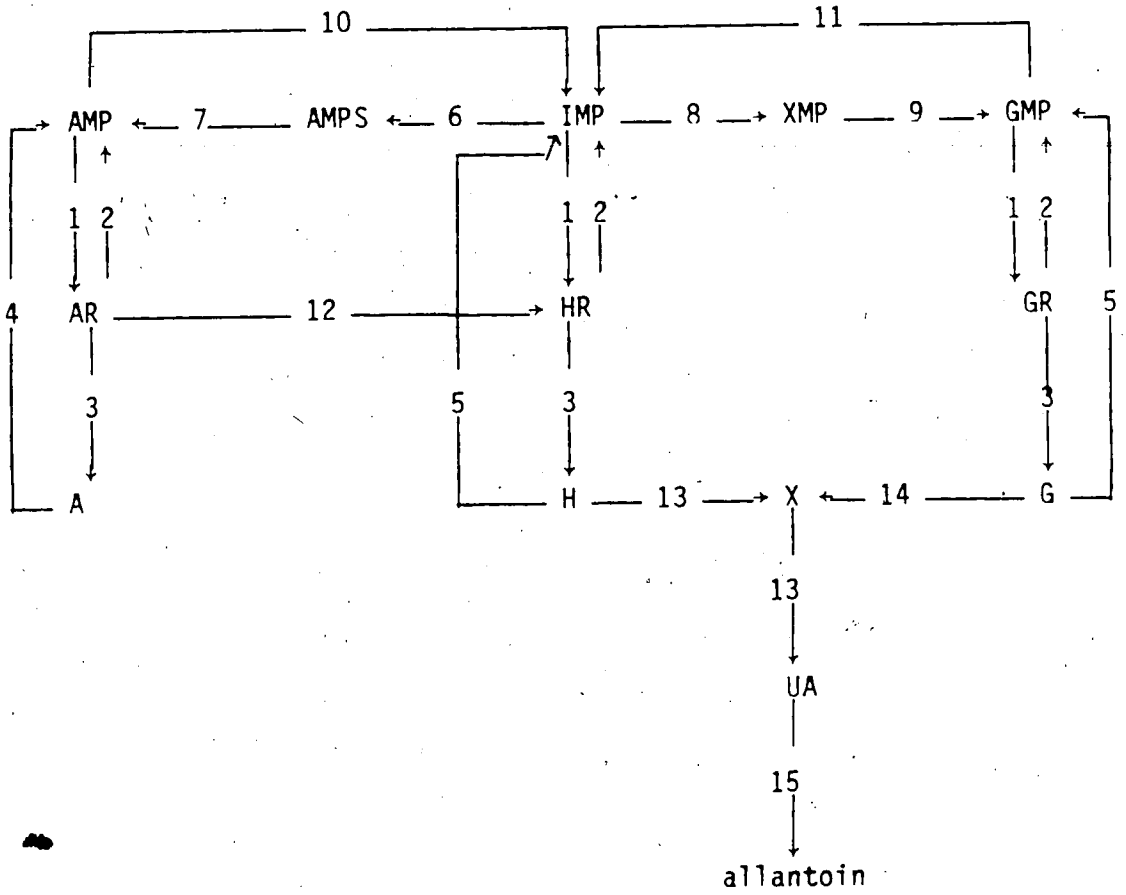
In humans, at least, *adeA* and *adeB* are on different chromosomes and presumably encode separate polypeptide chains. The activities encoded by *adeA* (PRPP amidotransferase) and *adeB* (phosphoribosylformylglycineamidine synthetase) both require glutamine, which may, in some manner, be the basis of their co-regulation. In the second case, *adeC* and *adeG* are syntenic in humans and coordinately regulated in Chinese hamster cells. Thus there is a possibility that these activities, phosphoribosylglycineamide synthetase and phosphoribosylaminoimidazole synthetase, exist in a multifunctional polypeptide, as has been suggested for *Schizosaccharomyces pombe*. Some of the enzymes of purine biosynthesis partially copurify, which, combined with this instance of coordinate regulation and synteny, suggests that, like those of pyrimidine metabolism, the enzymes of *de novo* purine biosynthesis may exist in a limited number of multi-functional enzyme complexes.

### 3. Purine Interconversion and Catabolism

The enzymes of purine interconversion and catabolism (see Figure 2) have been studied in greater detail than those of *de novo* synthesis. Efforts to establish a defined growth medium for Drosophila (reviewed by Sang, 1978) produced indirect evidence for the presence of the salvage enzymes, insofar as addition of purine sources increased the rate of development. The occurrence of auxotrophs requiring purine supplements provides qualitatively similar evidence. Subsequently, verification of the activities has

FIGURE 2: Pathways of Purine Interconversion in Drosophila

The numbers in the Figure refer to the list of enzymes involved in the interconversion and catabolism of purines. References which can be consulted for further information are indicated by the lower case letters. Additional references are available in Nash and Henderson (1982).




| | Enzyme Name | |
|---|---|---|
| 1 | 5' nucleotidase | f |
| 2 | nucleoside kinase | b,f,h |
| 3 | purine nucleoside phosphorylase | f,g |
| 4 | APRT | a,f |
| 5 | HGPRT | d,f,h |
| 6 | AMPS synthetase | f |
| 7 | AMPS lyase | f |
| 8 | IMP dehydrogenase | b,f |
| 9 | GMP synthetase | b,f |
| 10 | AMP deaminase | b,f |
| 11 | GMP reductase | b,f |
| 12 | AR deaminase | b,c,f |
| 13 | XDH | b,f,g |
| 14 | G deaminase | b,e,f,g |
| 15 | uricase | e,f |

References


a. Becker, 1974a.
b. Becker, 1974b.
c. Becker, 1975.
d. Becker, 1978.
e. Hodge and Glassman, 1967.
f. Johnson, Nash and Henderson, 1980a,b,c.
g. Morita, 1964.
h. Wyss, 1977.

come from *in vitro* enzyme assays (See Figure 2 and Nash and Henderson (1982) for specific references.) and from radioactive tracer studies *in vivo* (Johnson, *et al.*, 1980a, b, c).

In Drosophila, the conversion of IMP to AMP occurs at twice the rate of the conversion of IMP to GMP (Johnson, *et al.*, 1980a, b, c). Similar differential production of the two major nucleotide species has also been observed in mammalian systems (McFall and Magasanik, 1960). There evidence for the presence, in Drosophila, of 5'-nucleotidases and kinases, although the importance of adenosine kinase is questionable because the activity of adenosine deaminase is high. Purine nucleoside phosphorylase activity is low and acts preferentially in the direction of nucleoside cleavage (Becker, 1974b). Adenine phosphoribosyltransferase (APRT) activity is high. On the other hand, hypoxanthine-guanine phosphoribosyltransferase (HGPRT) activity is very low *in vivo* because the catabolic enzymes, xanthine dehydrogenase (XDH) and guanine deaminase, actively deplete the substrate pools. The adenine pool, primarily through adenosine deaminase, although adenylate deaminase is also present, can be converted to IMP and thence to GMP. There is conflicting evidence regarding the presence (Becker, 1974b) or absence (Hodge and Glassman, 1967a) of adenine deaminase. Because the *in vivo* activity of GMP reductase is low, guanine nucleotides cannot act as the sole purine source in Drosophila. (References for the above are to be found in Figure 2.)

Although better defined biochemically, the salvage and catabolic pathways in Drosophila are like the *de novo* pathway in being poorly defined genetically. The *rosy* (*ry*; 3-52.0) locus is the only known structural gene, encoding the enzyme, XDH. XDH is also required in pteridine metabolism and *ry* was initially identified because of its effect on the adult eye colour rather than its importance in purine metabolism. (For a review of the *ry* locus, see for instance, O'Brien and MacIntyre, 1978.) One locus has been implicated in purine salvage; *aprt* (3-0.03) mutants were selected for resistance to

purine (7H-imidazo(4,5-d)pyrimidine) and shown to lack APRT activity. Isoelectric focussing and gene dose response experiments suggest that *aprt* is the structural gene for adenine phosphoribosyltransferase (Johnson and Friedman, 1983).

Genetic analysis of purine interconversion and salvage is more complete in mammalian cell lines. Thus, mutants in adenylosuccinate synthetase (*adeH*, Patterson, 1976a), adenylosuccinate lyase (*adeI*, Patterson, 1976b), purine nucleoside phosphorylase (Hoffee, 1979), APRT (Taylor, *et al.*, 1977) and HGPRT (Patterson and Jones, 1976) are known in Chinese hamster cells. Additionally, the enzymes, adenosine deaminase and adenylate kinase, have been examined and shown to be polymorphic while adenosine kinase, APRT and purine nucleoside phosphorylase are known to be monomorphic (Stallings and Siciliano, 1981).

Although the genes encoding some of the *de novo* synthetic enzymes have been located in ans by somatic cell hybridization, the only defects which manifest clinically involve purine salvage and catabolism. These include enhanced activity of PRPP synthetase (which is considered a "salvage" enzyme because of its importance to phosphoribosyltransferase reactions) and deficiencies of APRT, muscle adenylate deaminase, adenosine deaminase, purine nucleoside phosphorylase, 5'-nucleotidase, xanthine oxidase and HGPRT. The symptoms associated with these biochemical defects include immunodeficiencies, gout and neurological symptoms. Some of the various hypotheses as to the origins of these pleiotropic effects are discussed by Boss and Seegmiller (1982).

**4. Pteridine Metabolism**

The wild-type eye-colour of *Drosophila melanogaster* adults arises from a combination of two classes of compounds, brown ommochromes and red pteridines. The pteridines are known collectively as "drosopterins", although the term also refers more specifically to a subclass of pigments which, along with sepiapterin, isoxanthopterin and

pterin form the major pteridine species in Drosophila (Evans and Howells, 1978; Fan *et al.*, 1976). Although the existence of the two types of pigments has been known for over 40 years, only the ommochromes are well characterized. Neither the chemistry, nor the biosynthesis of pteridines is understood. However, recent successes in purifying the enzymes and identifying the genetic components involved (reviewed by Phillips and Forrest, 1980; Nash and Henderson, 1982) suggest that this situation may soon be remedied.

The synthesis of pteridines is initiated with the conversion of GTP to dihydroneopterin triphosphate catalyzed by the enzyme, GTP cyclohydrolase (Brown, *et al.*, 1979). Recent work by MacKay and O'Donnell (1983) suggests that *Punch* (*Pu*; 2-97) is the structural gene encoding this enzyme in Drosophila. This claim rests on the observation that GTP cyclohydrolase activity varies in proportion to the number of wild-type copies of the *Pu* locus. This response is generally considered diagnostic of structural loci in Drosophila (Hodgetts, 1975). A number of loci, including *prune* and *raspberry*, have tissue-specific or age-specific effects on GTP cyclohydrolase activity (Evans and Howells, 1978; MacKay and O'Donnell, 1983) and are candidates for regulatory loci.

Dihydroneopterin triphosphate, the first intermediate, does not normally accumulate to any extent (Phillips and Forrest, 1980). It serves as the substrate for sepiapterin synthetase A. The product of this reaction, after non-enzymatic rearrangement, is converted to sepiapterin, a yellow compound, by sepiapterin synthetase B (Fan, *et al.*, 1975; Krivi and Brown, 1979). The first of these two enzymes is encoded by the *purple* (*pr*; 2-54.5) locus (Yim, *et al.*, 1977).

Depending on its oxidized state, sepiapterin is converted to either biopterin or dihydrobiopterin. The latter can be converted to biopterin by the action of dihydropterin oxidase, which non-specifically oxidizes dihydropterins to their pterin counterparts (Fan and Brown, 1979). The genetic basis of these reactions is unknown.

Only one other reaction in the synthesis of pteridines is known, although its position in the sequence is not known. XDH, encoded by *ry*, as mentioned previously, in addition to its role in purine catabolism also catalyzes the production of isoxanthopterin from 2-amino, 4-hydroxypteridine. Mutants of three other genes, *maroon-like* (X chromosome, map position 64.8), *cinnamon* (X-0.0) and *low xanthine dehydrogenase* (3-33.0) also have reduced XDH activity. However, since two other enzymes, pyridoxal oxidase and aldehyde oxidase, are similarly affected in these mutants, the effect is most likely indirect, perhaps through a shared cofactor (Finnerty, 1976).

It is obvious from this discussion, that large gaps remain to be filled before pteridine biosynthesis is completely understood. Of the major pteridine species found in Drosophila, the synthesis of sepiapterin is fully defined and that of isoxanthopterin partially defined, but the synthesis of the drosopterins (and remaining pteridines) is not defined at all.

A number of other aspects of pteridine metabolism are equally obscure. For instance, pteridine derivatives are important in a variety of metabolic processes, but it is not clear if they are produced endogenously in Drosophila. Some, like riboflavin and folic acid, clearly are not, since they are required components of the diet. However, there are indications from genetic studies that some endogenously produced pteridines are essential metabolites (Nash and Henderson, 1982). As another example, the biological importance of isoxanthopterin remains controversial. Ribosomal RNA synthesis is reduced in Drosophila embryos in the presence of isoxanthopterin and lethal, or near-lethal, effects are known for a number of genes (for instance, *deep orange* and *cinnamon*) which alter isoxanthopterin levels. However, *ry* homozygotes, which lack XDH activity, do not normally appear to be affected by the resulting lack of isoxanthopterin (Phillips and Forrest, 1980).

It is also difficult, in most cases, to correlate mutant eye-colours with biochemical defects. Two examples will suffice to illustrate this problem. The mutant

eye-colour of *ry* homozygotes cannot be a direct result of the absence of XDH since neither isoxanthopterin nor 2-amino, 4-hydroxypteridine are coloured. Instead, the phenotype apparently results from a secondary reduction in the drosopterins. In *sepia* (*se*; 3-26.0) mutants, the second example, sepiapterin accumulates and the drosopterins are absent. This would usually be interpreted to mean that sepiapterin is a direct precursor of drosopterins. However, the chemistry of the drosopterins, a group of bicyclic red-orange pteridines (Rokos and Pfleiderer, 1975) suggests that the pathway branches before sepiapterin and that the drosopterins derive instead from dihydroneopterin (Phillips and Forrest, 1980; Brown, 1971).

## 5. The Project

This work examines three auxotrophic mutants in light of the foregoing information about the metabolism of purines and pteridines in Drosophila. Genetically, the loci are discrete elements on the second chromosome and appear to have their major effects at different intervals during development. Phenotypically, mutations at two of the loci, *ade2* and *ade3*, share a requirement for an A source and *bur* mutants require G. In addition, *ade2* and *bur* are each associated with defects in adult eye-colour. Biochemically, specific lesions in purine metabolism, suggested by the nutritional phenotype are examined in *in vitro* enzyme assays and the pteridine defects suggested by the eye-colours are examined chromatographically.

# II. MATERIALS AND METHODS

## 1. Stocks

Three mutants of *Drosophila melanogaster*, auxotrophic for purine nucleosides, and the stocks used for their maintenance and experimental manipulation are described in Table 2.

## 2. Axenic Culture

In order to study *in vivo* nutritional requirements, reduce the risk of microbial contaminants in *in vitro* biochemical tests, and, in the case of *ade3-1* which has no visible phenotype, identify the mutant, all stocks are maintained in axenic culture. Axenic cultures are initiated by surface sterilizing 0-12 hour embryos through immersion in a saturated calcium hypochlorite solution for 20 minutes. This is followed by extensive washing with sterile Drosophila Ringers solution (NaCl 7.5 gm., KCl 0.35 gm., CaCl2 0.21 gm., H2O to 1000 ml., Ephrussi and Beadle, 1936) at room temperature. The sterilized eggs are placed in vials containing sterile yeast-sucrose medium (Table 3A) and maintained at 25°C. All operations are performed in sterile chambers. Periodically, cultures are checked for microbial contaminants by streaking a small amount of medium onto microbial testing medium (Table 3B) kindly supplied by Dr. R.C. von Borstel.

## 3. Nutritional Characterization

Auxotrophs are functionally defined by their inability to survive to adulthood on Sang's (1956) medium as modified by el Kouni and Nash (1974) (see Table 3C). The nature of the auxotrophic requirement is determined by culturing the mutants on this medium supplemented with RNA (4.0 mg/ml) and, for more precise characterization, a series of the purine or pyrimidine nucleosides (usually 3.2mM). These concentrations were shown by Sang (1956) to optimize Drosophila development.

TABLE 2: Description of *Drosophila melanogaster* Stocks Used

| Stock | Description | Source |
|---|---|---|
| Amherst | Inbred derivative of Oregon-R (see Stock 1, Amherst College, Drosophila Information Service, (1968); wild type | Amherst College |
| *ade2-1* | Purine nucleoside auxotroph supplemented by AR and HR; eye colour diluted pink | Naguib, 1976 |
| *ade3-1* | Purine nucleoside auxotroph supplemented by AR and HR; "leaky"; no visible phenotype | Nash, unpublished |
| *bur* | Purine nucleoside auxotroph supplemented by GR; eye colour brown | Cal. Tech. |
| *bur gua2-1* | Second allele of *bur* | Nash, unpublished |
| *SM5/Sp* | In(2LR)*SM5*, $al^2$ *Cy* $lt^v$ $cn^2$*/Sp*; second chromosome balancer | Cal. Tech. |
| *rdo hk pr* | *rdo: reduced ocelli* (2-53); *hk: hooked* (2-53.9); *pr: purple* (2-54.5) | Cal. Tech. |
| *pr cn* | *cn: cinnabar* (2-57.5) | Cal. Tech. |
| *cl spd* | *cl: clot* (2-16.5); *spd: spade* (2-21.9) | R. MacIntyre |
| *CyO*/Df(2L)clot-7 *b pr cn bw* | *CyO* second chromosome balancer; Df(2L)clot-7 exposes 25D7-E1; 26A7-8 | R. MacIntyre |
| *CyO*/Df(2L)clot-1 *cn bw* | 25D7-E1; 25E6-F3 | R. MacIntyre |
| *CyO*/Df(2L)GdhA $dp^{ov}$ | 25D7-E1; 26A8-9 | R. MacIntyre |
| Df(2R) *bur cn/SM5* | 42B1-2 | This study |

Further description of stocks 4 and 6-9 can be found in Lindsley and Grell (1968).

TABLE 3: Composition of Media

A. Yeast-Sucrose Medium

| | |
|---|---|
| Brewer's Yeast | 12.5 g |
| Sucrose | 10.0 g |
| Granulated Agar | 2.0 g |
| Streptomycin* | 25.0 mg |
| Penicillin* | 25,000 I.U. |
| Propionic Acid* | 1.0 ml |
| Water | 100.0 ml |

B. Microbial Testing Medium

| | |
|---|---|
| Agar | 1.5 g |
| Yeast Extract | 1.0 g |
| Peptone | 2.0 g |
| Dextrose | 2.0 g |
| Water | 100.0 ml |

C. Sang's Defined Medium

| | |
|---|---|
| Agar (Oxoid No. 3) | 2.50 g |
| Casein (vitamin-free) | 5.50 g |
| Sucrose | 0.75 g |
| Cholesterol | 0.03 g |
| Lecithin | 0.40 g |
| Thiamine | 0.20 mg |
| Riboflavin | 0.10 mg |
| Ca Panthothenate | 1.60 mg |
| Pyridoxine | 0.25 mg |
| Biotin | 0.16 mg |
| Folic Acid | 0.30 mg |
| $NaHCO_3$ (anhydrous) | 140.00 mg |
| $KH_2PO_4$ (anhydrous) | 183.00 mg |
| $K_2HPO_4$ (anhydrous) | 189.00 mg |
| $MgSO_4$ (anhydrous) | 62.00 mg |
| Streptomycin* | 13.20 mg |
| Penicillin* | 25,000 I.U. |
| Water | to 100.00 ml |

*Added after autoclaving

For the mutations under consideration here, the cross mutation/mutation female x mutation/*SM5* male, and its reciprocal, were used. These crosses should generate homozygous and heterozygous progeny, in equal numbers. Since the heterozygotes carry the dominant wing morphology mutation, *Curly* (*Cy*, 2-6.1), these serve as an internal control of the adequacy of the defined medium, as well as giving an estimate of the expected number of mutant homozygotes. The crosses were initiated on yeast-sucrose medium and the resulting cultures scored to estimate the mutant viability under permissive conditions. The parental flies were transferred to the appropriate defined medium. At least five replicates of each cross were set under all test conditions and the emerging progeny scored daily. All cultures were tested for microbial contamination and only axenic cultures retained.

**4. Recombination Mapping**

Preliminary mapping work using dominant markers (Naguib, 1976; Nash, unpublished) had established that *ade2-1* and *ade3-1* are both on the left arm of chromosome 2, near *Sternopleural* (*Sp*, 2-22.0) and that *bur-gua2-1* is near *Tuft* (*Tft*, 2-53.2). The protocol used to estimate the map positions of the three mutants more precisely is given in Figure 3. The recessive markers used in mapping *ade2-1* and *ade3-1* were *clot* (*cl*, 2-16.5) and *spade* (*spd*, 2-21.9). Initial attempts to map *bur-gua2-1* used the recessive mutations *reduced ocelli* (*rdo*, 2-53) *hook* (*hk*, 2-53.9) and *purple* (*pr*, 2-54.5). When these experiments indicated that *bur-gua2-1* is to the right of *pr*, the mutant was mapped using *pr* and *cinnabar* (*cn*, 2-57.5).

**5. Complementation Tests**

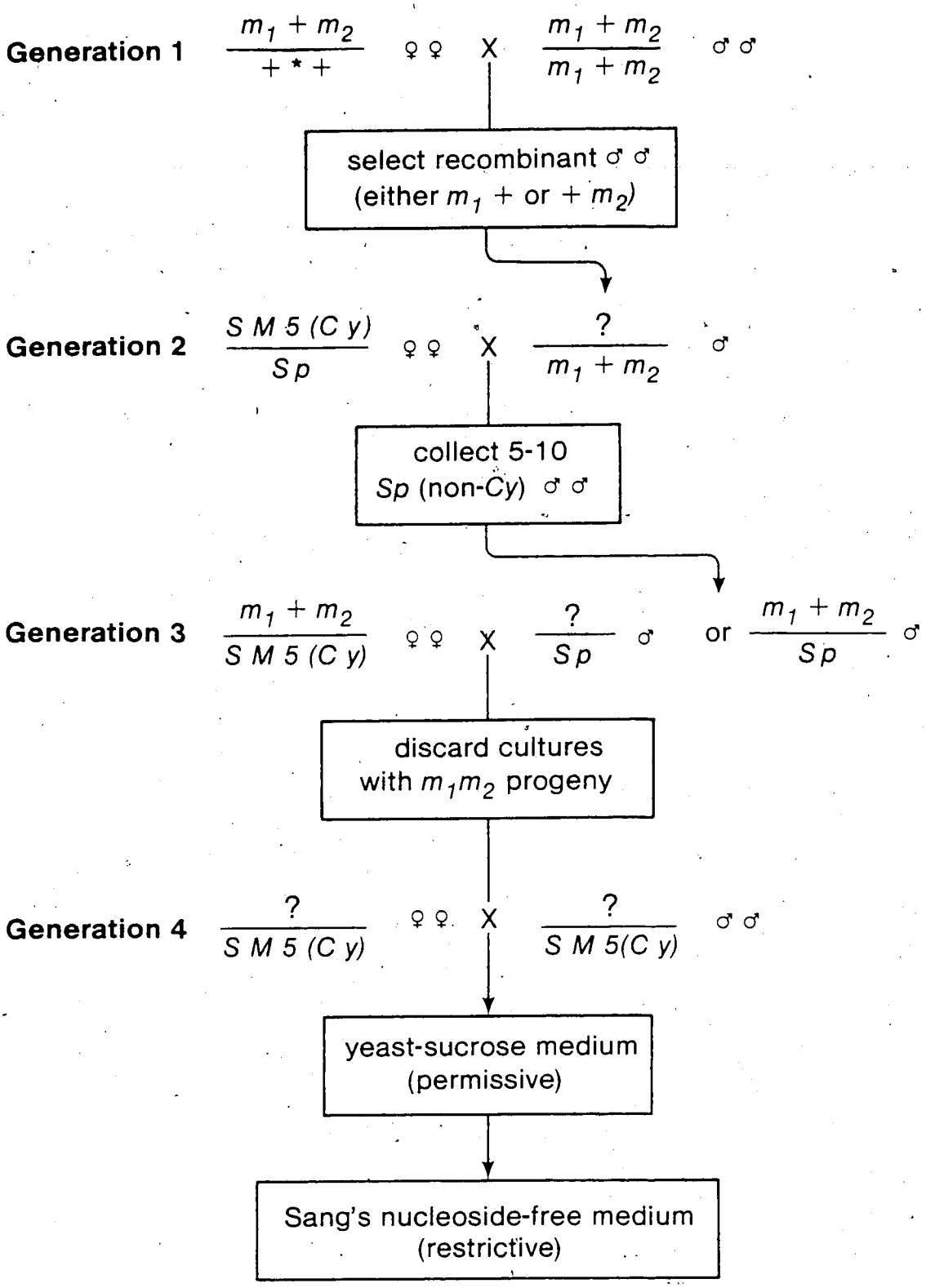
Tests to determine allelism between *ade2-1* and *ade3-1* were done, because of their proximity to each other and phenotypic similarity, by crossing homozygous females to males heterozygous for the other auxotrophy. Similar tests between *bur* and

FIGURE 3: Protocol Used in Mapping Second Chromosome Auxotrophs

Recombinants between $m_1$ and $m_2$ are identified among the progeny of Generation 1. The remainder of the protocol is devoted to identifying whether the recombinant contains the auxotroph (*). The unknown chromosome (?) in the parents of the second generation may thus be $m_1$*+ or $m_1$++ or +*$m_2$ or ++$m_2$.

Selected $F_2$ males which contain the unknown chromosome (?) allow confirmation, in the $F_3$, of the original recombination event and give appropriate test individuals for the fourth generation.

The progeny of the fourth generation are expected to include ?/*SM5* and ?/? individuals in a 2:1 ratio. *SM5/SM5* is a lethal combination.


Generation 1
$\frac{m_1 + m_2}{+ * +}$ ♀♀ X $\frac{m_1 + m_2}{m_1 + m_2}$ ♂♂
select recombinant ♂♂ (either $m_1$ + or + $m_2$)
Generation 2
$\frac{S\ M\ 5\ (C\ y)}{S\ p}$ ♀♀ X $\frac{?}{m_1 + m_2}$ ♂
collect 5-10 *Sp* (non-*Cy*) ♂♂
Generation 3
$\frac{m_1 + m_2}{S\ M\ 5\ (C\ y)}$ ♀♀ X $\frac{?}{S\ p}$ ♂ or $\frac{m_1 + m_2}{S\ p}$ ♂
discard cultures with $m_1 m_2$ progeny
Generation 4
$\frac{?}{S\ M\ 5\ (C\ y)}$ ♀♀ X $\frac{?}{S\ M\ 5 (C\ y)}$ ♂♂
yeast-sucrose medium (permissive)
Sang's nucleoside-free medium (restrictive)

*bur-gua2-1* involved heterozygous females and homozygous males since the *bur* chromosome also carries a recessive female sterility mutation (*fs(2)E1*, 2-57.6).

## 6. Generation of a *bur* Deficiency

The protocol for generating deficiencies which expose the region of *bur* is outlined in Figure 4. 0-2 day old homozygous *cn* males were irradiated with gamma rays at 5000r from a Cobalt-60 source, then mass-mated to *b bur cn* females. In the F1 both male and female progeny exhibiting the *bur cn* eye colour most probably indicated either a new *bur* mutation or a deficiency uncovering *bur*. Crossing these flies to *b bur cn/SM5* provided immediate confirmation of the mutant phenotype and protected the irradiated chromosome against recombination. However, the irradiated chromosome from females carrying a putative deficiency was not protected from recombination in the F1. Crosses of F2 progeny generated putative *Df(2R)bur,cn/SM5* progeny which were crossed to determine the viability of the putative deficiency homozygotes. These were expected to be inviable if a deficiency had been induced. In this case, *SM5* progeny were used to establish a balanced stock.

## 7. Deficiency Mapping

Cytogenetic mapping was performed by crossing females carrying either one or two copies of the auxotrophic mutation to males heterozygous for the deletion and a balancer second chromosome. The absence of auxotroph/deficiency progeny surviving on Sang's defined medium indicated that the deficiency exposed the mutation.

## 8. Nutritional Shift Experiments

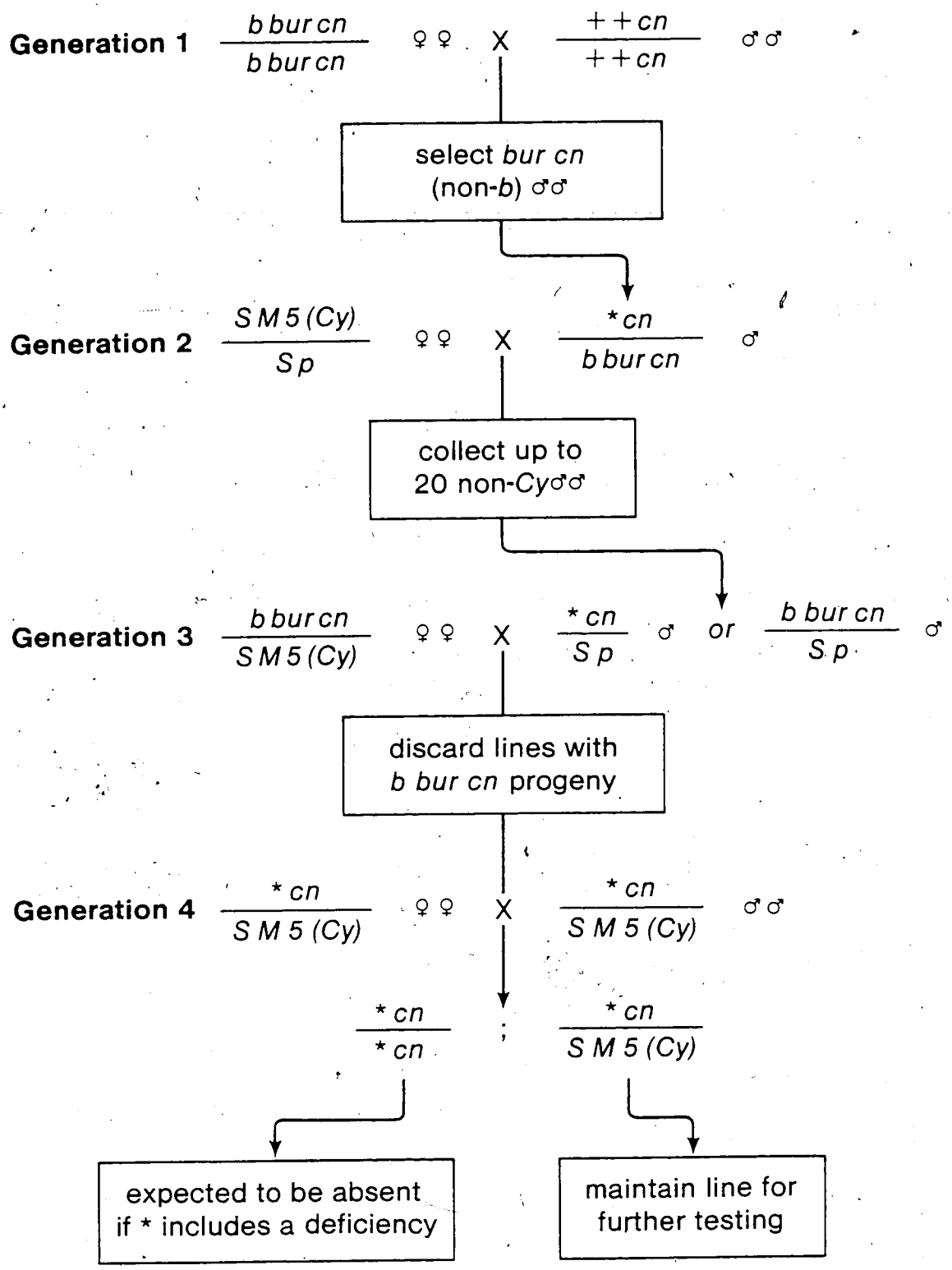
In order to determine the period of dependence on exogenously supplied purines, nutritional shift experiments were undertaken. To do this, between 10 and 20 replicate crosses of 10 heterozygous females and 5 homozygous males were initiated and

FIGURE 4: The Protocol for Generating a *bur* Deficiency

The male parents in Generation 1 were treated with 5000r gamma rays. Potential rearrangements are induced in a *cn* background to facilitate identification of *bur* mutations; the *bur cn* combination is more easily identified than *bur* alone. Phenotypically *bur cn* progeny in the F1 carry the treated chromosome (*) which may include new alleles of *bur*, deficiencies for *bur* or dominant eye-colour mutations. The remainder of the protocol identifies probable deficiencies.

Progeny of selected F2 males carrying the treated chromosome (*) confirm the original aberrant phenotype and provide the parents for Generation 4. In the F4, *SM5/SM5* progeny are lethal, **cn/*cn* are expected to be lethal if a deficiency is present and */SM5 can be used for stock maintenance and further testing.

The selected F1 progeny may be ♀, in which case the reciprocal to the Generation 2 cross is used. In this case, the treated chromosome (*) is not balanced against recombination, but untoward effects of recombination should become evident in the F3 when related sublines behave dissimilarly.


Generation 1
b bur cn / b bur cn ♀♀ X ++cn / ++cn ♂♂
select bur cn (non-b) ♂♂
Generation 2
SM5 (Cy) / Sp ♀♀ X *cn / b bur cn ♂
collect up to 20 non-Cy♂♂
Generation 3
b bur cn / SM5 (Cy) ♀♀ X *cn / Sp ♂ or b bur cn / Sp ♂
discard lines with b bur cn progeny
Generation 4
*cn / SM5 (Cy) ♀♀ X *cn / SM5 (Cy) ♂♂
*cn / *cn ; *cn / SM5 (Cy)
expected to be absent if * includes a deficiency
maintain line for further testing

maintained on sterile yeast-sucrose medium for 3 days. The replicates were then amalgamated in a sterile 1/2 pint milk bottle, capped with a 15mm petri plate containing either sterile yeast-sucrose or sterile unsupplemented Sang's medium, and the flies allowed to lay eggs for 8 hours. At the end of this period, the petri plate was removed and 150-200 eggs transferred axenically with a scalpel to another petri plate containing the same medium. At intervals of 24 hours, the eggs or larvae were axenically transferred with a scalpel to sterile vials containing the opposite medium, where they were allowed to complete development. A record was maintained of the initial number of eggs, the number of larvae transferred and the number and phenotype of eclosing adults. The egg dishes were retained until it was clear that all larvae had been transferred. All transfers were done in triplicate and any culture showing microbial infection was discarded.

**9. IMP dehydrogenase Assays**

Axenic cultures of homozygous third instar larvae grown on yeast-sucrose medium were collected and washed extensively in ice cold Ringer's solution and either used immediately or maintained at -35°C until use. A crude extract was made, at 0-4°C, by homogenizing three parts by weight of larvae in 1 part by volume of Buffer (100mM Tris, 100mM KCl, 1mM EDTA, 20% v/v glycerol, pH 7.5) in a glass tissue grinder. The homogenate was centrifuged in a Sorvall RC-2B centrifuge at 27,000x g for 20 minutes. The supernatant was removed with minimal disturbance to the overlying lipid layer and applied to a 1.5x60cm column (Pharmacia) gravity-packed with Sephadex G-50 fine, pre-equilibrated with the Buffer. The extract was eluted at 0-4°C with the same Buffer and six 2 millilitre fractions collected for assay.

Examination of the column eluant showed that the protein eluted primarily in two well-separated bands. The first, encompassed in about 8 ml., contained all of the measurable enzyme activity. To ensure the inclusion of all of the activity in these

determinations, two fractions flanking the protein and activity peak were routinely collected and assayed. As seen in other systems (Anderson and Sartorelli, 1968; Lagerkvist, 1958; Udaka and Moyed, 1963), freezing the extract, either before or after fractionation, for short periods did not affect the measured activity.

IMP dehydrogenase activity was assayed spectrophotometrically at 340nm by following the reduction of $NAD^+$ as IMP is converted to XMP (Magasanik, *et al.*, 1957). The reaction mixture consisted of 100mM Tris, 100mM KCl, 2.5mM IMP and 0.3mM $NAD^+$ (pH 7.9) in a volume of 0.8ml. The reaction was initiated by addition of 0.2ml of the column fraction and is measured against a blank containing no IMP (Anderson and Sartorelli, 1968). The protein content was determined by the method of Lowry *et al.* (1951). The results from all six fractions were pooled and specific activity expressed as micromoles of NADH produced per minute per milligram of protein.

**10. Estimation of Fertility**

Relative fertility was estimated roughly by setting crosses of 5 virgin 0-3 day old females to 5 males of the same age. The mating was allowed to proceed for 5 days and the progeny counted. Four types of crosses were set:

1. homozygous female x homozygous male
2. homozygous female x heterozygous male
3. heterozygous female x homozygous male
4. heterozygous female x heterozygous male

Heterozygotes carried the *SM5* balancer chromosome.

Where two recombinant lines show similar sterility patterns, they were tested for allelism by crossing line1/line2 females and males and counting the progeny produced.

**11. Guanine deaminase Assays**

The collection of third instar larvae was described for the IMP dehydrogenase assay. The larvae were homogenized 1.5:1 (w/v) in 0.1M Tris (pH 7.0) at 0-4°C. The homogenate was centrifuged for 20 minutes in a Sorvall RC-2B centrifuge at 27,000x g. The supernatant was adjusted to pH 5.1 with 1.0M acetic acid and centrifuged at 27,000x g for 5 minutes. The resulting supernatant was filtered through glasswool and adjusted to pH 8.0 with 1.0M KOH. Activated charcoal was added at 50 mg/ml and the mixture allowed to sit on ice for 1 hour with occasional stirring. The mixture was then centrifuged twice for 10 minutes at 27,000x g and passed through glass wool to remove the charcoal, and used immediately (Hodge and Glassman, 1967a,b).

In the assay, guanine deaminase activity is linked to xanthine oxidase. (Xanthine oxidase (XO) and xanthine dehydrogenase (XDH) catalyze the same reactions, but differ in the identity of their final oxygen receptors, a difference which is correlated with source. XO, the enzyme used in this assay, uses oxygen as the receptor; the endogenous Drosophila enzyme, XDH, uses $NAD^+$ in this role (Glassman and Mitchell, 1959).) The amount of X produced from G, is measured by following its oxidation to uric acid (UA). The production of UA was measured spectrophotometrically at 290nm in a reaction mixture consisting of 100mM Tris, pH 8.0, 62mM G, 25 mU xanthine oxidase (Kalckar, 1947). The reaction was started by adding extract to a total volume of 1.0 ml. and measured against a blank containing no extract. The presence of endogenous X can be checked by measuring the production of UA in the absence of added G. Endogenous uricase activity can be determined by adding UA to a reaction mixture lacking xanthine oxidase and measuring the decrease in absorbance at 290nm. Although it is a less sensitive assay (Shuster, 1955) the enzyme activity can also be measured at 270nm following the production of X. Protein content was determined by the method of Lowry *et al.* (1951) and the specific activity expressed as nmoles of UA produced per minute per milligram of protein.

## 12. Pteridine Determination

The pteridine content of whole flies was qualitatively determined by a modification of the method of Fan *et al.* (1976). 10 parts of adult flies were homogenized in 1 part (w/v) of solvent containing equal volumes of 1% $NH_4OH$ and chloroform in a glass tissue grinder. The homogenate was centrifuged at 27,000x g for 20 minutes in a Sorvall RC-2B centrifuge. The protein was precipitated from the supernatant by addition of a cold solution of TCA to a concentration of 5% followed by centrifugation at 27,000x g for 20 minutes at 0-4°C. The pteridines in this extract were separated using descending chromatography in the dark. 100 μlitres of the extract was spotted in 5 μl aliquots on Whatmann #1 filter paper. The chromatograph was developed in n-propanol: 1% $NH_4OH$ (2:1 v/v) for 8 hours after equilibrating overnight in 10% $NH_4OH$. Standard spots of isoxanthopterin, pterin and xanthopterin dissolved in equal volumes of 1% $NH_4OH$ and 10% TCA were included on every chromatograph. Spots were visualized under ultraviolet light and photographed.

Occasionally, flies were decapitated and heads squashed directly onto the filter paper then developed as usual.

## 13. Chemicals

The chemicals used in the culture media were obtained from the following sources: Dispensaries Wholesale Ltd.: Penicillin (1,000,000 I.U.). Fisher Scientific Co.: Magnesium sulphate (anhydrous); sodium carbonate (anhydrous); potassium phosphate dibasic (anhydrous); potassium phosphate monobasic (anhydrous); proprionic acid. Fison: Oxoid agar No. 3; casein (vitamin-free). ICN Pharmaceuticals Ltd.: Brewer's yeast; granulated agar. MacDonalds Consolidated Ltd.: Sucrose. Sigma Chemicals Co.: All nucleic acid bases, nucleosides and nucleotides; allopurinol (4-hydroxypyrazole-(3,4-d)-pyrimidine); d-Biotin; calcium pantothenate; cholesterol; folic acid; lecithin; niacin; pyridoxine HCL; riboflavin; RNA (type V sodium salt);

streptomycin sulphate; thiamine HCL.

Compounds used in the enzyme assays were obtained as follows: Fisher Scientific Co.: Acetic acid (glacial); ethylene diaminetetraacetic acid (EDTA); glycerol; potassium chloride; potassium hydroxide; Tris-(hydroxymethyl)-aminomethane (THAM). Pharmacia Canada Ltd.: Sephadex G-50 (fine). Sigma Chemical Co.: Activated charcoal; $\beta$-nicotinamide adenine dinucleotide ($\beta$-$NAD^+$); uric acid; xanthine oxidase (Grade III from buttermilk).

The compounds used in protein determinations, bovine serum albumin, cupric sulphate, Folin phenol reagent and sodium bicarbonate, were all purchased from Fisher Scientific Company.

The supplies for pteridine determinations were from the following sources: Canlab: Whatmann #1 filter paper. Fisher Scientific Co.: Ammonium hydroxide; chloroform; n-propanol; trichloroacetic acid. Sigma Chemicals Co.: Isoxanthopterin (2-amino-4,7-dihydroxypteridine); pterin (2-amino-4-hydroxypteridine); xanthopterin (2-amino-4,6-pteridinedione).

# III. RESULTS

## Nutritional Characterization

All three of the mutants considered in this study were initially selected as RNA-requiring auxotrophs and had already been partially characterized. The nutritional results presented here confirm the originally described mutant phenotypes and extend the testing to include other potential supplements.

### 1. *ade2-1*

When initially characterized by Naguib (1976) (*ade2-1* was then designated C2-10) it was found that *ade2-1/ade2-1* was as viable as its heterozygous siblings on yeast-sucrose medium; the relative viability of *ade2-1/ade2-1* compared to *ade2-1/SM5* was 108.9%. The relative viability on Sang's medium supplemented with 4mg/ml RNA (99%), Sang's plus 3.2mM AR (81.8%) and Sang's plus 3.2mM HR (153%) was also high. Since no maternal effect appears to be associated with *ade2-1*, the survival rates given are taken from crosses of homozygous females to heterozygous males. Similar results were obtained in other crosses.

On unsupplemented Sang's only 1.8% of the expected homozygotes survived and their development was delayed by 3.5 days compared to the heterozygous siblings. Homozygotes were only 3.3% as viable as heterozygotes when supplemented with 3.2mM GR and had a developmental delay of 11.8 days. In all of these results, there was a possibility of misclassification since the *ade2-1* eye-colour had not been recognized. This difficulty did not arise in the present study since either the mutant eye-colour, or one of the visible markers acquired on the chromosome during the recombination mapping was sufficient for unequivocal classification.

As shown in Table 4, the results of retesting the nutritional requirements of *ade2-1/ade2-1* confirm the previous work., Table 4 includes results for supplements not

TABLE 4: Response of *ade2-1* to Various Nutritional Conditions

| Medium | Survivors m/m | Survivors m/*SM5* | Relative Viability | Delay |
|---|---|---|---|---|
| Yeast-sucrose | 445 | 466 | 95.5 | 0.2 |
| Sang's + RNA | 163 | 228 | 71.5 | 1.0 |
| Sang's | 0 | 263 | 0 | --- |
| Sang's + AR | 288 | 352 | 81.8 | 0.1 |
| Sang's + HR | 250 | 225 | 111.1 | 0 |
| Sang's + GR | 6 | 213 | 2.8 | 5.1 |
| Sang's + UR | 0 | 311 | 0 | --- |
| Sang's + A | 77 | 177 | 43.5 | 1.6 |
| Sang's + H | 0 | 199 | 0 | --- |
| Sang's + G | 0 | 385 | 0 | --- |
| Sang's + C | 0 | 214 | 0 | --- |
| Sang's + U | 0 | 253 | 0 | --- |

The data are generated from crosses of *ade2-1/ade2-1* ♀ x *ade2-1/SM5* ♂. Results are pooled from at least 5 replicate cultures, each containing 5 parents of each sex.

The first column gives the culture medium. RNA was present at 4.0 mg/ml, the concentration which is optimum for the survival of *D. melanogaster* (Sang, 1956). Nucleosides and bases were present at the equivalent concentration of 3.2 mM.

The second and third columns give the number of adults eclosing under each nutritional condition. Homozygous mutant *(ade2-1/ade2-1=m/m)* data are shown first, the control data *(ade2-1/SM5=m/SM5)* second. *SM5* carries *Cy*, a dominant visible mutation.

"Relative viability" expresses the survival of m/m as a function of m/*Cy*. The theoretical expectation is for a relative viability of 100%.

"Delay" represents the difference, in days, between the mean developmental time to eclosion of homozygous mutant and heterozygous control flies.

considered in Naguib's work. Pyrimidine bases and their ribonucleosides do not support the growth of *ade2-1* homozygotes. Nor do any of the purine bases with the exception of A. It supports the survival of approximately 50% of the expected homozygotes although their development is delayed by 1.6 days. Since APRT is more active in *D. melanogaster* than HGPRT (Johnson, *et al.*, 1980a,c), it is not surprising that A but not H acts as a supplement.

2. *ade3-1*

In the preliminary characterization of *ade3-1* (Nash, unpublished) it was fairly leaky with up to 11% homozygous survivors developing with a 4 to 8 day delay under apparently restrictive conditions. Since *ade3-1* has no visible phenotype, and the culture conditions can affect the expression of *Cy*, the possibility of misclassifying this phenotype was high. Consequently, further nutritional characterization was deferred until recombinant lines carrying visible markers became available. Since recombinant lines with strong auxtrophic phenotypes were deliberately selected, the data in Table 5A, which otherwise verify the original analysis, show few escapers. Over time, the frequency of escapers occurring among the progeny of these recombinants tended to increase to the originally observed levels. Although the escapers occur only as the progeny of heterozygous mothers in these data, suggesting a maternal effect, this is not always observed.

Comparison of the relative viabilities of *ade3-1/ade3-1* on yeast-sucrose and RNA-supplemented Sang's medium reveals one feature of this auxotrophic phenotype commonly observed on permissive media. On RNA, only 50-70% of the expected homozygotes survive compared to over 90% on yeast-sucrose medium. Although the viability of the mutant is reduced, the development time of the homozygote is similar to that of the heterozygote.

TABLE 5: Response of *ade3* to Various Nutritional Conditions

**A**

| Cross | Medium | Survivors m/m | Survivors m/*SM5* | Relative Viability | Delay |
|---|---|---|---|---|---|
| m/m x m/*SM5* | Yeast-sucrose | 654 | 718 | 91.1 | 0.3 |
| | Sang's + RNA | 353 | 501 | 70.5 | 0.2 |
| | Sang's | 0 | 413 | 0 | --- |
| m/*SM5* x m/m | Yeast-sucrose | 250 | 254 | 98.4 | -1.5 |
| | Sang's + RNA | 93 | 185* | 50.3 | -0.2 |
| | Sang's | 5 | 78* | 6.4 | 4.2 |

**B**

| Medium | Survivors m/m | Survivors m/*SM5* | Relative Viability | Delay |
|---|---|---|---|---|
| Sang's + AR | 306 | 455 | 67.3 | -0.3 |
| Sang's + HR | 253 | 300 | 84.3 | 1.0 |
| Sang's + GR | 39 | 362 | 10.8 | 6.6 |
| Sang's + XR | 0 | 148 | 0 | --- |
| Sang's + UR | 0 | 229 | 0 | --- |
| Sang's + CR | 139 | 524 | 26.5 | 7.3 |
| Sang's + A | 181 | 273 | 66.3 | 0.6 |
| Sang's + H | 176 | 473 | 37.2 | 7.6 |
| Sang's + G | 40 | 849 | 4.7 | 5.4 |
| Sang's + U | 31 | 549 | 5.6 | 7.8 |
| Sang's + C | 27 | 707 | 3.8 | 6.8 |

**C**

| | | | | |
|---|---|---|---|---|
| Sang's + GR | 268 | 322 | 83.2 | 6.0 |
| Sang's + U | 232 | 422 | 55.0 | 6.6 |
| Sang's + C | 179 | 478 | 37.4 | 8.1 |

TABLE 5 (continued)

---

The data are generally drawn from 5 replicate cultures, each containing 5 parents of each sex. Exceptions (*) involve 2 or 3 replicate cultures.

A Results of reciprocal crosses between homozygous (m/m) and heterozygous (m/SM5) parents are shown.

B Results are from crosses of homozygous (m/m)♀ x heterozygous♂ (m/SM5) .

C The data represent unusual results of the cross used in B. The aberrant survival of *ade3-1/ade3-1* in these, and similar apparently restrictive conditions, does not arise from either microbial contamination or improperly labelled culture medium.

The data are represented as in Table 4. The theoretical expectation is for a relative viability of 100%.

Negative values for "Delay" indicate that the homozygous mutant flies developed faster than the heterozygous control flies.

The data in Table 5B show that the survival of *ade3-1/ade3-1* is strongly supported by AR (67%), HR (84%) and A (66%). With these three supplements, there is little developmental delay. H increases relative viability considerably, to 37%, although the intermediate level of survival and the developmental delay of over 7 days again suggest an effect of the low levels of HGPRT activity. Surprisingly, CR, but not UR, supports the survival of 26% of the homozygotes, although development is again, delayed by more than 7 days. Of the other nucleosides and bases tested, none increases survival spectacularly. Occasionally, *ade3-1* homozygotes do survive on these other media (Table 5C) but very high developmental delays (6-8 days) are observed.

3. *bur-gua2-1*

Initial characterization of *bur-gua2-1* (Nash, unpublished) (See RESULTS section 3-2 for a discussion of the naming of this allele.) showed that the homozygote survived only when grown on yeast-sucrose, RNA-supplemented Sang's or GR-supplemented Sang's media. The data in Table 6 confirm these results. Table 6A presents the results of reciprocal crosses between homozygous and heterozygous parents. The homozygous progeny are completely inviable on unsupplemented Sang's medium and well supplemented by 4 mg/ml RNA (30-50%) with little developmental delay. The reduced productivity of homozygous mothers, compared to the productivity of an equal number of heterozygous mothers in the other crosses, is a reflection of their low fertility rather than a maternal effect of the mutation. As homozygous males are normally fertile, the remainder of the nutritional tests were carried out using homozygous males and heterozygous females as parents.

Of the purines and pyrimidines tested, only GR supports the survival of *bur-gua2-1/bur-gua2-1* (Table 6B). The relative viability is high (120%) and the development of the homozygote is not delayed in the presence of 3.2mM GR. Although GR at 1mM supplements poorly (2.7%) and allopurinol, at the same concentration, not

TABLE 6: Response of *burgua2-1* to Various Nutritional Conditions

**A**

| Cross | Medium | Survivors m/m | Survivors m/SM5 | Relative Viability | Delay |
|---|---|---|---|---|---|
| m/m x m/SM5 | Yeast-sucrose | 64 | 82 | 78.0 | 0 |
| | Sang's + RNA | 16 | 55 | 29.1 | 0.6 |
| | Sang's | 0 | 55 | 0 | --- |
| m/*SM5* x m/m | Yeast-sucrose | 241 | 356 | 67.7 | 0 |
| | Sang's + RNA | 143 | 271 | 52.8 | 0.8 |
| | Sang's | 0 | 246 | 0 | --- |

**B**

| Medium | Survivors m/m | Survivors m/*SM5* | Relative Viability | Delay |
|---|---|---|---|---|
| Sang's + AR | 0 | 251 | 0 | --- |
| Sang's + HR | 2 | 212 | 0.9 | 4.1 |
| Sang's + GR | 350 | 288 | 121.5 | -0.1 |
| Sang's + XR | 2 | 355 | 0.6 | 5.5 |
| Sang's + UR | 0 | 519 | 0 | --- |
| Sang's + CR | 0 | 232 | 0 | --- |
| Sang's + A | 3 | 209 | 1.4 | 6.8 |
| Sang's + H | 0 | 131 | 0 | --- |
| Sang's + G | 0 | 322 | 0 | --- |
| Sang's + U | 0 | 96 | 0 | --- |
| 1mM GR | 6 | 226 | 2.7 | 1.9 |
| 1mM allopurinol | 0 | 151 | 0 | --- |
| 1mM GR + 1mM allopurinol | 61 | 72 | 84.7 | 0.4 |
| 1mM allopurinol + 3.2mM G | 0 | 96 | 0 | --- |

TABLE 6 (continued)

---

The data are pooled from 5 replicate cultures, each containing 5 parents of each sex.

A The results of reciprocal crosses of *bur gua2-1/bur gua2-1* (m/m) and *bur gua2-1/SM5* (m/SM5) parents are shown. The reduced productivity of crosses involving homozygous mutant mothers is discussed in the text.

B The data are generated from crosses of *bur gua2-1/SM5* ♀ x *bur gua2-1/bur gua2-1* ♂. Allopurinol is a specific inhibitor of the catabolic enzyme, xanthine dehydrogenase. At the concentration used, it is somewhat toxic (Nash, unpublished).

The results are represented as in Table 4. The theoretical expectation for relative viability is 100%.

Negative values for "Delay" indicate that the homozygous mutants develop faster than the heterozygous controls.

at all, simultaneous supplementation with both supports the survival of 85% of the expected homozygotes. Allopurinol does not enhance the value of G as a supplement. Allopurinol (4-hydroxypyrazole-(3,4-d)-pyrimidine) is a specific inhibitor of XDH, an enzyme which forms part of the pathway of guanine catabolism. Again, the low effective activity of HGPRT *in vivo* (Johnson, *et al.*, 1980a,c) probably explains why G is an ineffective supplement.

**Recombination Mapping**

1. *ade2-1*

In mapping the auxotroph, *ade2-1*, relative to *cl* and *spd*, a total of 51 males recombinant between the two recessive markers were recovered among 1200 $F_1$ males examined. After following the protocol outlined in Figure 3, the recombinants were self-crossed as heterozygotes and tested in duplicate on unsupplemented Sang's medium to determine which allele of *ade2* was present. If the homozygotes are viable, they should theoretically represent one-third of the progeny of this cross. (*SM5/SM5* flies die.) The data in Table 7 show the viability of each homozygous recombinant relative to its heterozygous sibling, and its relative development time on restrictive medium.

Six of the *cl* and 12 of the *spd* recombinants are completely inviable on Sang's medium. In addition, only these *spd* recombinants express the eye colour previously associated with *ade2-1*. These 6 *cl* recombinants have an eye-colour less extreme than *cl/cl* suggesting that the *ade2-1* eye-colour defect is epistatic to that of *cl*. The remainder of the recombinants, with relative viabilities, as homozygotes, of at least 18% and developmental times within 1.4 days of the heterozygotes on Sang's medium, appear to carry the wild-type allele of *ade2-1*. (Although the relative viability occasionally seems low, the absence of a developmental delay strongly suggests that these recombinants are *ade2*$^+$.) These *spd* recombinants have wild-type, and the *cl*

TABLE 7: *ade2-1* Recombination Mapping

A. Relative Viabilities of $Cy^+$ Segregants

| Genotype | Yeast | Sang's | Delay |
|---|---|---|---|
| *cl + +* | 41 | 55 | 0.8 |
| | 54 | 34 | 1.0 |
| | 46 | 30 | 0.3 |
| | 48 | 49 | 0.9 |
| | 45 | 35 | 1.1 |
| | 56 | 48 | 0.9 |
| | 50 | 50 | 0.3 |
| | 51 | 47 | 1.1 |
| | 41 | 28 | 0.4 |
| | 47 | 55 | 1.4 |
| | 42 | 58 | -2.5 |
| | 45 | 46 | 1.0 |
| | 43 | 57 | 0.2 |
| | 44 | 55 | 0.6 |
| | 33 | 32 | 0.3 |
| | 43 | 47 | 0.4 |
| | 57 | 52 | 0.5 |
| | 58 | 98 | -1.5 |
| | 41 | 62 | 0.8 |
| | 45 | 124 | -1.9 |
| | 53 | 33 | 1.2 |
| *cl ade2-1 +* | 61 | 0 | --- |
| | 42 | 0 | --- |
| | 57 | 0 | --- |
| | | 0 | --- |
| | 56 | 0 | --- |
| | 48 | 0 | --- |
| *+ + spd* | 42 | 30 | 0.2 |
| | 40 | 50 | 0.7 |
| | 57 | 85 | -2.3 |
| | 44 | 47 | 0.7 |
| | 45 | 51 | 0.1 |
| | 27 | 31 | 0.9 |
| | 43 | 50 | 0.2 |
| | 49 | 18 | 0.6 |
| | 39 | 55 | 0 |
| | 62 | 40 | 0.2 |
| | 50 | 32 | 0.6 |
| | 50 | 68 | 0.4 |
| *+ ade2-1 spd* | 46 | 0 | --- |
| | 46 | 0 | --- |
| | 62 | 0 | --- |
| | 44 | 0 | --- |

TABLE 7 (continued)

| Genotype | Yeast | Sang's | Delay |
|---|---|---|---|
| *+ ade2-1 spd* | 53 | 0 | --- |
| | 60 | 0 | --- |
| | 41 | 0 | --- |
| | 42 | 0 | --- |
| | 50 | 0 | --- |
| | 39 | 0 | --- |
| | 39 | 0 | --- |
| | 56 | 0 | --- |

B. Summary of Recombinants

| | |
|---|---|
| *cl + +* | 21 |
| *cl ade2-1 +* | 6 |
| *+ ade2-1 spd* | 12 |
| *+ + spd* | 12 |
| Total | 51 |

The recombinants were generated by following the protocol outlined in Figure 3 mapping *ade2-1* in relation to *cl* and *spd*. In the final generation, the parents of both sex are heterozygous for the recombinant chromosome and *SM5(Cy)*. The crosses are initiated on yeast-sucrose medium and then transferred to nucleoside-free Sang's medium to determine which allele of *ade2* is present.

A. The numbers represent the relative viability of homozygous *(Cy+)* recombinants. (The comparison is to the heterozygous siblings.) The theoretical expectation is for a relative viability of 50%.

"Delay" represents the difference, in days, between the mean developmental time to eclosion of homozygous recombinant and heterozygous *(Cy)* control flies.

Negative values indicate that the homozygotes developed faster than the controls.

B. The data in A. are summarized.

recombinants, typically *clot* eyes. Given the consistent cosegregation of the eye-colour and the auxotrophy, either the eye-colour mutation is a second locus very close to the auxotrophy or both phenotypes are products of a single mutation.

Standard calculation of the map position locates *ade2* 1.9 mu to the right of *cl* at 2-18.4. Although the data appear to contain substantial asymmetries between theoretically equally frequent classes of recombinants, $X^2$ analyses show that none are significant. The 95% confidence interval around the map position, calculated by the method of Nash (1963) locates *ade2* between 2-17.8 and 2-19.2. These results confirm the preliminary determination that *ade2* was close to, and probably to the left of, *Sp* (2-22.0) (Naguib, 1976).

2. *ade3-1*

Examination of 1500 $F_1$ male progeny yielded 43 males recombinant between *cl* and *spd* (see Figure 3). The results of the duplicate final generation tests of relative viabilities on yeast-sucrose and unsupplemented Sang's media are presented in Table 8. Once again, the expectation is that where the homozgyotes are viable, they should be half as frequent as the heterozygotes.

The leaky expression of the *ade3-1* auxotrophy, which was evident in the nutritional characterization, coupled with the absence of a visible phenotype, complicates the interpretation of the results. Some justification is needed for the assignment of genotypes: consideration of both the relative viability and the development time of the homozygote on Sang's medium generally gives a clear distinction between *ade3-1* and its wild-type allele. Eight of the *cl* + and 16 of the + *spd* recombinants with relative viabilities of 17 to 217% and developmental delays of less than 0.7 days are clearly *ade3-1*$^+$ (see Table 8). Likewise, that 11 *cl* + and 4 + *spd* recombinant lines carry *ade3-1* is indicated by the low viability on Sang's medium (0-9%) and high developmental delay (1.9-10.5 days). In these designations, where the relative viability

Table [illegible] *ade3-1* Recombination Mapping

A. Relative Viabilities of *Cy+* Segregants

| Genotype | Yeast | Sang's | Delay |
|---|---|---|---|
| *cl + +* | 51 | 39 | 1.5* |
| | 38 | 40 | 1.8* |
| | 33 | 7 | -1.4* |
| | 90 | 51 | -0.6 |
| | 53 | 26 | -0.2 |
| | 46 | 17 | 0.2 |
| | 53 | 35 | 0.2 |
| | 48 | 53 | 0.7 |
| | 46 | 30 | -0.5 |
| | 55 | 36 | 0.7 |
| | 40 | 35 | 0.6 |
| | 57 | 11 | -0.8* |
| *cl ade3-1 +* | 61 | 0 | --- |
| | 42 | 9 | 3.2 |
| | 37 | 1 | 5.6 |
| | 54 | 2 | 6.7 |
| | 45 | 1 | 1.9 |
| | 52 | 0 | --- |
| | 60 | 4 | 5.4 |
| | 46 | 1 | 9.1 |
| | 33 | 4 | 6.7 |
| | 48 | 1 | 10.5 |
| | 38 | 0 | --- |
| *+ + spd* | 41 | 24 | -0.1 |
| | 44 | 56 | -0.1 |
| | 58 | 34 | 0 |
| | 56 | 29 | 0.1 |
| | 41 | 86 | -2.9 |
| | 63 | 20 | -0.1 |
| | 49 | 32 | 0.4 |
| | 40 | 34 | 0.2 |
| | 41 | 33 | 0.3 |
| | 39 | 22 | -0.2 |
| | 41 | 59 | -0.1 |
| | 62 | 29 | 0.3 |
| | 4 | 29 | -0.3 |
| | - | 41 | -0.2 |
| | 3, | 217 | -2.2 |
| | 41 | 37 | -0.4 |
| *+ ade3-1 spd* | 34 | 2 | 8.2 |
| | 43 | 0 | --- |
| | 41 | 0 | --- |
| | 53 | 0 | --- |

TABLE 8 (continued)

B. Summary of Recombinants

| | |
|---|---|
| *cl + +* | 11 |
| *cl ade3-1 +* | 11 |
| *+ ade3-1 spd* | 4 |
| *+ + spd* | 16 |
| Total | 42 |

The recombinants were generated by following the protocol outlined in Figure 3 mapping *ade3-1* in relation to *cl* and *spd*. In the final generation, the parents of both sex are heterozygous for the recombinant chromosome and *SM5(Cy)*. The crosses are initiated on yeast-sucrose medium and then transferred to nucleoside-free Sang's medium to determine which allele of *ade3* is present.

A. The numbers represent the relative viability of homozygous recombinants *(Cy+)*. The theoretical expectation is for a relative viability of 50%.

"Delay" represents the difference, in days, between the mean developmental time to eclosion of homozygous recombinant and heterozygous *(Cy)* control flies.

Negative values indicate that the homozygotes developed faster than the controls.

Ambiguous results (*) are discussed in the text.

B. The data in A. are summarized.

seems extreme for the genotype (high for *ade3-1* or low for *ade3-1*$^-$), the assignment is justified by the development time (very long for *ade3-1* and short for *ade3-1*$^-$).

The 4 remaining *cl* + recombinants are less clear cut. In two cases (Table 8, *cl*+ +, line 1 and 2), the designation *ade3-1*$^-$ is applied on the basis of high viability on Sang's medium (39 and 40% respectively), although both develop rather slowly (delays of 1.5 and 1.8 days) compared to the other recombinants wild-type for *ade3-1*. In the nutritional tests, *ade3-1/ade3-1* escapers generally developed between 4 and 8 days more slowly than heterozygotes on restrictive media, and of the clearly *ade3-1* recombinants recovered in this analysis, only one developed with as short a delay as 1.9 days. The delay for the remainder ranged from 3.2 to 10.5 days. Consequently, the designation of *cl ade3-1*$^-$ + in these two cases seems appropriate.

The third *cl* + recombinant requiring mention had a relative viability, on Sang's medium, of only 7% (line 3) but the homozygotes developed 1.4 days faster than the heterozygotes. The data represent one replicate with only 30 total progeny (28 *Cy*:2 *Cy*$^+$). Attempts to reset the cross failed and the line was lost. In this case, with such a low yield, the relative viability on Sang's is probably misleading and the designation of *ade3-1*$^-$ was based on the developmental time.

The final questionable *cl* + recombinant (line 12) also had low viability on restrictive medium (11%) and developed quickly (0.8 days faster than the heterozygote). In this case, the data are pooled from two replicates which gave conflicting results. In one cross the homozygote was completely inviable on Sang's medium (51 *Cy* : 0 *Cy*$^+$) and in the second the relative viability of the homozygote was 13% (203 *Cy*:27 *Cy*$^+$). Since the line was lost before it could be retested, the two results remain incompatible and this recombinant was disregarded in the mapping calculations.

Standard calculations, based on 42 recombinants, locate *ade3* at 2-20.0 in a 95% confidence interval of 2-19.2 to 2-20.7. The asymmetries in the frequencies of reciprocal recombinant classes are not significant at 5%. Although the 95% confidence

intervals meet, *ade3* appears to be to the right of *ade2*. This agrees with the order suggested by the preliminary mapping with dominant markers.

3. *bur-gua2-1*

*bur-gua2-1* was mapped using the visible markers *pr* (*purple*) and *cn* (*cinnabar*) (see Figure 3). Table 9 gives the data from the final generation tests, done in duplicate, of the surviving 19 (of 25) recombinant males recovered among 2200 $F_1$ male progeny. The offspring are expected in the ratio of 2 heterozygotes to 1 homozygote where the siblings are equally viable. Of the 7 recombinants carrying *bur-gua2-1*, 6 were completely inviable and 1 nearly so (0.3%) on Sang's medium. The escapers developed with a delay of 2 days. The 12 *bur*$^+$ recombinants survived well on restrictive medium (10-44%) with developmental delays of less than 1 day. As usual, if the relative viability was low in the "wild-type" recombinants, the homozygotes developed with little delay compared to the heterozygotes.

The eye-colour associated with *bur-gua2-1* interacts with *cn* to produce a yellow-orange colour which was visible in the 3 *cn* recombinants carrying the auxotrophy. The *bur-gua2-1* eye-colour is not detectable in combination with *pr*, but when the *pr* recombinants are made heterozygous with *bur-gua2-1*,(*pr*$^+$), only those carrying the auxotrophy express the associated eye-colour. In the unsuccessful mapping of *bur-gua2-1* against *rdo hk pr*, the eye-colour and auxotrophy also cosegregated in nearly 40 recombinants. Hence, it appears probable that both phenotypes result from a single mutation.

Based on the data in Table 9, which contain no significant asymmetries, *bur-gua2-1* is located at 2-55.8 in a 95% confidence interval of 2-55.2 to 2-56.4. This location is in agreement with the preliminary mapping work.

TABLE 9: *bur gua2-1* Recombination Mapping

A. Relative Viabilities of *Cy+* Segregants

| Genotype | Yeast | Sang's | Delay |
|---|---|---|---|
| *+ + cn* | 56 | 10 | -0.2 |
| | 68 | 23 | -0.5 |
| | 41 | 32 | -0.4 |
| | 39 | 11 | -0.5 |
| *+ bur cn* | 30 | 0.3 | 2.0 |
| | 30 | 0 | --- |
| | 34 | 0 | --- |
| *pr + +* | 40 | 38 | 0.2 |
| | 42 | 33 | 0.6 |
| | 32 | 32 | 0.7 |
| | 29 | 22 | 0.3 |
| | 41 | 44 | 0 |
| | 47 | 36 | 0.3 |
| | 47 | 35 | 0 |
| | 39 | 34 | 0.2 |
| *pr bur +* | 26 | 0 | --- |
| | 25 | 0 | --- |
| | 22 | 0 | --- |
| | 3 | 0 | --- |

B. Summary of Recombinants

| | |
|---|---|
| *+ + cn* | 4 |
| *+ bur cn* | 3 |
| *pr bur +* | 4 |
| *pr + +* | 8 |
| Total | 19 |

The recombinants were generated by following the protocol outlined in Figure 3 mapping *bur gua2-1* in relation to *pr* and *cn*. In the final generation, the parents of both sex are heterozygous for the recombinant chromosome and *SM5(Cy)*. The crosses are initiated on yeast-sucrose medium and then transferred to nucleoside-free Sang's medium to determine which allele of *bur* is present.

A. The numbers represent the relative viability of homozygous recombinants *(Cy+)*. The theoretical expectation is for a relative viability of 50%.

TABLE 9 (continued)

"Delay" represents the difference, in days, between the mean developmental time to eclosion of homozygous recombinant and heterozygous *(Cy)* control flies.

Negative values indicate that the homozygotes developed faster than the controls.

B. The data in A. are summarized.

*bur gua2-1* is abbreviated to *bur*.

## Complementation Tests

1. *ade2-1* and *ade3-1*

The nutritional phenotypes of the two AR-requiring mutants are very similar and as the 95% confidence intervals about their map positions meet, *ade2-1* and *ade3-1* were tested for allelism. When homozygous *ade2-1* females were crossed to *ade3-1/SM5* males, 306 *Cy* and 252 *Cy*$^+$ progeny survived on restrictive medium. The reciprocal cross yielded 415 *Cy* and 441 *Cy*$^+$ flies. Heterozygous *ade2-1/ade3-1* adults, which had wild-type eye-colour, developed 1.4 and 3.6 days, respectively, faster than their *Cy* siblings. Therefore all three criteria, eye-colour, viability and the developmental rate on restrictive medium, suggest that *ade2-1* and *ade3-1* are not alleles of a single locus.

2. *bur-gua2-1*

A mutation called *burgundy* (*bur*), located at 2-55.7 (Lindsley and Grell, 1968), was described by Meyer and Edmundson in 1949 with an eye-colour similar to *bur-gua2-1*. Complementation tests between *bur* and *bur-gua2-1* showed that the eye-colours are allelic. As shown in Table 10, *bur/bur-gua2-1* flies are inviable on restrictive medium in the absence of GR. Homozygous *bur/bur* adults also fail to survive on unsupplemented Sang's medium. In accordance with the conventions of naming alleles of a single locus in *Drosophila melanogaster*, (Falk and Nash, 1974a) the allele, which had previously been called *gua2-1*, was renamed *bur-gua2-1*.

## Deficiency Mapping

1. *ade2-1* and *ade3-1*

A series of deficiencies partially exposing the region between *cl* at 25D7-E4 and *spd* at 26E4-27E1 (Kotarski, *et al.*, 1983) were obtained from R. MacIntyre. Females

TABLE 10: Complementation Between *bur* and *bur gua2-1*

| Medium | + | *Cy* | Relative Viability | Delay |
|---|---|---|---|---|
| Yeast-sucrose | 121 | 267 | 45.3 | 0.2 |
| Sang's | 0 | 332 | 0 | --- |
| Sang's + RNA | 171 | 380 | 45.0 | 0.6 |
| Sang's + AR | 0* | 27 | 0 | --- |
| Sang's + XR | 1 | 235 | 0.4 | 1.4 |
| Sang's + HR | 0 | 134 | 0 | --- |
| Sang's + GR | 78 | 185 | 42.2 | 0.7 |

Crosses of *bur gua2-1/SM5* ♀ x *bur fs(2)E1/SM5* ♂ were set using 5 parents of each sex and the results pooled from 5 replicate cultures. Exceptions (*) involve 2 or 3 replicates.

The "+" survivors are *bur gua2-1/bur fs(2)E1*. These flies have the mutant *bur* eye color.

The *"Cy"* survivors are either *bur gua2-1/SM5* or *bur fs(2)E1/SM5*.

The theoretical expected relative viability of "+" is 50%.

"Delay" is the difference, in days, between the mean developmental time to eclosion of "+" and "Cy" flies.

RNA was present at 4.0 mg/ml and nucleosides at 3.2mM.

homozygous for either *cl ade2-1* or *cl ade3-1* were crossed to deficiency/*CyO* males. Table 11 gives the relative viabilities of the deficiency heterozygotes on yeast-sucrose and unsupplemented Sang's media. Both of the auxotrophs have high relative viabilities and insignificant developmental delays on restrictive medium when heterozygous with any of the deficiencies. In addition, the deficiencies do not expose the *ade2-1* eye-colour. Since both *ade2-1* and *ade3-1* are to the right of the deficiencies tested and to the left of *spd*, they are located in the cytological region from 26A7 to 27E1.

2. *bur-gua2-1*

The protocol for generating a deficiency exposing *bur* (Figure 4, Materials and Methods) takes advantage of the interaction between *bur* and *cn* to allow easier identification of the putative deficiencies. Nearly 70,000 progeny of irradiated males were examined and 56 aberrant phenotypes observed. The majority of these aberrations were phenotypically *b* (16), *Minute* (14) or *brown-Dominant* (11). Various morphological defects were observed in 10 flies and there were two apparent reversions of *cn*. None of these defects were characterized further.

Only 3 of the 56 aberrations (5.4%) were putative *bur* deficiencies. Only one putative deficiency was successfully maintained, *Df*(2R) MJ. It proved to be homozygous lethal, as expected of a deficiency, and exposes the *bur* eye-colour. In crosses of *bur-gua2-1*/SM5 to Df(2)MJ/SM5, expected to yield *Cy* and *bur*/Df progeny in a 2:1 ratio, the relative viability of the deficiency heterozygote was 45% on yeast-sucrose medium and 0% on Sang's medium (Table 11). Thus, the deficiency exposes the auxotrophy as well as the eye-colour defect. Cytological examination of polytene chromosomes from third instar larvae heterozygous for the deficiency shows that it spans 42B1-2 at the proximal end of 2R.

TABLE 11: Deficiency Mapping the Auxotrophs

| *Cy*+ Segregant | Relative Viability | | |
|---|---|---|---|
| | Yeast-Sucrose | Sang's | Delay |
| *ade2-1*/Df(2L)Gdh A | 103 | 58 | 0.2 |
| *ade2-1*/Df(2L)clot-7 | 134 | 195 | -0.3 |
| *ade2-1*/Df(2L)clot-1 | 103 | 313 | -0.5 |
| *ade3-1*/Df(2L)Gdh A | 102 | 64 | 0.2 |
| *ade3-1*/Df(2L)clot-7 | 107 | 118 | 0 |
| *bur*/Df(2R)MJ | 45 | 0 | --- |

The data derive from five replicate cultures each containing five parents of each sex. Crosses are initiated on yeast-sucrose medium, then transferred to nucleoside-free Sang's medium. The deficiencies are described in Table 2.

For *ade2-1* and *ade3-1*, homozygous auxotrophic mutant ♀ were crossed to ♂ heterozygous for the deficiency and the second chromosome balancer, *CyO*. The deficiencies expose regions of the second chromosome between *cl* and *spd* .

The "+" survivors, heterozygous for either *ade2-1* and *ade3-1*, and the deficiency, are expected to be absent on Sang's medium if the deficiency exposes the auxotrophy. Otherwise their expected relative viability is 100%.

In the case of $bur^{gua2-1}$ (abbreviated to *bur* in the Table), the reduced fertility of homozygous ♀ necessitated the use of $bur^{gua2-1}$/*SM5* flies as the ♀ parent. These were crossed to ♂ heterozygous for the deficiency and *SM5*. Hence, the expected relative viability of $bur^{gua2-1}$ deficiency heterozygotes is only 50%.

"Delay" is the difference, in days, between the mean developmental time to eclosion of *"Cy+"* and *"Cy"* flies. Negative values indicate that the non-*Cy* flies develop faster than the controls.

**Nutritional Shift Experiments**

In this series of experiments, a synchronized population of larvae is allowed to commence development on either complete (yeast-sucrose) or restrictive (unsupplemented Sang's) medium. After defined intervals, the larvae are transferred to the other medium where they complete development. By analogy with temperature shift experiments involving temperature-sensitive mutations, these experiments should provide information concerning the period during which the mutant homozygotes depend upon exogenously supplied purines and yield clues as to the time of wild-type gene activity.

Transferring larvae after defined times rather than at specific larval stages imposes a difficulty which must be borne in mind when interpreting the results: the larvae do not develop at the same rate on yeast-sucrose and unsupplemented Sang's media. Development is slower on unsupplemented Sang's than it is on yeast-sucrose medium, (even for flies without unusual dietary requirements). To avoid repetition later, the chronology of larval development under the experimental conditions is outlined here. This description of larval development is based on observations of size without reference to other criteria.

Oviposition was allowed for 8 hours on a non-nutritive medium. The resulting larval populations are expected to include equal numbers of homozygous and heterozygous progeny deriving from *mutant/SM5* female and *mutant/mutant* male parents. Twenty hours after the termination of oviposition, assuming it to be uniform in time, an average individual would be one day old. After a further 24 hours, the population is undergoing the first larval apostasis. Differences in the rate of larval development on the two media, if they exist, are not evident at this time.

On yeast-sucrose medium, the second larval apostasis begins at 96 hours. By 144 hours, two of the experimental populations, containing either *ade2-1* or *bur-gua2-1* mutants, begin to pupariate. In the *ade3-1* experiment this stage is not reached until 192 hours.

On Sang's medium, all three experimental populations reach the second larval moult at 144 hours. After 192 hours, the *ade2-1* and *bur-gua2-1* populations begin to pupariate. This stage begins in the *ade3-1* population by 240 hours.

The descriptions given above are based on the subpopulation of larger larvae which becomes visible on Sang's medium after 72-96 hours. Since homozygous individuals of all three of the auxotrophic mutants are known to develop more slowly than their heterozygous siblings when confronted with poor nutritional conditions, it is probable that the larger larvae represent the faster-developing heterozygotes. The effect on the mutant homozygotes of the experimental manipulations will be discussed for each auxotroph individually and is in addition to the developmental delay on Sang's medium already described.

The *ade3-1* populations generally develop more slowly than either of the other mutant populations. Since the larvae derive from heterozygous mothers, this is unlikely to represent a maternal, but rather a dominant effect of the *ade3-1* mutation.

The experimental effects on the embryos and the larvae can be separated. However, since the separated effects are similar for the three mutants (manipulation is most traumatic for embryos and very young larvae), the results considered here are the cumulative effects.

A lethal period for larvae raised on restrictive medium, indicated by a decreased survival until the second transfer, was observed only in the *bur-gua2-1* experiment. Homozygous *ade2-1* and *ade3-1* larvae are apparently present throughout the experiment.

The heterozygous segregants normally represent half of the experimental populations and thus serve as internal controls.

1. *ade2-1*

a. Controls

The results of the conditional shift experiments on *ade2-1* are presented in Table 12. Under standard conditions, that is, without transfer, the relative viability of *ade2-1/ade2-1* is 77%, with no developmental delay, on yeast-sucrose (complete) medium (Table 12, line 1) and 0% on Sang's (restrictive) medium (line 2). Under experimental conditions, 33% of eggs which were not subjected to further manipulation following transfer to yeast-sucrose medium yielded *ade2-1/SM5* (*Cy*) adults (Table 12, Transfer from Sang's to Yeast at t=0, line 3) and 31% of those transferred directly to Sang's medium yielded *Cy* segregants (Table 12, Transfer from yeast-sucrose to Sang's at t=0, line 10). Thus, in this experiment, up to 40% of the *ade2-1/SM5* heterozygotes die, as a result either of handling or intrinsic inviability. In comparable samples transferred a second time at t=24 hours (lines 4 and 11), recovery of *Cy* adults decreased by a further 40% indicating that the transfer is traumatic to young larvae. With increasing larval age at the time of the second transfer the trauma is somewhat reduced. The decreased survival of heterozygotes transferred after 144 hours on yeast-sucrose medium (line 16) is probably due to damage to prepupae and pupae. When initial development is on Sang's medium, the effect of larval age at transfer is less consistent.

b. Response of *ade2-1/ade2-1* when transferred from Sang's to yeast-sucrose medium

33% of the embryos transferred to yeast-sucrose medium at t=0 yield *Cy* (*ade2-1/ade2-1*) adults. This represents 100% relative viability (line 3) (or rather greater than the 77% in the untransferred controls, line 1), indicating either that the experimental conditions favour survival of *ade2-1* homozygotes compared with standard conditions, or that homozygotes are less subject to the effect of transfer, or both.

The relative viability of homozygotes which develop on Sang's medium for 24 hours before transfer decreased slightly, to 86% (line 4). After a further 24 hours on

TABLE 12: Response of *ade2-1* to Nutritional Shifts

A. Pre-test Without Transfer

| Medium | Relative Viability | Delay |
|---|---|---|
| Yeast-sucrose | 77.4 | -0.7 |
| Sang's | 0 | --- |

B. Cultures Initiated on Sang's and Transferred to Yeast-sucrose after x Hours

| x | Viability of m/SM5 | Relative Viability of m/m | Delay |
|---|---|---|---|
| 0 | 32.9 | 100.7 | -0.4 |
| 24 | 18.9 | 85.9 | -0.2 |
| 48 | 23.3 | 57.1 | -0.3 |
| 72 | 20.0 | 104.4 | 1.3 |
| 96 | 22.9 | 105.0 | 1.6 |
| 120 | 16.1 | 162.0 | 1.7 |
| 144 | 23.1 | 37.9 | 3.2 |

C. Cultures Initiated on Yeast-sucrose and Transferred to Sang's after x Hours

| | | | |
|---|---|---|---|
| 0 | 30.7 | 0 | --- |
| 24 | 17.8 | 1.3 | 5.6 |
| 48 | 17.8 | 13.8 | 5.8 |
| 72 | 18.9 | 78.8 | 3.9 |
| 96 | 20.9 | 103.2 | 2.4 |
| 120 | 26.9 | 86.0 | 0.3 |
| 144 | 23.1 | 97.3 | -0.1 |

TABLE 12 (continued):

Ten to 20 replicate crosses of 10 *ade2-1/SM5* (m/*SM5*)♀ and 5 *ade2-1/ade2-1* (m/m)♂ were initiated on yeast-sucrose medium. Five of these replicates were transferred to nucleoside-free Sang's mediur and he progeny emerging in these 10 vials scored to show that the auxotrophic behaviour was present under standard conditions (A).

The crosses were amalgamated and oviposition allowed for 8 hr. Eggs were collected in batches of 150 and placed on either Sang's (B) or yeast-sucrose (C) medium. Thereafter, at 24 hr intervals, the surviving larvae were transferred to the opposite medium to complete development. Each batch of eggs represents one replicate culture (of 3, usually) at each time point.

A. The numbers in the second column give the relative viability of m/m compared to m/*SM5* when reared under standard conditions (i.e. neither eggs nor larvae are manipulated). The expectation is for a relative viability of 100%. Delay is the difference in days, between the developmental time to eclosion of m/m compared to m/SM5. A negative value indicates that the homozygous mutants developed faster than the heterozygous controls.

B.& C. The first column lists the time of transfer. The second expresses, as a percentage, the number of m/SM5 adults as a function of the number of eggs initiating the culture. The expectation is for a viability of 50%. The third column gives the viability of m/m compared to m/SM5 adults. The theoretically expected relative viability is 100%. The last column gives the developmental delay of m/m.

restrictive medium, the relative viability again decreased, to 60% (line 5). At all of these transfer times, the homozygous flies developed marginally faster than the heterozygotes. Although the relative viability of the homozygotes increased following transfer during second instar (lines 6-8), development is slowed considerably.

Prior to 72 hours, the range of sizes among the larvae is consistent with their age range. By 96 hours, some of the larvae are appreciably smaller and this difference persists through the remainder of the experiment. Since the first appearance of the smaller larvae coincides with the onset of the delayed development of *ade2-1/ade2-1*, it seems likely that they are the homozygotes.

After 144 hours, when the larger larvae, presumably *ade2-1*/SM5, are entering third instar on restrictive medium, the relative viability of the homozygote drops below 40% and their developmental delay doubles (line 9).

These data show that dietary restriction for up to 120 hours has little effect on subsequent survival of the homozygote. By the time the presumed heterozygotes are entering 3rd instar, a large proportion of the homozygotes can no longer be rescued.

c. Response of *ade2-1/ade2-1* when transferred from yeast-sucrose to restrictive medium

Embryos transferred to Sang's at t=0 (Table 12, line 10), yielded no *ade2-1/ade2-1* adults. Even when development on complete medium is allowed for part or all of 1st instar (up to 48 hours) very few homozygous adults survive and those eclose almost 6 days after their heterozygous siblings (lines 11 and 12). Extending the supplementation into 2nd instar improves the relative viability of the homozygotes to equal the standard conditions. However, the development remains delayed (lines 13 and 14). Only when the *ade2-1/ade2-1* larvae receive a complete diet until 3rd instar (lines 15 and 16), do the relative viability and the development time both fall within the ranges commonly observed under standard supplementing conditions.

d. Summary

Homozygous *ade2-1* larvae are not affected by either supplementation or restriction of their diet during first instar. Restriction of their diet during early to mid-second instar, while not reducing the relative viability of the homozygotes, slows their development. Supplementation during the same period improves the survival of the homozygote. Homozygous larvae which do not receive exogenous purines by late second instar can no longer reach adulthood, although they are still alive at the transfer time. Those which continue to be supplemented into third instar are unaffected by later dietary restrictions.

Supplementation during second instar is necessary for the survival of *ade2-1/ade2-1*, and may indeed be sufficient. However, the latter statement cannot be made with confidence on the basis of these data alone. It might be confirmed in a similar experiment which offered each nutritional condition for timed pulses throughout development.

2. *ade3-1*

a. Controls

Under standard conditions (without manipulation), the relative viability of homozygous *ade3-1* adults was 95% on yeast-sucrose medium (complete) and 22% on unsupplemented Sang's medium (restrictive) (Table 13, lines 1 and 2). The frequency of homozygous escapers under restrictive conditions, while high, was not excessive for *ade3-1* (see Nutritional Characterization) and the developmental delay of more than 6 days was consistent with previous observations.

The yield of *ade3-1/SM5 Cy* adults from embryos transferred to yeast-sucrose medium to complete development was 33% (line 3). Eggs similarly transferred to Sang's

TABLE 13: Response of [illegible]-1 to Nutritional Shifts

A. Pre-test Without Transfer

| Medium | Relative Viability | Delay |
|---|---|---|
| Yeast-sucrose | 94.8 | 0.2 |
| Sang's | 22.0 | 6.4 |

B. Cultures Initiated on Sang's and Transferred to Yeast-sucrose after x Hours

| x | Viability of m/SM5 | Relative Viability of m/m | Delay |
|---|---|---|---|
| 0 | 32.9 | 96.6 | -0.3 |
| 24 | 27.2 | 100.0 | -0.1 |
| 48 | 29.3 | 81.1 | -0.1 |
| 72 | 30.0 | 82.2 | 0.8 |
| 96 | 28.9 | 94.6 | 0.9 |
| 120 | 32.1 | 87.4 | 1.4 |
| 144* | 31.3 | 76.6 | 2.0 |
| 168* | 23.1 | 136.0 | 1.7 |

C. Cultures Initiated on Yeast-sucrose and Transferred to Sang's after x Hours

| | | | |
|---|---|---|---|
| 0 | 29.3 | 71.2 | 5.0 |
| 24 | 13.1 | 93.1 | 3.9 |
| 48 | 22.4 | 65.3 | 3.8 |
| 72 | 24.9 | 75.0 | 4.2 |
| 96 | 25.6 | 73.9 | 2.8 |
| 120 | 32.6 | 84.3 | 2.2 |
| 144 | 33.5 | 74.5 | 1.2 |
| 168* | 35.0 | 82.9 | 0.4 |

TABLE 13 (continued):

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The culture conditions and representation of the data are defined in Table 12.

The cross was *ade3-1/SM5* ♀ x *ade3-1/ade3-1* ♂.

The relative viability of m/m is expected to be 100%.

Viability of m/SM5 is expected to be 50%.

"*" indicates that only 1 or 2 replicates were done.

medium yielded 29% *Cy* adults (line 11). Thus, as in the *ade2-1* experiment, nearly 40% of the heterozygotes died as a result of handling or intrinsic inviability. When the developing populations were transferred a second time after 24 hours, the viability of *ade3 '/SM5* decreased a further 20-50% (lines 4 and 12).

The effect of the second transfer remained fairly constant, regardless of larval age, when the populations were transferred to yeast-sucrose medium (lines 4 to 10). When the transfer was to restrictive medium, the reduction in heterozygote viability was greatest for very young larvae and decreased with increasing larval age (lines 12 to 18). Why the initial growth medium should affect the response to experimental manipulation is not clear. It is unlikely to represent an auxotrophic response since, with the possible exception of t=168 (line 10), development on Sang's medium prior to transfer did not decrease heterozygote viability.

b. Response of *ade3-1/ade3-1* when transferred from Sang's to yeast-sucrose medium.

The 97% relative viability of *ade3-1/ade3-1* embryos which developed entirely on complete medium (line 3), matched the control result (line 1). Although, somewhat variable, the relative viability of the *ade3-1* homozygotes which commenced development on purine-free medium remained high regardless of when the larvae were transferred to complete medium (lines 4 to 10).

Development of homozygotes transferred to yeast-sucrose medium after either 24 or 48 hours was as fast as the heterozygotes. However, after the elapse of 72 hours or more before transfer to yeast-sucrose medium, development of the homozygote was increasingly delayed. At the same time, it was observed that the larvae could be classified into two discrete size classes. The size differential continued to be visible at least until the last transfer at 168 hours.

c. Response of *ade3-1/ade3-1* to transfer from yeast-sucrose to Sang's medium

The relative viability of homozygous *ade3-1* adults developing from eggs transferred to Sang's medium was 71% (line 11). Although survival was unusually high, the homozygotes developed almost as slowly as under standard conditions (compare lines 11 and 2). It was observed during the nutritional characterization of *ade3-1*, that the homozygotes occasionally survived well under conditions known to be restrictive, but in these cases, their slower development appeared to be a reliable indicator of the adequacy of the nutritional conditions. The relative viability of *ade3-1/ade3-1* adults increased somewhat after the population remained on complete medium for 24 hours.

Increasing time spent on complete medium prior to transfer had little effect on the relative viability of the homozygote. However, the same conditions resulted in increasingly fast development, until after spending 168 hours on complete medium, the homozygote developed almost as fast under experimental conditions as under standard conditions (lines 18 and 1).

d. Summary

Considering only the relative viability data, it seems that *ade3-1* homozygotes survive if their diet is supplemented anytime during larval development. However, since the relative viability of *ade3-1/ade3-1* was so high after transfer to restrictive medium as embryos, it is apparent that the reliability of this conclusion is suspect.

The time required for the homozygote to complete development, a phenotype which more consistently reflects the nutritional conditions, did depend on the duration of dietary supplementation. Homozygotes which developed on Sang's medium for 48 hours, until the beginning of 2nd instar, developed as fast as their heterozygous siblings. However, when the diet was restricted for more than 48 hours, development of *ade3-1* adults was increasingly delayed.

In the reciprocal transfers, individuals which received a complete diet for the first 72 hours (mid-2nd instar) or less, developed only slightly faster than completely

unsupplemented larvae. Longer periods of supplementation resulted in significantly faster development. Homozygotes developed as fast as heterozygotes only when the supplementation extended into 3rd instar.

With the exception of the 1st larval instar during which the diet is unimportant, it appears that *ade3-1* homozygotes require continuous supplementation to completely satisfy the auxotrophic requirements.

3. *bur-gua2-1*

a Controls

The results of nutritional shift experiments involving *bur-gua2-1* are presented in Table 14. The relative viability of homozygotes which developed under standard conditions (without transfer) on yeast-sucrose medium (complete) was 43% (line 1). The relative viability of *bur-gua2-1* homozygotes is commonly lower than the theoretically expected 100% (see Nutritional Characterization). However, development of the homozygote is not delayed in these cases. No homozygous progeny survived under standard conditions on unsupplemented Sang's medium (restrictive) (line 2).

Under normal conditions, heterozygotes of *ade2-1* and *ade3-1* were equally viable on yeast-sucrose and Sang's media, but this does not appear to be true of *bur-gua2-1/SM5 (Cy)*. Only 62% as many heterozygotes survived on Sang's medium as on complete medium despite equal numbers of parents and equal oviposition time. This will be discussed below.

When transferred eggs were allowed to complete development on yeast-sucrose medium (line 3), the viability of the heterozygote was only 23%. Embryos similarly transferred to Sang's medium yielded only 13% heterozygotes (line 10). Thus, under conditions which are generally found to be satisfactory, 50% of *bur-gua2-1* heterozygotes die as a result of experimental conditions and intrinsic inviability. On Sang's medium, this figure approaches 75%. Heterozygotes of both *ade2-1* and *ade3-1* were equally

TABLE 14: Response of *burgua2-1* to Nutritional Shifts

A. Pre-test Without Transfer

| Medium | Relative Viability | Delay |
|---|---|---|
| Yeast-sucrose | 42.6 | 0.1 |
| Sang's | 0 | --- |

B. Cultures Initiated on Sang's and Transferred to Yeast-sucrose after x Hours

| x | Viability of m/SM5 | Relative Viability of m/m | Delay |
|---|---|---|---|
| 0 | 22.9 | 56.3 | 0.6 |
| 24 | 12.1 | 61.4 | 0.4 |
| 48 | 17.1 | 27.3 | 1.0 |
| 72 | 23.1 | 0 | --- |
| 96 | 15.3 | 0 | --- |
| 120 | 15.8 | 0 | --- |
| 144* | 12.0 | 0 | --- |

C. Cultures Initiated on Yeast-sucrose and Transferred to Sang's after x Hours

| | | | |
|---|---|---|---|
| 0 | 12.9 | 0 | --- |
| 24 | 6.7 | 0 | --- |
| 48 | 8.5 | 0 | --- |
| 72 | 14.4 | 12.3 | 4.0 |
| 96 | 11.6 | 5.8 | 4.3 |
| 120 | 22.2 | 44.0 | 0.5 |
| 144 | 20.4 | 75.0 | 0 |

TABLE 14 (continued):

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The culture conditions and representation of the data are defined in Table 12.

The cross was *bunguа2-1/SM5* x *bunguа2-1/bunguа2-1*.

The relative viability of m/m is expected to be 100%.

Viability of m/SM5 is expected to be 50%.

"*" indicates that only 1 or 2 replicates were done.

viable on yeast-sucrose and Sang's media under these conditions.

The viability of the heterozygote was further decreased by almost 50% following a second transfer after 24 hours to either yeast-sucrose (line 4) or Sang's medium (line 11). Thereafter, the viability of the heterozygote increased with increasing time on complete medium until the final transfer (lines 12 to 16). This accords with the previous experiments in which increasing larval age mitigated the apparent trauma due to transfer.

When development began on Sang's medium, the viability of *Cy* adults also increased with increasing larval age, but only until 72 hours (lines 4 to 6). Later transfers to complete medium (lines 7 to 9) supported increasingly fewer heterozygote until their viability matched that of heterozygotes which developed entirely on Sang's medium (compare lines 9 and 10). The fluctuations in the heterozygote viability may represent variations around a constant viability. However, as will be seen, nutritional deprivation for the length of time which began to reduce heterozygote viability was lethal to the homozygotes. This, coupled with the generally reduced viability of the heterozygote on restrictive medium, suggests that *bur-gua2-1* is partially dominant.

b. Response of *bur-gua2-1/bur-gua2-1* to transfer from Sang's to yeast-sucrose medium

When eggs were transferred to yeast-sucrose medium to complete development (line 3) the relative viability of *bur-gua2-1/bur-gua2-1* was 56.3%. Since this is higher than the control result on complete medium (line 1), it appears that the experimental conditions favour the homozygote although their development was slightly delayed (0.6 day). A similar result was obtained with transfers made after 24 hours (line 11).

By 48 hours, when the larvae were beginning to enter 2nd instar on Sang's medium (line 5), the relative viability after transfer decreased to half and the developmental delay doubled. Development for 72 hours or more on Sang's medium (lines 6 to 9) was lethal to the homozygote.

Between 72 and 120 hours, there were two discernible sizes among the larvae. Although the larger larvae continued to develop normally, the smaller larvae appeared to remain in 1st instar until their disappearance at 120 hours. The proportion of the larvae which were able to survive on Sang's medium until the time of transfer decreased somewhat while these size sub-populations were visible, and decreased even further after 120 hours. Hence, it is probable that the smaller larvae, which are most likely *bur-gua2-1* homozygotes, are actually dying during this interval.

c. Response of *bur-gua2-1/bur-gua2-1* to transfer from yeast-sucrose to Sang's medium.

Homozygous *bur-gua2-1* embryos transferred to Sang's medium do not complete development (line 10). Indeed prior to 72 hours (line 13), no homozygotes survived transfer to Sang's medium.

When transfer took place at 72 hours, the relative viability of *bur-gua2-1/bur-gua2-1* increased to 12% (line 13). After larvae were transferred at 96 hours, the relative viability of homozygotes was 6% (line 14). In both cases development of the homozygote was delayed by 4 days.

After 120 hours on complete medium prior to transfer, the relative viability of homozygous individuals increased to 44% and the developmental delay decreased to 0.5 days (line 15). The relative viability of *bur-gua2-1* adults was further increased to 75% and the developmental delay decreased to 0 if the larvae developed on complete medium for 144 hours before their diet was restricted (line 16). The results obtained after 120 hours on complete medium are comparable to the results obtained when development was entirely on yeast-sucrose medium (compare lines 15 and 3).

d. Summary

The high relative viability and nearly normal developmental rate of homozygotes which spent their first 24 hours on Sang's medium argue that *bur-gua2-1/bur-gua2-1* do

not require supplementation during the earliest part of the 1st larval instar. By 48 hours, when the larvae normally begin 2nd instar, the lack of supplementation reduces the survival of the homozygotes. The restricted diet is lethal to the homozygotes by 72 hours.

Between 72 and 120 hours, an exceptional sub-population of the larvae developing on Sang's medium retains the appearance of 1st instar larvae. The total survival of larvae until transfer from restrictive medium is high until 72 hours, at which time it begins to drop. After 120 hours, the unusual larvae disappear and larval survival decreases markedly. Because of the coincidence of the onset of lethality and the appearance of the sub-population of larvae, it seems reasonable to conclude that the developmentally stalled larvae are *bur-gua2-1/bur-gua2-1* individuals. If so, it is possible that the mutant larvae are unable to moult in the absence of a source of guanosine.

Larvae which developed on yeast-sucrose medium entered 2nd instar after 48 hours and 3rd instar after 96 hours. Homozygotes supplemented only during 1st instar were inviable and those supplemented during 2nd instar nearly so. Only homozygotes which entered 3rd instar in the presence of guanosine survived.

These results can be interpreted in two ways. Either, *bur-gua2-1* requires supplementation continuously from the middle of 1st instar until 3rd instar, or the homozygotes require guanosine to undergo the larval moults. These possibilities can be distinguished in a similar experiment in which the larvae are returned to the original medium after defined intervals. If the purines are not necessary continuously, such an experiment could define the interval or intervals when supplementation is critical.

**IMP Dehydrogenase Assays**

The dietary requirements of *bur-gua2-1* homozygotes are satisfied only by GR. Classically, this supplementation pattern would be interpreted to mean that one of the two enzymatic steps uniquely involved in the production of GMP is affected by the *bur*

mutation. Although the ineffectiveness of XR as a supplement would usually indicate a defect in the conversion of XMP to GMP by GMP synthetase, it has been suggested that XR cannot be utilized by larvae of *D. melanogaster* (el Kouni and Nash, 1977). As well, if GMP synthetase activity is absent in *bur-gua2-1* homozygotes, XMP is expected to accumulate. This was not observed in radioactive tracer studies on homozygous first instar larvae (Johnson, 1978) suggesting that *bur-gua2-1* is blocked in the previous enzymatic step catalyzed by IMP dehydrogenase.

The procedures used to extract and assay IMP dehydrogenase activity were described in Materials and Methods. The degree of purification achieved cannot be stated accurately since, as in other systems (Anderson and Sartorelli, Atkinson, *et al.*, 1963), IMP dehydrogenase activity could not be measured in extract. However, since the fractions used for enzymatic measurements contained 50%, or more, of the total protein in the crude extract, the purification was less than 5-fold.

The activity analyzed in this work was heat-denaturable. The observed reduction of $NAD^+$ was dependent on the presence of IMP. Neither H nor HR were active as substrates. The measurable activity was not affected by preincubation with 6-chloroIMP, which is a powerful inhibitor of IMP dehydrogenase in some systems (Hampton, 1963; Brox and Hampton, 1968; Sartorelli, *et al.*, 1968). Glutathione, which is sometimes added to enhance the enzyme activity (Magasanik, 1963; Weinbaum and Suhadolnik, 1964), was nonetheless found to promote inhibition by 6-chloroIMP (Hampton, 1963). Its absence in the assays reported here may have been decisive in preventing a demonstration of inhibition. However, IMP dehydrogenase of Drosophila has not been shown to be inhibited by 6-chloroIMP.

The results of enzyme assays on wild-type and homozygous *bur-gua2-1* larvae are shown in Table 15. Comparison of lines 1 and 2 shows that in the original isolate of *bur-gua2-1*, the activity of IMP dehydrogenase was only 8% of the wild-type activity. The apparent fertility of homozygous females of this line was originally very low, but

TABLE 15: IMP dehydrogenase Activities *in vitro*

| Extract | Specific Activity |
|---|---|
| wild-type | 40.0 (± 4.5) |
| *burgua2-1* original | 3.3 (± 1.5) |
| *burgua2-1* selected | 45.3 (±10.3) |
| *burgua2-1* frozen | 0.8 * |
| wild-type frozen | 35.3 * |
| *burgua2-1* "F" | 6.2 (± 0.8) |
| *burgua2-1* selected 3 months | 12.5 (± 1.9) |
| *burgua2-1* selected 8 months | 28.5 |
| *burgua2-1* selected 18 months | 21.0 (± 0.6) |

Specific activity is expressed as μmoles of NADH produced/min/mg protein. It is calculated as a function of the total change in absorbance at 340nm after 10 min and the total protein. The reaction usually remains linear for at least 10 min. The extinction coefficient for NADH at pH 8.0 is $6.22 \times 10^3$ (Anderson and Sartorelli, 1968).

Values are the average of at least 2 independent determinations, except where marked (*).

The extracts are described in the text.

was observed to increase over the generations of collecting large amounts of tissue needed to assay the enzyme. When larvae from the fertile line were assayed (line 3), it was shown that the IMP dehydrogenase activity was wild-type, although both the mutant eye-colour and the auxotrophic requirement for guanosine were still expressed.

Two experiments were done to determine the validity of the initial observation of reduced IMP dehydrogenase activity. In the first, a sample of homozygous *bur-gua2-1* larvae which had been collected and preserved at -40°C for several months was assayed. As shown in line 4 of Table 15, the enzyme activity detected was only 2% of wild-type. Line 5 is the result of an assay of wild-type larvae which had been similarly preserved and shows that prolonged freezing of the tissue did not destroy the enzyme activity.

The second experiment was a determination of the IMP dehydrogenase activity in an independently maintained line of *bur-gua2-1* homozygotes which still exhibited relatively low fertility. The result in line 6 shows that the enzyme activity was nearly an order of magnitude lower than the wild-type activity. When this stock was maintained for several generations under the conditions which had previously resulted in increased fertility and IMP dehydrogenase activity, it was again found that both the fertility and the enzyme activity (compare lines 6 and 9) increased.

The correlated increases in relative fertility (This will be discussed in the next section.) and IMP dehydrogenase activity suggest that they may be related. Either a single reverse mutation, or an accumulation of modifiers, each affecting a small change, could have been responsible for the alteration of both phenotypes. The probability of the former idea, a single reversion, is limited by at least two considerations. It requires the presence, in the original isolate of *bur-gua2-1*, of two mutations which were coincidentally capable of producing the same phenotype; furthermore the progressive increase in IMP dehydrogenase is an unlikely response to a single mutation. Nonetheless, since a variety of recombinants, generated when *bur-gua2-1* was mapped genetically, were available, they were examined for the presence of a segregating

mutation affecting the enzyme activity (Table 16). None of the recombinants express levels of activity as low as originally seen, regardless of either relative fertility (discussed in the next chapter) genotype with respect to *bur-gua2-1*. This argues against, but does not disprove existence of two mutations in the original isolate of *bur-gua2-1*. A number of generations elapsed between the loss of the IMP dehydrogenase defect and the generation of the recombinants during which, the postulated reversion, if it exists, could have been fixed in the *bur-gua2-1* line used in the mapping experiment.

## Fertility Tests

As already mentioned, the initial isolate of *bur-gua2-1* was semi-sterile. This defect was noted but not investigated until later results suggested that fertility and IMP dehydrogenase activity were associated with each other, but not with the auxotrophy. A number of related stocks were generated during the recombination mapping of *bur-gua2-1*. Representatives of six of these genotypes were examined: *b pr cn* (stocks A10, A14, A26) and *b bur-gua2-1* (B1, B2, B4, B5) are non-recombinant in the region adjacent to *bur*; *+ pr + +* (D3,D4,D5,D6, D8, D10, D12, D13) and *b + bur-gua2-1 cn* (C5, C7, C8) are recombinant to the right of *bur*; *+ pr bur-gua2-1 +* (D2, D7, D9, D11) and *b + + cn* (C1, C2, C3, C4, C6) are recombinant in the region to the left of *bur*.

The fertility patterns were determined in four crosses set with each of these 26 stocks. Comparison of Type 1 (homozygous female x homozygous male) and Type 2 (homozygous female and heterozygous male) crosses to Type 3 (heterozygous female x homozygous male) and 4 (heterozygous female x heterozygous male) gives the fertility of homozygous females relative to heterozygous females. The fertility of homozygous males is similarly indicated by comparing crosses 1 and 3 to 2 and 4. The effect, if any, of male genotype on female fertility is indicated by comparing crosses 1 and 2, or 3 and 4. The results of these crosses, expressed as the number of progeny produced per female

TABLE 16: IMP dehydrogenase Activities in Recombinants

| Extract | Relative Fertility | Specific Activity |
|---|---|---|
| D3+ | high | 20.9 (± 6.5) |
| D10+ | high | 19.3 (± 0.1) |
| D12+ | high | 28.7 * |
| D13+ | high | 14.3 (± 1.9) |
| D8+ | moderate | 12.9 (± 3.7) |
| D6+ | moderate | 27.1 * |
| C7 | moderate | 12.1 (± 3.8) |
| [illegible]8 | moderate | 23.8 (± 1.5) |
| D2 | poor | 14.6 (± 0.8) |
| D11 | poor | 21.4 (± 8.3) |

The relative fertility and IMP dehydrogenase activity of homozygous recombinants are shown. The recombinants were recovered during the recombination mapping of *bur*$^{gua2-1}$ and are included in Table 9 except II-8. This stock was generated during an earlier unsuccessful attempt to map *bur*$^{gua2-1}$ against *rdo*, *hk* and *pr*.

The estimation of fertility is discussed in more detail in the next chapter. For the purposes of this table, "high" relative fertility means that the recombinants are equally fertile as homozygotes or heterozygotes. "Moderate" means the homozygous recombinants are less than half as fertile as the heterozygotes, but the line can be maintained easily in homozygous condition. Recombinants with the "poor" fertility rating are nearly sterile. Tissue for the enzyme assays of these stocks was accumulated over a period of time before a sufficient amount was available. The two "poor" fertility stocks included in this table eventually became completely sterile as homozygotes.

Specific activity, expressed as μmoles of NADH produced/min/mg protein, was calculated as described in Table 15. Except where noted (*), values are the average of at least two independent determinations.

per day, are presented in Table 17.

Almost half of the 26 stocks were sterile as homozygotes and among these, there were three patterns of sterility. Homozygous females were sterile in six stocks (A10$^-$, A14$^-$, A26$^-$, D2$^-$, D7$^-$ and C2$^-$). (In these stock designations, the superscripted "+" and "-" indicate, respectively, that a wild-type or mutant allele of *bur* is present.) In three stocks (C1$^-$, C6$^-$ and D11$^-$) both females and males were homozygous sterile. Homozygotes of 3 stocks (D9$^-$, B1$^-$ and B2$^-$) were sterile when they were the only parents, but the sterility was rescued if either parent was heterozygous.

Table 18 presents the results of complementation tests among these homozygous sterile stocks. As shown, the sterilities fall into at least four complementation groups. C1$^-$, C6$^-$ and D11$^-$, which are sterile as both male and female homozygotes, fail to complement each other and have been assigned to complementation group A. D11$^-$ also fails to complement group B which includes the six female sterile stocks. Two members of group B, D2$^-$ and D7$^-$, fail to complement D9$^-$, a rescuable homozygous sterile stock (group C). Among the rescuable sterile stocks, D9$^-$ and B1$^-$ complement, as do B1$^-$ and B2$^-$. Since B2$^-$ was not tested for complementation with D9$^-$, it is not known whether there are two (C and D) or three (C, D and E) complementation groups governing the rescuable sterility pattern.

The simplest, but not the only, interpretation of the complementation patterns requires one locus conferring both male and female fertility (complementation group A), one which confers female fertility (B) and two, or three, which confer a rescuable sterility (C, D and possibly, E). In this interpretation, D11$^-$ must carry defects in both the male and female fertility (A) and the female fertility (B) complementation groups. D2$^-$ and D7$^-$ must be defective in the female fertility (B) and the rescuable group C factors. The remaining stocks need carry only a single sterility mutation.

If there is any relationship between the *bur* locus and fertility in these recombinants, it is obscure. Neither allele of *bur* is correlated with any specific fertility

TABLE 17: Fertility Estimations

| Stock | Genotype | Cross 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| B1 | *b + bur +* | 0 | 0.3 | 2.1 | 4.0 |
| B2 | *b + bur +* | 0 | 1.1 | 0.8 | 3.3 |
| D9 | *+ pr bur +* | 0 | 0.8 | 1.1 | 2.6 |
| A10+ | *b pr + cn* | 0 | 0 | 3.7 | 4.6 |
| A14+ | *b pr + cn* | 0 | 0 | 4.0 | 4.6 |
| A26+ | *b pr + cn* | 0 | 0 | 4.0 | 3.1 |
| C2+ | *b + + cn* | 0 | 0 | 6.9 | 5.6 |
| | *+ pr bur +* | 0 | 0 | 1.8 | 2.7 |
| | *+ pr bur +* | 0 | 0 | 0.9 | 2.9 |
| C6+ | *b + + cn* | 0 | 0 | 0 | 4.1 |
| | *b + + cn* | 0 | 0 | 0 | 4.3 |
| D11 | *+ pr bur +* | 0 | 0 | 0 | 2.0 |
| D5+ | *+ pr + +* | 0.02 | 0.08 | 0.9 | 3.1 |
| C5 | *b + bur cn* | 0.4 | 0.5 | 3.6 | 3.0 |
| C7 | *b + bur cn* | 0.3 | 0.6 | 3.3 | 3.2 |
| C8 | *b + bur cn* | 0.3 | 0.6 | 2.4 | 3.7 |
| D4+ | *+ pr + +* | 1.1 | 1.3 | 4.8 | 4.1 |
| D6+ | *+ pr + +* | 0.4 | 0.6 | 3.8 | 3.9 |
| B4 | *b + bur +* | 0.6 | ND | ND | 4.1 |
| B5 | *b + bur +* | 0.6 | 1.1 | 1.4 | 3.5 |
| D8+ | *+ pr + +* | 1.7 | 2.2 | 2.6 | 3.9 |
| C4+ | *b + + cn* | 6.3 | 5.7 | 6.2 | 6.0 |
| D3+ | *+ pr + +* | 3.6 | 3.5 | 4.0 | 3.2 |
| D10+ | *+ pr + +* | 2.7 | 2.7 | 3.3 | 3.8 |
| D12+ | *+ pr + +* | 5.8 | 5.4 | 5.3 | 3.9 |
| D13+ | *+ pr + +* | 4.0 | 4.6 | 4.6 | 4.2 |

The stocks discussed here are recombinants recovered during the genetic mapping of *burgua2-1* against *pr* and *cn* (see Table 9). The numbers give the number of progeny produced per ♀ per day from crosses involving 5 parents of each sex. Crosses were done at least in duplicate and oviposition was allowed for 5 days.

TABLE 17 (continued)

The cross numbers indicate which of the following crosses was set:

Cross 1: homozygous ♀ x homozygous ♂
Cross 2: homozygous ♀ x heterozygous ♂
Cross 3: heterozygous ♀ x homozygous ♂
Cross 4: heterozygous ♀ x heterozygous ♂

ND - not determined

*bur*$^{qua2-1}$ is abbreviated as *bur*.

TABLE 18: Complementation Tests Involving Sterile Stocks

| | C6+ | C1+ | D11 | A10+ | A14+ | A26+ | C2+ | D2 | D7 | D9 | B1 | B2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C6+ | - | - | - | + | + | + | + | + | + | + | + | ND |
| C1+ | | - | - | + | + | + | + | + | + | + | + | ND |
| D11 | | | - | - | - | - | - | - | - | + | + | ND |
| A10+ | | | | - | - | - | - | - | - | + | + | ND |
| A14+ | | | | | - | - | - | - | - | + | + | ND |
| A26+ | | | | | | - | - | - | - | + | + | ND |
| C2+ | | | | | | | - | - | - | + | + | ND |
| D2 | | | | | | | | - | - | - | + | ND |
| D7 | | | | | | | | | - | - | + | ND |
| D9 | | | | | | | | | | - | + | ND |
| B1 | | | | | | | | | | | - | + |
| B2 | | | | | | | | | | | | + |

The stocks are homozygous sterile recombinant lines described in Table 17. Parents were made heterozygous for two of the recombinant chromosomes and crossed, in duplicate, using 5 parents of each sex. Oviposition was allowed for 5 days and the resulting progeny, if any, counted.

"-" indictes that the parents were sterile; "+" that they were fertile. The fertile crosses usually produced 3 or more progeny/♀/day.

ND - not determined.

phenotype, and even representatives of a single genotype do not consistently express a single fertility pattern. Thus, among those of the B⁻ stocks which are homozygous sterile, two different rescuable sterilities are indicated. Likewise, only two of the three sterile C⁻ recombinant lines share the same defect. Finally, although all of the D⁻ lines are homozygous sterile, three complementation groups are represented and the situation is further complicated by the appearance of multiple lesions in some of these recombinants.

Only the non-recombinant A⁻ stocks, which all fall into complementation group B, and the C⁻ and D⁻ recombinant lines, all of which are homozygous fertile, behave consistently. Since C⁻ and D⁻ are the reciprocal genotypes generated by crossovers to the right of *bur*, they would normally be expected to express different fertility phenotypes if a locus affecting fertility were located in this region. However, this is not the case and the allele present at the *bur* locus appears to be largely irrelevant to the degree of fertility in these recombinants.

The degree of fertility among the remaining fourteen stocks varied. In five stocks (C4⁻, D3⁻, D10⁻, D12⁻ and D13⁻), all of which are *bur*, the homozygotes and heterozygotes were equally fertile. Homozygotes of the final nine stocks were less fertile than their heterozygous siblings. Comparing the productivity of the four crosses revealed patterns of fertility reminiscent of the previously described sterility patterns. Homozygous D8⁻, B4⁻ and B5⁻ were moderately fertile when self-crossed (cross 1). Like the rescuable sterile lines, the productivity of the cross increased if either parent was heterozygous (crosses 2 and 3) and was greatest when both parents were heterozygous (cross 4). Female homozygotes of six stocks, D4⁻, D6⁻, C5⁻, C7⁻ and C8⁻, were moderately fertile, and, like the female sterile stocks, their fertility was unaffected by the genotype of the male parent. In the final stock, D5⁻, which is suggestive of the male and female sterile lines, the fertility of homozygous females was extremely low, and homozygous males were poorly fertile.

The similarities in the patterns of fertility observed in the homozygous sterile and moderately fertile stocks suggests that the latter carry less extreme alleles of the already described fertility mutations. However, the notion remains largely unconfirmed, since the appropriate complementation tests were only done with D5$^{-}$, the least fertile of the fertile stocks. If the hypothesis is correct, D5$^{-}$ is expected to complement all of the mutants except the homozygous male and female sterile (complementation group A) lines. Heterozygotes of D5$^{-}$ and representatives of complementation group B (female sterile) are fertile (# progeny per female per day: D5$^{-}$/A26$^{-}$ = 4.5; D5$^{-}$/C2$^{-}$ = 3.0). D5$^{-}$ also complements with group C (D5$^{-}$/D2$^{-}$ = 1.3, D5$^{-}$/D7$^{-}$ = 1.1, D5$^{-}$/D9$^{-}$ = 1.5) and group D (D5$^{-}$/B1$^{-}$ = 1.3) (both rescuable sterile). Heterozygotes of D5$^{-}$ and group A (male and female sterile) (C6$^{-}$ and D11$^{-}$ were tested) are sterile. This accords with the idea that D5$^{-}$ flies carry a less extreme allele of the male and female fertility mutation (group A).

Speculation regarding the locations of the factors affecting fertility is generally unenlightening because so little of the genetic constitution of these flies was controlled. The stocks were isogenized only with respect to their second chromosomes. In two of the + *pr bur-gua2-1*$^{s}$ + stocks (D2$^{-}$ and D11$^{-}$) it was clear that the fertility factors (a total of three factors with only one common to both stocks) were not on chromosome 2: homozygotes of these stocks were originally fertile, but became sterile after several months of routine maintenance in heterozygous form.

The difficulty of localizing the fertility factors is enhanced, because the second chromosome, itself, was not defined genetically except in the *pr* - *cn* region. Fertility factors located near the ends of the second chromosome (as well as those on other chromosomes) could assort independently during the recombination mapping. This could account for the variety of fertility phenotypes associated with a single genotype. (For example, B1$^{-}$ and B2$^{-}$ are complementing rescuable sterile stocks and B4$^{-}$ and B5$^{-}$ are fertile.) There was only one consistently observed association of genotype and fertility

phenotype. Homozygotes were fully fertile only in the presence of *bur*.

It appears that the fertility in these 26 stocks is governed by a number of factors, at least some of which are not on the second chromosome and none of which are closely linked to *bur*. Interestingly, such a variety of fertility phenotypes remains unique, among the purine auxotrophs included in this work, to the *bur* stocks. All stocks related to either *ade2-1* or *ade3-1*, including the original isolates and all of the sublines generated in the recombination mapping experiments, were equally fertile as homozygotes and heterozygotes. Thus, the possibility still remains, that *bur-gua2-1* either reduces fertility, or interacts with other factors to reduce fertility.

**Guanine Deaminase Assays**

Guanine deaminase is the first enzyme involved in the catabolism of G. By its action G, is converted to X which is further catabolized to UA by XDH. UA can be converted to allantoin by uricase, although it is, itself, a major excretion product in adults (Hodge and Glassman, 1967a). Guanine deaminase has been shown to degrade 84% of G fed to wild-type larvae (Johnson, 1978).

As originally hypothesized (Naguib, 1976), the nutritional phenotype of *ade2-1* suggests that it is defective in *de novo* biosynthesis of purines. However, radioactive tracer studies on homozygous *ade2-1* larvae (Johnson, 1978) showed that *de novo* synthesis is normal in this mutant. Instead, the data suggested that guanine deaminase activity was reduced in *ade2-1/ade2-1*. If so, the concentration of the guanine nucleotide pool could increase to toxic levels. Since the toxicity of GR to cultured Drosophila cells can be counteracted by A, AR and HR (Wyss, 1977), and these are the substrates which support *ade2-1* homozygotes, reduced guanine deaminase activity is a potential explanation of the observed auxotrophic phenotype. Since *ade3-1* homozygotes are nutritionally very similar to *ade2-1/ade2-1*, the same explanation could account for this auxotrophy, as well.

Radioactive tracer studies on *bur-gua2-1/bur-gua2-1* larvae (Johnson, 1978) showed that catabolism of H, HR and GR was increased over wild-type catabolism. At that time, since the bulk of the evidence supported the notion that the auxotrophy results from defective synthesis of GMP, the increased catabolism was simply considered another aspect of the generally unhealthy phenotype of the homozygotes. When it became clear that IMP dehydrogenase, one of the two enzymes solely committed to GMP synthesis, is not responsible for the auxotrophic phenotype, the data from the radioactive tracer studies were re-examined. These studies, now, appear to suggest that guanine deaminase activity might be enhanced. The resulting increase in catabolism could result in a mutant requirement for exogenous GR.

The extraction and assay procedures for determination of guanine deaminase activity have been described. The extract excluded uricase activity but sometimes retained low levels of xanthine oxidase (XO) activity. The guanine deaminase activity obtained was thermolabile, and, as found by Hodge and Glassman (1967b), the preparation deaminated G but not GR. These workers also found that presumed tyrosinase activity retained in the extract exhibited guanine deaminase activity. The extracts used in this study all exhibited this activity, indicated by blackening of the extract, and lost activity progressively, when held above freezing for prolonged intervals.

The assay detected guanine deaminase activity by measuring the production of UA in a linked reaction mediated by XO. The activity could be detected more directly, but with reduced sensitivity (Shuster, 1955), by measuring the degradation of G at 245nm or the synthesis of X at 270nm. Observations at these wavelengths showed the expected disappearance of G and appearance of X.

The reaction was routinely measured for 10 minutes during which it remained linear. After this period, the increase in absorbance at 290nm generally ceased, indicating that the production of UA probably stopped. Further synthesis could be stimulated by the addition of G, but not by additional aliquots of either XO or the

larval extract.

The amount of activity observed was proportional to the amount of protein in the assay if the protein concentration was relatively low. High protein levels (more than 3mg/ml) were slightly inhibitory, possibly due to the concommitantly high levels of tyrosinase (see above). However, the nature of the "inhibitor(s)" was not investigated. Instead, the results presented in Table 19 were generated in experiments in which the protein concentrations were adjusted prior to assay to ensure that they fell well below the inhibitory levels. This procedure has the added advantage of ensuring that the protein levels are similar in all of the extracts. Hence the guanine deaminase activities are directly comparable. Results of a large number of assays in which the protein concentrations were not controlled also support the conclusions drawn below.

The results of guanine deaminase assays on wild-type, *ade3-1 spd*, *ade2-1 spd*, and *b bur-gua2-1* are presented in Table 19. Homozygotes of both *ade3-1* and *bur-gua2-1* have levels of guanine deaminase activity which fall within the range of wild-type activities. The guanine deaminase activity detected in *ade2-1* extracts, is only 15% of the wild-type, or 19% of *ade3-1* and *bur-gua2-1* activities.

These assays showed that *ade3-1* and, contrary to expectations, *bur-gua2-1* have wild-type levels of guanine deaminase activity. The approximately 6x reduction in the activity measured in homozygous *ade2-1* larvae supports the hypothesis that its auxotrophy may be a result of reduced catabolism of G.

**Pteridine Determination**

Homozygotes of two of the mutants included in this study (*ade2-1* and *bur-gua2-1*) have visibly altered eye-colours. Since pteridines are derived from purines, pteridine content of all three mutants was surveyed.

The pteridines were examined by comparing the intensities of spots on the chromotographs. Since the accuracy of visual comparisons is likely to suffer at low

TABLE 19: Guanine Deaminase Activities *in vitro*

| Extract | Specific Activity |
|---|---|
| wild-type | 6.87 (±1.94) |
| *b bur gua2-1* | 5.20 (±0.24) |
| *ade3-1 spd* | 5.37 (±0.56) |
| *ade2-1 spd* | 1.00 (±0.15) |

Guanine deaminase activities were measured in extracts of homozygous third instar larvae. The preparation of the extracts, and the assay procedures are described in Materials and Methods.

Specific activity is expressed as nanomoles of UA produced/min/mg protein.

The extinction co-efficient of UA at pH 8.0 is $12.15 \times 10^6$. All values are the average of at least 3 determinations.

XO was present at approximately 30 mU/assay.

The protein concentration (excluding XO) was from 0.1 to 0.7 mg/ml.

intensities, minor pteridine components were ignored and the drosopterins were considered as a unit. Standard solutions included on every chromatograph allowed the identification of isoxanthopterin, pterin and xanthopterin. Sepiapterin was identified by its presence at high concentrations in extracts of *cl* homozygotes (Hadorn and Mitchell, 1951; Nolte, 1954). In addition, identifications were aided by the characteristic colours of the pteridines. The drosopterins fluoresce red, isoxanthopterin deep violet, pterin blue, xanthopterin green-blue and sepiapterin yellow when visualized under ultraviolet light. The colours are particularly valuable in discriminating isoxanthopterin and xanthopterin, and sepiapterin and pterin which do not separate well in this system. Rfs were not calculated because the extraction procedure was modified from the literature making it unlikely that the Rfs would conform with published values.

The results are presented in figures comprised of 3 elements. Representative chromatographs were visualized under ultraviolet light and photographed. Unfortunately, the photographs are not completely accurate portrayals of the chromatographs. In particular, the resolution based on fluorescent colours is lost, and the photographic representation of intensities is sometimes misleading. The remaining elements of the figures serve to mitigate these difficulties (which will be mentioned, where appropriate, in the text). Trace outlines, presented to the right of the photographs, show the actual resolution of the spots visible under U.V. light. The problem of intensity is dealt with in the third part of the figure which presents a numerical estimate of the relative intensity of each spot. This is, of course, highly subjective and is only intended to be consistent within each chromatograph.

1. *ade2-1*

Extracts of *ade2-1*, *cl ade2-1*, *cl* and "Amherst" (wild-type) adults are compared in the four leftmost lanes of the chromatograph in Figure 5. The three lanes to the right carry the standards, isoxanthopterin, pterin and xanthopterin and illustrate an

FIGURE 5: Pteridine Analysis of *ade2-1*

Adults homozygous for the eye colour aberrations given in the figure (C) were collected and aged for ten days. Pteridine extracts were prepared and examined using descending chromatography. The protocols are described in detail in Materials and Methods.

A. The chromatograph was visualized with U.V. light and photographed.

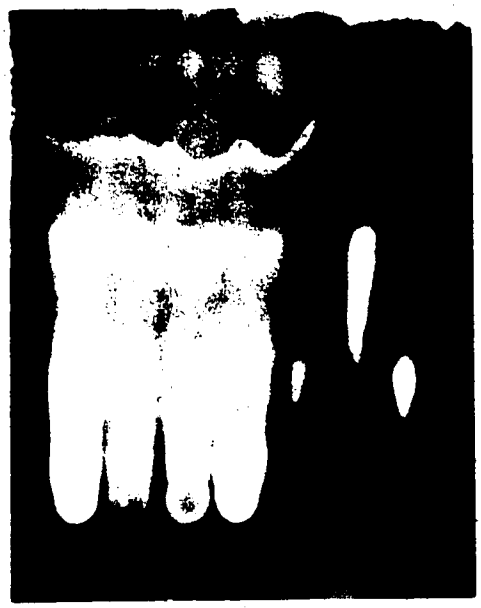
B. Because equally intense, but differently coloured spots are indistinguishable in A, a trace outline of the spots visible under U.V. light was prepared.

C. The relative intensities of the spots under U.V. light were estimated on a scale from 1 to 8.

The spots are identified in the centre between A and B.
PT = pterin SP = sepiapterin XP = xanthopterin
IXP = isoxanthopterin DP = drosopterins O = origin

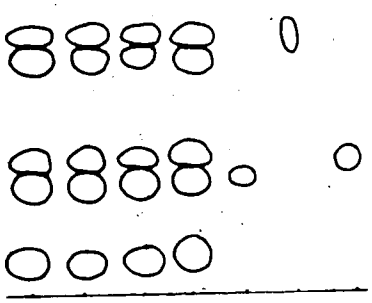
Lanes a, b, c and d contain the experimental extracts identified in C and discussed in the text.
+ is the wild-type.
Lanes e, f and g contain the standards.
e = isoxanthopterin f = pterin g = xanthopterin

A.



a b c d e f g

B.




C.

| lane | extract | DP | IXP | XP | SP | PT |
|---|---|---|---|---|---|---|
| a | *cl* | 4 | 2 | 4 | 8 | 2 |
| b | *ade 2-1* | 2 | 8 | 1 | 2 | 2 |
| c | *cl ade 2-1* | 1 | 4 | 1 | 3 | 2 |
| d | + | 8 | 7 | 2 | 2 | 2 |

important feature of the figures in this section. Although all of the standards are spotted in similar quantities, their fluorescent intensities are quite disparate. This can be important to an interpretation of the figures. As an example, isoxanthopterin and xanthopterin, which are distinguishable on the basis of colour, can have a disproportionate effect on the apparent intensity, in the photograph, of the "spot" which includes both pteridines.

The extract in lane a, *cl/cl*, is included both as a standard, to identify sepiapterin, and as an experimental lane, to examine the interaction observed between the *cl* and *ade2-1* eye-colours. As illustrated (lane a), *cl/cl* accumulates sepiapterin and has reduced amounts of drosopterins and isoxanthopterin. (Note that neither of these reductions are very obvious in the figure.) The remaining pteridines are present in quantities similar to wild-type (lane d).

The pteridine content of adult *ade2-1* homozygotes is shown in lane b. The drosopterins are strongly reduced and xanthopterin is somewhat reduced (see first paragraph for comment on the intensity of this spot). With the possible exception of isoxanthopterin, which may be slightly increased, the remaining pteridines in *ade2-1/ade2-1* are comparable to wild-type. The combined *cl* and *ade2-1* effects are depicted in lane c. The amounts of pteridines present in the double mutant are generally intermediate between the two parent mutants, but moderated in the direction of *ade2-1*. Thus, the accumulation of sepiapterin and reduction of isoxanthopterin characteristic of *cl/cl* is largely blocked in *cl ade2-1/cl ade2-1*. The exception is the drosopterin group which is even more severly reduced in the double muntant than in either parent mutant.

2. *ade3-1*

The results of chromatographing *cl*, *cl ade3-1*, *ade3-1* and "Amherst" (wild-type) are presented in Figure 6. The three standards are present in the righthand lanes. The

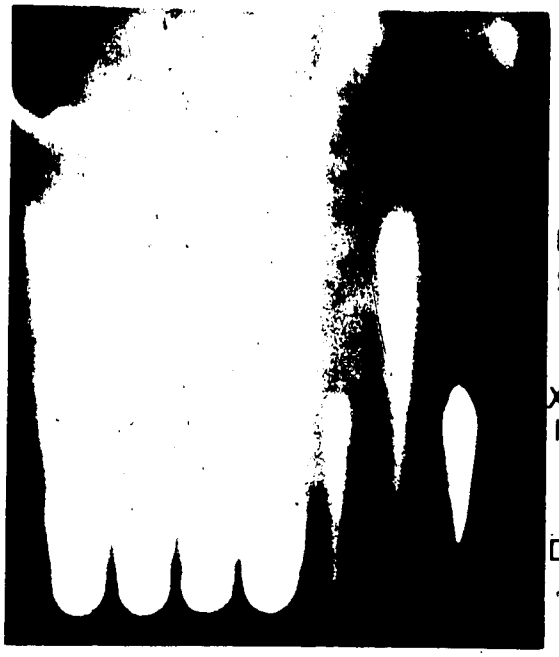
FIGURE 6: Pteridine Analysis of *ade3-1*

The figure is described in the legend to Figure 5.

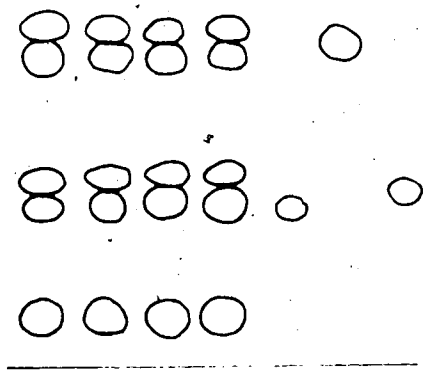
PT = pterin SP = sepiapterin XP = xanthopterin
IXP = isoxanthopterin DP = drosopterins O = origin

Lanes a, b, c and d contain the experimental extracts identified in C and discussed in the text.
+ is the wild-type.
Lanes e, f and g contain the standard solutions.
e = isoxanthopterin f = pterin g = xanthopterin

A.




B.




C.

| lane | extract | DP | IXP | XP | SP | PT |
|---|---|---|---|---|---|---|
| a | *cl* | 4 | 2 | 4 | 8 | 1 |
| b | *cl ade,81* | 2 | 2 | 3 | 8 | 1 |
| c | *ade 81* | 6 | 8 | 2 | 2 | 1 |
| d | + | 6 | 8 | 2 | 2 | 1 |

pteridine composition of *cl/cl* is described in the previous section.

The *ade3-1/ade3-1* extract appears in lane c. In this figure, it appears deficient in all pteridines. However, this is an unusual observation, and since all of the pteridines are affected equally, it is probably an artifact of this extraction. The results in part C of Figure 6 reflect those commonly obtained. The pteridine content of *ade3-1* homozygotes is directly comparable to the wild-type content (lane d). When *cl ade3-1/cl ade3-1* flies are constructed, the adult eye-colour is indistinguishable from *cl/cl* alone. This is reflected in the pteridine content of *cl ade3-1* (lane b) and *cl* (lane a) homozygotes which are identical within the limits of the technique. In keeping with the observable eye-colour, then, the pteridine content of *ade3-1/ade3-1* appears to be wild-type.

3. *bur-gua2-1*

The chromatographic analysis of the pteridines in *bur-gua2-1* homozygotes is presented in Figure 7. The standard solutions and an extract of *cl/cl* (previously described) are included in the four righthand lanes for the usual purpose of identification.

An examination of the pteridine content of *bur-gua2-1/bur-gua2-1* adults (lane b) shows no obvious changes from the wild-type pattern (lane a). In view of the visibly mutant eye-colour in these adults, this is an unexpected result. However, it has been confirmed in numerous repetitions. Occasionally, the amount of the drosopterins or sepiapterin appears reduced, but neither change is consistently observed.

When *bur-gua2-1* and *cn* are present together, the adult eye-colour in the resulting homozygote is light yellow. Since *cn* blocks ommochrome synthesis, and *bur* was assumed to affect pteridine biosynthesis, the eye-colour of the double mutant indicating deficiencies of both ommochromes and pteridines, was not surprising. However, the apparently wild-type pteridine composition of *bur-gua2-1* throws doubt on

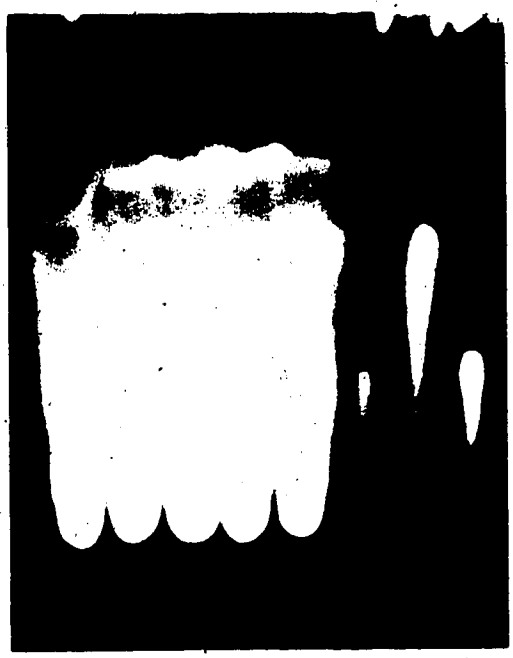
FIGURE 7: Pteridine Analysis of *bur$^{gua2-1}$*

The figure is described in the legend to Figure 5.

PT = pterin SP = sepiapterin XP = xanthopterin
IXP = isoxanthopterin DP = drosopterins O = origin

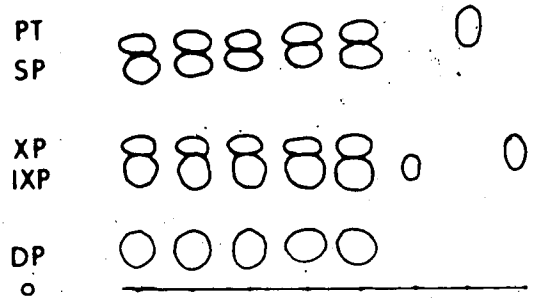
Lanes a, b, c, d and e contain the experimental extracts identified in C and discussed in the text.
+ is the wild-type.
*bur$^{gua2-1}$* is abbreviated to *bur*
Lanes f, g and h contain the standards.
f = isoxanthopterin g = pterin h = xanthopterin

A.



a b c d e f g h

B.




C.

| lane | extract | DP | IXP | XP | SP | PT |
|---|---|---|---|---|---|---|
| a | + | 8 | 6 | 3 | 2 | 1 |
| b | *bur* | 8 | 6 | 3 | 2 | 1 |
| c | *bur cn* | 8 | 6 | 2 | 3 | 1 |
| d | *cn* | 8 | 6 | 2 | 3 | 1 |
| e | *cl* | 4 | 2 | 4 | 8 | 1 |

this interpretation. Examination of *bur-gua2-1 cn/bur-gua2-1 cn* does not clarify the situation. The pteridine content of *cn/cn* (lane d), as expected, is similar to wild-type. The extract from the double mutant homozygote (lane c) is virtually identical to *cn/cn*. (In Figure 7, the extracts in lanes c and d, both appear unusually intense. This is probably an artifact arising out of slight errors of concentration during preparation.)

The *bur/bur* eye-colour defect is more striking in newly eclosed flies than in the older adults included in the extracts discussed so far. The heads of young flies were examined to see whether this was reflected in the pteridine composition. Heads of *bur-gua2-1/bur-gua2-1* adults examined within 2-4 hours of eclosion, are severely deficient in the drosopterins and slightly deficient in sepiapterin. These deficiencies are reduced over time, until by 30 hours post-eclosion, sepiapterin is present in wild-type amounts. The amount of drosopterin present increases as well, but even in 10 day-old flies remains somewhat below wild-type when only heads are examined. In this procedure, the comparisons are between the pteridines present in similar numbers of heads. The eyes of *bur-gua2-1* homozygotes are sometimes small and deformed which may be responsible for the apparent reduction in drosopterin content. However, such an effect should also be seen in the extracts. On the other hand, the mutant eye-colour and the strong interaction between *cn* and *bur-gua2-1* would be in accordance with a reduction in drosopterins.

The differences between the observations from whole-body extracts and head-only squashes might ordinarily suggest that *bur-gua2-1* flies have a tissue-specific pteridine defect. However, the results so far are not completely satisfying. While the simultaneous, though unequal, reduction in sepiapterin and the drosopterins in young flies is perplexing (As mentioned in the INTRODUCTION, although the biosynthesis of pteridines is not known in detail, sepiapterin and the drosopterins do not appear to be direct chemical relatives.) the apparent reduction in the drosopterin levels in older flies seems insufficient to account for the mutant eye-colour. Although Gregg and Smucker

(1965) found that there was often little correlation between eye-colour and pteridine content, it seems likely that changes not detectable in this system must be present in the pteridine composition of *bur-gua2-1* homozygotes.

# IV. DISCUSSION

Because the same series of experiments was usually done with each mutant in this study, the results of each experiment have been presented for the three auxotrophs sequentially. This facilitates comparisons among the mutants but does not encourage formation of an integrated picture of each mutant individually. This chapter commences with an attempt to remedy this situation and concludes with more general comments arising from consideration of the mutants.

## 1. *ade2-1*

*ade2-1* is an auxotroph which responds to AR, HR or A, but not to H, GR or G. The utilization of AR and HR requires the activity of either adenosine kinase or inosine kinase, or both. Known levels of activity of APRT (high) compared with HGPRT (low) are sufficient to explain why A, but not H, can satisfy the requirement for an exogenous purine source (Johnson, *et al.*, 1980a, c). Because GMP reductase activity is very low in Drosophila, neither GR nor G can act as the sole purine source (Johnson, *et al.*, 1980c). These considerations (see INTRODUCTION for other references) led to the proposal that the mutant defect in *ade2-1* probably lies in *de novo* IMP biosynthesis (Naguib, 1976).

Tracer studies in which homozygous larvae of *ade2-1* were fed radioactive purines or purine precursors (Johnson, 1978) showed, unexpectedly, that *de novo* synthesis appears normal in this mutant. However, radioactivity is accumulated in the guanine and guanosine pools suggesting that guanine deaminase activity is reduced. She suggested that the accumulation on one side of the purine pathway could, through a regulatory effect on *de novo* synthesis, create an imbalance in the nucleotide pools. In the absence of GMP reductase activity, this imbalance could only be redressed by feeding an adenosine source, resulting in the observed auxotrophy. Support for this hypothesis derives from the studies of Wyss (1977). The present study supports Johnson's

hypothesis by direct enzyme assay. However, the enzyme studies meet none of the criteria for suggesting that the *ade2-1* locus is the structural gene for guanine deaminase: given that the reduction in activity is quantitative rather than absolute, while the mutant *ade2-1* is a rather "clean" auxotroph, there remains a real possibility that the guanine deaminase deficiency is a secondary mutant defect.

The interpretation of experiments such as those in which larvae are transferred between media at various times is never easy. It is all the less so when the molecular basis of the phenotype being studied is unknown, but suspected of being complex and perhaps indirectly related to immediate gene action.

The nutritional definition of the situation with *ade2-1* is clear. Supplementation is required in the second instar. An ambiguity remains; either it is not required at other times, or additional supplementation either in first or in third instar serve equally well to support normal development.

Although the period of nutritional dependency is early in development, the lethal effect of the *ade2-1* mutation is not felt until much later. Unsupplemented *ade2-1* homozygotes die during third instar after slow, but otherwise normal, larval development.

It is the biochemical interpretation of the results which gives cause for thought. Even if we accept the Johnson (1978) hypothesis, the question remains as to whether the nutritionally sensitive period represents a time, for example, when (a) guanine deaminase is critically active, (b) the accumulation of guanine derivatives becomes critical, (c) supplies of adenine derivatives become depleted or (d) demand for adenine derivatives becomes unusually high. This list by no means exhausts the possibilities and merely serves as a reminder that experiments of this sort cannot, of themselves, define the biochemical basis of a mutant phenotype.

The *ade2-1* phenotype includes, in addition to the nutritional requirement, a defect in adult eye-colour which is visible whenever *ade2-1* homozygotes survive. It seems unlikely that two closely linked lesions, affecting two such closely related areas of

metabolism, would be coincidentally isolated in a single mutant strain. However, the eye-colour phenotype appears anomalous in the context of the accumulation of guanine derivatives: GTP is the direct precursor of the pteridines, so it might be expected that some of this excess would be channeled into pigment formation. None-the-less, *ade2-1/ade2-1* adults have significantly reduced levels of drosopterin, and *ade2-1* even blocks pteridine accumulation in *cl/cl* eyes. This result is consistent with the eye-colour of *ade2-1/ade2-1* but incongruent with the postulated elevated level of pteridine precursor.

Given the currently incomplete understanding of drosopterin synthesis and its regulation, the resolution of this paradox can only be speculative. The pteridine content of *ade2-1* and *cl ade2-1* homozygotes suggests that either the availability of GTP or its access to the pteridine pathways is limited. The first possibility is opposed by the accumulation of G and GR observed in *ade2-1* larvae (Johnson, 1978).

Given that there is probably only one mutation in *ade2-1*, the second possibility implies that the pteridine pathways leading to eye-pigmentation are metabolically sequestered from the general purine pools. This could be a simple geographic isolation within the developing adult, or temporal isolation, since pigment formation occurs largely at the end of the nutritionally-closed pupal phase. By that time, the nutritionally derived nucleotides could have been depleted.

Either hypothesis, spatial or temporal isolation implies that purine biosynthesis *de novo* is a prerequisite for eye-pteridine formation. Then, if elevated levels of G do suppress *de novo* synthesis, the accumulated G in *ade2-1* larvae may have the paradoxical effect of reducing the levels of GTP available for pigment formation. Note that, by citing G as the antagonist of purine biosynthesis, it is possible to avoid the objection that it could, itself, act as a precursor, there being little HGPRT activity. However, other, less elegant, hypotheses can be generated which would allow, GR, for example, to operate in the same manner.

2. *ade3-1*

The auxotrophic requirements of *ade3-1* homozygotes are best satisfied by either AR or HR. Adenine, too, is an adequate supplement for *ade3-1/ade3-1* and, as expected (see INTRODUCTION), supports the survival of a significantly greater proportion of the mutant homozygotes than hypoxanthine. Both bases promote the survival of *ade3-1* better than they do *ade2-1*. However, the severe developmental delay with H places it in doubt as a compound which genuinely remedies the defect in *ade3-1*. Indeed, the only compounds which promote survival accompanied by near normal development rates are those which rescue *ade2-1* suggesting in this case too, that the mutant may be defective in the *de novo* biosynthesis of purines.

There are a number of compounds other than H which promote survival of the mutant without supporting a normal rate of development. Naguib and Nash (1976) have argued that survival under such conditions results from the generally improved nutritional conditions which reduce other, unrelated, barriers to survival without specifically addressing the cause of the auxotrophy.

One curious feature of the auxotrophic phenotype is the response of *ade3-1/ade3-1* to supplementation with RNA. RNA was tested at a concentration which provides each of its component bases at a concentration of approximately 3 mM. This amount of either AR or HR alone, supports the survival of a greater proportion of the mutant homozygotes than the equivalent amount of RNA. Similar behaviour is observed with both *ade2-1* and *bur-gua2-1*. However, because the RNA used is not of the highest purity, it is possible, and perhaps even likely, that this behaviour is a response to toxic contaminants in the RNA.

The auxotrophic phenotype of *ade3-1* is not very stringently expressed. The same conditions which usually do not permit the survival of *ade3-1* homozygotes, sometimes support significant numbers of these individuals. For instance, GR, U and C usually support fewer than 10% of the *ade3-1* homozygotes, but in rare cases, as many

as 80% eclosed.

Attempts to establish cultures in which the auxotrophy was more strictly observed were unsuccessful. The data presented in the nutritional characterization of *ade3-1* derive from an early generation of one such line. Initially, fewer than 6% of the homozygotes survived to adulthood on Sang's medium, but by the time of the transfer experiments, which used the same line, the proportion had increased to over 20%. The inability to permanently reduce the occurrence of escapers, suggests the presence of a modifier or modifiers in the original isolate of *ade3-1*. A mutation which could reduce the individual's reliance on exogenous purines would have an obvious advantage in some conditions. Since the requirement for supplementation seems to be marginal in *ade3-1*, the effect of the modifier(s) need not be large. Such a mutation, segregating in the *ade3-1* lines, might quickly become common.

These problems are offset by the discovery that the rate of development of the homozygotes is strongly dependent on diet. Generally, if the nutritional conditions always support the survival of large numbers of homozygotes, these individuals develop as fast as their heterozygous siblings. On the other hand, in conditions which usually support only a few homozygotes, the homozygous adults eclose several days later than the heterozygotes. Then, even in circumstances where unexplained improvement in survival occurred, the development rate remained low. It has been suggested (Falk and Nash, 1974b) that slow development can, itself, be considered an auxotrophic phenotype, and certainly in the case of *ade3-1*, the mutant phenotype cannot be reliably distinguished without reference to the development time. In all, the requirement of *ade3-1* for supplementation with an adenine source appears marginal, so that one might suspect a somewhat "leaky" mutation at the enzyme level.

The behaviour of *ade3-1* homozygotes after nutritional transfers further illustrates the importance of development time as a measure of the auxotrophic phenotype; whether the cultures were initiated on complete or restrictive medium, as long as access to a

purine source was allowed at some time (minimum 24 hours) during larval development, homozygous and heterozygous *ade3-1* adults were equally viable. This again suggests that survival of *ade3-1/ade3-1* individuals is only marginally dependent on exogenous purines. In contrast, the time required to complete development varies continuously, being measurably reduced by even limited supplementation, but remaining longer than wild-type unless supplement is received for the duration of the second and third larval instars. Like *ade2-1*, it appears that *ade3-1/ade3-1* embryos contain a source of purines which is sufficient for the needs of first instar, as measured by both survival and rate of development.

The nutritional shift experiments in which absolute, rather than relative, rates of development were measured, indicated a possible dominant effect of *ade3-1* on the rate of development. Even on complete medium, the development of *ade3-1/SM5* is delayed by two days compared to *ade2-1/SM5* or *bur-gua2-1/SM5*. On nucleoside-free Sang's medium, the delay is four days. In neither case is the survival of the heterozygotes affected.

It is characteristic of *ade3-1/ade3-1* that under many nutritional conditions, survival and rate of development appear to be largely unrelated. Since these aspects of the phenotype cannot logically be considered as separate, the lack of correlation is interesting. It suggests that the availability of purines functions in two ways as a critical determinant of development; first, a certain threshold supply is necessary for survival, and second, development is paced by purine availability.

Compounds which support survival, but not normal development rates (H, CR etc.) are apparently able, either directly or indirectly, to free a supply of purines sufficient only to meet the minimum threshold requirement. Whether these are two aspects of a single process or two distinct processes is not yet known.

The question arises as to whether the dual nature of the purine requirement is limited to *ade3-1*. Although, being less "leaky" than *ade3-1*, *ade2-1* and *bur-gua2-1*

provide fewer instances for comparison, the answer is that it seems to be a fairly general phenomenon. Indeed, wild-type flies raised on purine-free medium survive although their development time is extended (Sang, 1956). This has been taken to suggest that endogenous *de novo* IMP biosynthesis is limiting even in wild-type (Nash and Henderson, 1982). If so, it accords with the contention that attainment of optimal rate of development is nutritionally more demanding than simple survival.

None of the reported results conflict with the *a priori* proposal that the *ade3-1* defect lies in *de novo* purine biosynthesis. Unfortunately, the appropriate radioactive tracer studies have not been done. The occurrence of homozygous escapers suggests that *ade3-1* reduces the amount of purine biosynthesis without producing a complete block. This assumes that homozygotes cultured on restrictive medium would be unlikely to survive the complete absence of purines resulting if *ade3-1* produces a complete block. On the other hand, if synthesis is merely reduced, slow-growing excapers are not inconceivable, by analogy with wild-type larvae cultured without purines (Sang, 1956). In view of their auxotrophic phenotypes, the absence, in *ade3-1* homozygotes, of an eye-colour defect, is consistent with the *ade2-1* mutant eye-colour only if the *ade2-1* defect is presumed to have a much more severe effect on the pteridine pathways.

In light of this interpretation of the mutant behaviour, the cytological location of *ade3-1* becomes interesting. Henikoff *et al.* (1983) have examined a plasmid from Drosophila which includes the gene for phosphoribosylglycineamide formyltransferase, the enzyme catalyzing the third step of *de novo* purine biosynthesis. The plasmid is known to hybridize, as a unique sequence, to 27C on the Drosophila cytological chromosome map (Henikoff, *et al.*, 1981). Recent results (Kennison and Nash, unpublished) have localized *ade3-1* to a small region including 27C and preliminary experiments (Henikoff and Nash, unpublished) have shown *ade3-1* homozygotes to have little GAR-transformylase activity.

3. *bur-gua2-1*

The auxotrophic phenotype of *bur-gua2-1* is the "strongest" of the three mutants described in this study; there is an absolute requirement for GR. Unlike *ade3-1*, and even *ade2-1*, *bur-gua2-1* is effectively non-"leaky". The concentration of GR required is high; at 1mM neither the viability nor the rate of development of *bur-gua2-1/bur-gua2-1* compare to *bur-gua2-1/SM5*.

Cosupplementation with allopurinol, an inhibitor of XDH and hence of catabolism, does reduce the concentration of GR required by *bur-gua2-1/bur-gua2-1*. This indicates first, that the GR pool *per se* does appear to be deficient, and second, that in the presence of such a defect, catabolism is not suppressed sufficiently to compensate. Thus, it is not exclusively a barrier to entry which regulates the level of supplementation necessary. In fact, it is possible that a high rate of catabolism is the sole cause of the strict requirement.

The survival of *bur-gua2-1* homozygotes is not promoted by G at any concentration even in the presence of allopurinol. Presumably, the low HGPRT and high guanine deaminase activities (see INTRODUCTION) combine to block the utilization of the base.

The stringency of the *bur-gua2-1* nutritional requirement is also evident in the larval shift experiments; the mutant homozygotes are completely inviable unless an exogenous purine supply becomes available before the end of the first larval instar (prior to which, the maternally derived su sufficient) and remains available until the beginning of the third instar.

It was not possible, because of erimental design, to determine whether *bur-gua2-1* homozygotes require supplem continuously until third instar, or only during the larval moults. The develop *gua2-1* adults reflects the extent of the supplementation.

The initial response to dietary restriction takes the form of delayed moult. Following continued restriction, the homozygous larvae die, apparently without moulting. This is the only instance, in this work, of mutant larvae dying during the interval encompassed by the transfers.

Homozygous *bur-gua2-1* individuals survive to adulthood if GR is supplied until the early part of third instar. Supplementation need not be continued until pupariation.

It appears that the auxotrophic requirement for GR may be dominant; the same initial number of eggs yields less than 60% as many heterozygotes on Sang's medium as on yeast-sucrose medium. Their viability varies with nutritional conditions in the same way, although to a lesser extent, as the homozygote viability. What effect the nutritional conditions may have on the rate of development is not known since the heterozygotes form the basis of comparison in this calculation.

Homozygous *bur-gua2-1* adults have a deep red mutant eye-colour. In recombination mapping experiments, both the eye-colour defect and the auxotrophic requirement for GR were shown to be located at 2-55.8 on the genetic map. This location is shared by the previously known mutation, *burgundy* (*bur*), named for its eye-colour. When the two mutations were shown to be allelic, the allele which until then had been identified as *gua2-1* because of the guanosine requirement was redesignated *bur-gua2-1*. In addition to the mutant eye-colour, *bur* and *bur-gua2-1* also share the auxotrophic requirement for guanosine.

A single chromosome deficient for *bur* was recovered after irradiation of wild-type males. The deficiency, in which the cytological interval 42B1-2 is deleted, exposes both the mutant eye-colour and the nutritional requirement characteristic of *bur-gua2-1* homozygotes. Since the two phenotypes are associated with both known *bur* alleles, they cosegregated consistently in a substantial number of recombination experiments and are uncovered by a single small deficiency, there can be little doubt that both the eye colour and the auxotrophy are governed by a single lesion.

In view of the limited availability of GTP implied by the auxotrophic requirement, a pleiotropic effect on pteridine metabolism might be predicted. However, despite the visibly mutant eye-colour, the effect of the mutation on pteridine content is not very obvious; in both *bur-gua2-1* and *bur-gua2-1 cn* adults, the pteridine defect detectable in the chromatographic studies seems insufficiently severe to account for the visible eye-colour.

The major period of pteridine synthesis in the head occurs toward the end of the pupal phase (Phillips and Forrest, 1980). As judged by the transfer experiments, the auxotrophic requirement for GR in *bur-gua2-1* homozygotes is satisfied before the end of the third larval instar. Hence, the nutritionally closed puparia must have a large enough store of G, possibly in the form of nucleic acids in potentially dead cells, to support survival. However, the G store is either insufficient for, or depleted before, pigment formation.

The nutritional requirement of *bur* homozygotes is satisfied only by GR, a phenotype which suggests, *a priori*, that the mutant defect lies in one of the terminal steps of GMP synthesis, either IMP dehydrogenase or GMP synthetase. In radioactive tracer studies on homozygous *bur-gua2-1* larvae (Johnson, 1978), it appeared that GMP synthesis might be reduced. Since the intermediate product, XMP, was not accumulated, the data were compatible with reduced levels of the first activity, IMP dehydrogenase.

*In vitro* enzyme assays showed that in third instar larvae of the original isolate of *bur-gua2-1*, the activity of IMP dehydrogenase was an order of magnitude lower than in wild-type. This defect could explain the auxotrophic and, if the need for *de novo* GTP synthesis in pigmentation is assumed, the eye-colour phenotype. However, it was found that the enzyme activity could increase to wild-type levels in sublines of the mutant, without affecting either of these mutant phenotypes.

The probability of isolating a single mutant carrying two lesions, both capable of producing the same phenotype seems, to say the least, very low. Hence, the obvious

explanation is that the transient reduction in IMP dehydrogenase activity was an artifact. Satisfying as this would be, however, the idea is not really tenable. The observations were too repeatable and the reduced activity too consistently associated only with *bur-gua2-1* sublines to be artifactual.

One element of the original mutant phenotype, female fertility, was correlated with the IMP dehydrogenase activity. Initially semi-sterile, the apparent fertility of homozygous *bur-gua2-1* females increased during the interval in which IMP dehydrogenase activity increased. The fertility of a number of independent sublines of *bur-gua2-1* was examined and it was found that defects at at least four loci (complementation groups) could produce sterility. The varying degrees of fertility of the remaining sublines may also be governed by the same loci.

It would not be surprising if females with reduced ability to synthesize GMP were less able to satisfactorily provision the egg and hence were less fertile than normal. In such flies, any increase in GMP synthesis would likely improve their fertility. In this particular case, fertility and IMP dehydrogenase activity increased concommitantly. It is suggested that the enzyme activity, in fact, increased in response to selection for higher fertility levels. Since the enzyme activity increased gradually, it is probably affected by a number of factors, possibly even the same elements involved in fertility. However, although a correlation was observed between IMP dehydrogenase activity and fertility, a causal relationship has not been demonstrated.

In addition to a possible defect in GMP synthesis, the radioactive tracer studies also showed that purine catabolism was enhanced in *bur-gua2-1* homozygotes (Johnson, 1978). As long as a defect in biosynthesis was assumed to cause the requirement for GR, the high levels of catabolism were not considered significant. However, elevated guanine deaminase activity, a defect which is compatible with all of the data, might produce a dependence on exogenous GR by effectively depleting the *in vivo* pool of G nucleotides. When guanine deaminase was assayed in extracts of third instar larvae, the

activity found in *bur-gua2-1* homozygotes was similar to wild-type. Unless the expression of the biochemical defect, like the auxotrophic requirement for GR, does not persist until the late third instar and thus would not be detected in these assays, guanine deaminase activity does not appear to be high in *bur-gua2-1/bur-gua2-1*.

In biochemical terms, the simplest explanation of the *bur* auxotrophy is still a defect in one of the enzymes directly involved in either the catabolism or biosynthesis of guanine nucleotides. Two such enzymes, guanine deaminase and IMP dehydrogenase, now seem to be eliminated from consideration. The enzymes which remain as possibilities are XDH, in catabolism, and GMP synthetase, in biosynthesis. Neither possibility is particularly satisfying.

If the *bur* mutation enhances the activity of XDH, the auxotrophic requirement for GR can be explained, but the mutant eye-colour cannot. The difficulty here, is that *rosy* mutants, which lack XDH activity, have an eye-colour similar to *bur* homozygotes. It is unlikely that both high and low levels of the same activity would result in such similar eye-colours. However, it is worth remembering that despite the fact that the involvement of XDH in both purine and pteridine metabolism is well-defined, the relationship between the biochemical lesion and the adult eye-colour in *rosy* homozygotes is not clear.

On the other hand, if the *bur* defect blocks, or reduces, GMP synthetase activity, then the substrate, XMP, should accumulate. However, the radioactive tracer studies found only the usual amounts (Johnson, 1978). Unless the XMP is catabolized without reaching detectably increased levels, this defect in biosynthesis seems unlikely.

Thus far, the simple enzymatic explanations of the *bur* auxotrophy have proven unsatisfactory. This leaves the possibility that *bur* is, instead, involved in the regulation of purine metabolism. Because it is such an important area, the regulation of purine metabolism is likely to be extremely subtle and complex.

# V. CONCLUSIONS

In the early work with auxotrophy in prokaryotes, it was often possible to extrapolate from the observed mutant nutritional requirement directly to the biochemical lesion. As the mutants included in this study show, the problem is often not as simple in Drosophila. On the basis of nutritional needs alone, it would be predicted that both *ade2-1* and *ade3-1* have mutant defects in the *de novo* biosynthesis of purines and that *bur-gua2-1* is defective in the synthesis of GMP from IMP. The weight of the evidence favours the *a priori* hypothesis only in the case of *ade3-1*. Catabolism, rather than biosynthesis, seems to be reduced in *ade2-1* mutants. However, there is a discrepancy between the non-"leaky" nutritional behaviour of *ade2-1* homozygotes and the only partial reduction in guanine deaminase activity which leaves this conclusion open to change. The evidence is so far insufficient to identify the cause of the *bur* auxotrophy.

Despite being largely inconclusive in defining the biochemistry of the mutant defects, this study did shed some light on purine nucleoside auxotrophy in Drosophila. Whether this information will be applicable to fruit fly auxotrophs in general remains to be seen.

In genetic terms, the situation is fairly straight-forward; three auxotrophic mutants have been unambiguously mapped to distinct locations on the second chromosome. The guanosine-requirer, *bur-gua2-1*, has proven to be an allele of a previously described mutation and *ade2-1* and *ade3-1* are, each, the only known alleles of their respective loci. There is no reason, at present, to suppose that these loci are genetically complex. The proximity of the two adenosine-requirers is interesting in this context, but at a distance of 2 map units, the appearance of proximity is probably illusory.

The lack of "simple" success in the analysis of purine auxotrophy in Drosophila may be characteristic of eukaryotes in general. Auxotrophic mutations of purine biosynthetic genes known in mammalian cell cultures (see Puck and Kao, 1982),

demonstrate the existence and mutability of the relevant genes in higher eukaryotes. Despite their auxotrophic behaviour in cell culture, such mutations have not been identified in man. Instead, mutations of purine metabolism are limited to either "salvage" or catabolic enzymes (Boss and Seegmiller, 1982).

This disparity between the cellular and organismal identification of mutations may stem from the complex organization of animals. The same features of higher eukaryotes which may be acting to limit the range of viable mutations, multicellularity, size, specialized tissues and organs, interactive developmental processes, may also be acting to reduce the ease with which such mutants can be analyzed.

The nutritional shift experiments were the first ever performed with Drosophila auxotrophs and helped define both the genes, and the dynamics of purine synthesis, accumulation and utilization.

Contrary to expectations, the three mutants have dissimilar temporal patterns of nutritional requirement. If, as has been suggested on other grounds, *de novo* purine biosynthesis is already limiting (Nash and Henderson, 1982), it would be expected that any reduction would result in a continuous requirement for exogenous purines. However, within the limits of the metrics employed (survival and development time) only *ade3-1* requires continuous supplementation. The other two mutants each require only temporary supplementation. Their periods of dependence overlap, but are quite distinct.

There are various explanations of a temporary requirement for supplementation. These generally involve tissue or stage-specific nutritional needs or gene activities.

In addition to their periods of dependency, these auxotrophs also differ on the basis of associated dominant effects. Heterozygotes of *bur-gua2-1* suffer from reduced viability, and *ade3-1/SM5* individuals develop slowly when cultured without purines. The third auxotroph, *ade2-1*, behaves as a standard recessive mutation.

Auxotrophic mutations producing a dominant requirement for pyrimidines affect carbamyl phosphate synthetase, the rate-limiting enzyme of pyrimidine biosynthesis (Falk and Nash, 1974a). By analogy, auxotrophic mutations resulting in a dominant requirement for purines might also be expected to affect the rate-limiting enzymes of purine metabolism. However, this appears not to be true of *ade3-1* which is defective in GAR transformylase, an activity not thought to be rate-limiting in purine biosynthesis *de novo* (see INTRODUCTION). The *bur* defect has not been defined, and consequently cannot be discussed in this context.

It should be remembered that dominant effects are possible in other ways than this. Regulatory defects, for instance, are frequently dominant. While the regulation of *de novo* purine biosynthesis is not known in detail, the importance of this area of metabolism suggests it will be complex. Hence the possible range of mutational lesions may be lar

An additional consideration is that any step of a pathway may become limiting if it is sufficiently reduced. Any time that a single wild-type allele cannot supply enough enzyme activity to maintain the overall rate of the pathway, that gene may be expected to have dominant mutant alleles. The dominant effects, in such cases, may be allele-specific.

In contrast to these differences, a feature common to these auxotrophs is the sensitivity of development rate to nutritional conditions. Culture conditions sufficient for mutant survival are often completely inadequate to support a normal rate of development. Since development is a complex and interactive process, its rate will be paced by the most nutritionally demanding steps. When any of these three auxotrophs are cultured in sub-optimal, but not completely restrictive, conditions, development seems to "stall" temporarily. Presumably, this allows the individual to accumulate the nutrients required to proceed. Thus, survival is ensured, but since development occurs in "fits and starts", it is slower than usual.

Improvements in the understanding of pteridine metabolism arising from this study are largely hypothetical. On the one hand, the detailed biochemical relationship between purine and pteridine metabolism remains obscure. On the other hand, it is abundantly clear, that genetically the two are inextricable. With few exceptions, *ade3-1* being one, purine auxotrophs in Drosophila are associated with defective eye-colours. Within an overall theoretical framework linking purine auxotrophy and pteridine synthesis to the maintenance of balanced purine pools the eye-colours of these mutants can now be explained.

Despite a certain amount of progress in some areas, purine nucleoside auxotrophy in Drosophila still presents a fruitful area for future investigations.

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APPENDIX 1: Preliminary Mapping Data

I *ade2-1* (from Naguib, 1976)

Cross: $\frac{S\ Sp\ +}{+\ +\ ade2\text{-}1} \times \frac{+\ +\ ade2\text{-}1}{+\ +\ ade2\text{-}1}$

Results:

| | Genotype | | | |
|---|---|---|---|---|
| | *S Sp*$^+$ | *S*$^+$ *Sp* | *S*$^+$ *Sp*$^+$ | *S Sp* |
| *# segregants surviving on Sang's medium | 50 | 176 | 6 | 388 |

From the double recombinant class, *S*$^+$*Sp*$^+$, *ade2-1* is located between *S* and *Sp*. The number of recombination events between *S* and *ade2-1* (176+6) is 3x greater than between *ade2-1* and *Sp* (50+6). Hence *ade2-1* is much closer to *Sp*.

II. *ade3-1*

Cross: $\frac{S\ Sp\ +}{+\ +\ ade3\text{-}1} \times \frac{+\ +\ ade3\text{-}1}{+\ +\ ade3\text{-}1}$

Results:

| | Genotype | | | |
|---|---|---|---|---|
| | *S Sp*$^+$ | *S*$^+$ *Sp* | *S*$^+$ *Sp* | *S Sp* |
| *# segregants surviving on Sang's medium | 103 | 99 | 394 | 290 |
| Average Development Time | 19.3 | 14.0 | 18.9 | 13.6 |

The frequency of the recombinant classes must be considered in conjunction with the development time since alone, the frequencies suggest the genes are not linked. The *S Sp*$^+$ and *S*$^+$ *Sp*$^+$ classes, develop on average 5 days more slowly and probably include many homozygous escapers. In order to reconcile the frequencies of recombinant classes, the gene order is determined as *S Sp ade3-1*. Distance calculations are extremely unreliable because of the escapers.

*Recombinants homozygous for the auxotrophy are not expected to be represented among the survivors.

APPENDIX 1 (continued): Preliminary Mapping Data

III *bur gua2-1*

Cross: $\frac{+\ +\ Tft}{b\ bur\ +}$ x $\frac{b\ bur\ +}{b\ bur\ +}$

Results:

| | Genotype | | | |
|---|---|---|---|---|
| | *b Tft+* | *b+ Tft* | *b+Tft+* | *bTft* |
| *# segregants surviving on Sang's medium | 1 | 316 | 0 | 5 |

The low frequency of recombinants makes it difficult to reliably decide the gene order. However, it is clear that *bur* is close to *Tft*.

IV *bur gua2-1*

Cross: $\frac{rdo\ hk\ pr\ +}{+\ +\ +\ b\ u\ r}$ x $\frac{+\ +\ +\ bur}{+\ +\ +\ bur}$

Results:

| | Genotype | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *rdo hk +* | | *rdo + +* | | *+ hk pr* | | *+ + pr* | |
| | *bur* | + | *bur* | + | *bur* | + | *bur* | + |
| Recombinants recovered | 6 | 0 | 8 | 0 | 0 | 4 | 0 | 7 |

The recovery of no *pr bur* recombinants suggests that *bur gua2-1* is to the right of *pr*.

Map locations:

| | | | |
|---|---|---|---|
| S | 2-1.3 | rdo | 2-53 |
| Sp | 2-22.0 | hk | 2-53.9 |
| b | 2-48.5 | pr | 2-54.5 |
| Tft | 2-53.2 | | |

*bur gua2-1* is abbreviated to *bur*.

APPENDIX 2: Nutritional Shift Data

I *ade2-1*

A. Cultures initiated on Sang's and transferred to yeast-sucrose medium

| Time of Transfer | Replicate | # of Eggs Transferred | # of Larvae Transferred | # of *Cy* Adults Eclosed | # of *Cy*$^+$ Adults Eclosed | Development Delay of *Cy*$^+$ Adults |
|---|---|---|---|---|---|---|
| 0 | 1 | 150 | --- | 49 | 44 | 0 |
| | 2 | 150 | --- | 37 | 50 | 0 |
| | 3 | 150 | --- | 62 | 55 | 0 |
| 24 | 1 | 150 | 91 | 26 | 28 | 0 |
| | 2 | 150 | 106 | 30 | 24 | 0 |
| | 3 | 150 | 116 | 29 | 21 | 0 |
| 48 | 1 | 150 | 86 | 32 | 20 | 0 |
| | 2 | 150 | 101 | 44 | 16 | 0 |
| | 3 | 150 | 106 | 29 | 24 | 0 |
| 72 | 1 | 150 | 66 | 17 | 20 | 0 |
| | 2 | 150 | 107 | 32 | 33 | 1 |
| | 3 | 150 | 115 | 41 | 41 | 1 |
| 96 | 1 | 150 | 59 | 14 | 14 | 1 |
| | 2 | 220 | 195 | 63 | 67 | 1 |
| | 3 | 150 | 119 | 42 | 44 | 2 |
| 120 | 1 | 150 | 89 | 30 | 22 | 1 |
| | 2 | 150 | 96 | 35 | 22 | 3 |
| | 3 | 190 | 142 | 14 | 84 | 1 |
| 144 | 1 | 150 | 109 | 41 | 20 | 4 |
| | 2 | 150 | 70 | 36 | 14 | 2 |
| | 3 | 150 | 124 | 33 | 21 | 3 |

APPENDIX 2 (continued): Nutritional Shift Data

I *ade2-1*

B. Cultures initiated on yeast-sucrose and transferred to Sang's medium

| Time of Transfer | Replicate | # of Eggs Transferred | # of Larvae Transferred | # of *Cy* Adults Eclosed | # of *Cy*+ Adults Eclosed | Development Delay of *Cy*+ Adults |
|---|---|---|---|---|---|---|
| 0 | 1 | 150 | --- | 38 | 0 | |
| | 2 | 150 | --- | 39 | 0 | |
| | 3 | 150 | --- | 61 | 0 | |
| 24 | 1 | 150 | 101 | 34 | 0 | |
| | 2 | 150 | 66 | 20 | 0 | |
| | 3 | 150 | 103 | 26 | 1 | 7 |
| 48 | 1 | 150 | 54 | 12 | 2 | 6 |
| | 2 | 150 | 88 | 30 | 2 | 6 |
| | 3 | 150 | 124 | 38 | 7 | 5 |
| 72 | 1 | 150 | 82 | 27 | 10 | 4 |
| | 2 | 150 | 80 | 19 | 20 | 2 |
| | 3 | 150 | 109 | 39 | 37 | 2 |
| 96 | 1 | 150 | 59 | 16 | 10 | 1 |
| | 2 | 150 | 120 | 40 | 42 | 2 |
| | 3 | 150 | 119 | 38 | 45 | 1 |
| 120 | 1 | 150 | 77 | 34 | 20 | 0 |
| | 2 | 150 | 114 | 41 | 37 | 0 |
| | 3 | 150 | 121 | 46 | 47 | 0 |
| 144 | 1 | 150 | 46 | 13 | 14 | 0 |
| | 2 | 150 | 110 | 48 | 45 | 0 |
| | 3 | 190 | 149 | 52 | 51 | 0 |

APPENDIX 2 (continued): Nutritional Shift Data

II *ade3-1*

A. Cultures initiated on Sang's and transferred to yeast-sucrose medium

| Time of Transfer | Replicate | # of Eggs Transferred | # of Larvae Transferred | # of *Cy* Adults Eclosed | # of *Cy*+ Adults Eclosed | Development Delay of *Cy*+ Adults |
|---|---|---|---|---|---|---|
| 0 | 1 | 150 | --- | 33 | 42 | 0 |
| | 2 | 150 | --- | 53 | 49 | 0 |
| | 3 | 150 | --- | 62 | 52 | 0 |
| 24 | 1 | 150 | 93 | 38 | 26 | 0 |
| | 2 | 170 | 151 | 49 | 53 | 0 |
| | 3 | 150 | 119 | 41 | 49 | 0 |
| 48 | 1 | 150 | 90 | 38 | 30 | 0 |
| | 2 | 150 | 110 | 52 | 37 | 0 |
| | 3 | 150 | 107 | 42 | 40 | 1 |
| 72 | 1 | 150 | 124 | 54 | 37 | 0 |
| | 2 | 150 | 95 | 37 | 30 | 0 |
| | 3 | 150 | 123 | 44 | 44 | 1 |
| 96 | 1 | 150 | 126 | 43 | 42 | 1 |
| | 2 | 150 | 109 | 39 | 41 | 1 |
| | 3 | 150 | 136 | 48 | 40 | 1 |
| 120 | 1 | 170 | 155 | 50 | 57 | 2 |
| | 2 | 150 | 109 | 48 | 29 | 1 |
| | 3 | 150 | 132 | 53 | 46 | 2 |

APPENDIX 2 (continued): Nutritional Shift Data

II *ade3-1*

A. Cultures initiated on Sang's and transferred to yeast-sucrose medium

| Time of Transfer | Replicate | # of Eggs Transferred | # of Larvae Transferred | # of *Cy* Adults Eclosed | # of *Cy*+ Adults Eclosed | Development Delay of *Cy*+ Adults |
|---|---|---|---|---|---|---|
| 144 | 1 | 150 | 92 | 32 | 26 | 2 |
| | 2 | 150 | 125 | 62 | 46 | 2 |
| | 3* | | | | | |
| 168 | 1 | 108 | 73 | 25 | 34 | 3 |
| | 2* | | | | | |

*Culture lost to microbial contamination

APPENDIX 2 (continued): Nutritional Shift Data

II *ade3-1*

B. Cultures initiated on yeast-sucrose and transferred to Sang's medium

| Time of Transfer | Replicate | # of Eggs Transferred | # of Larvae Transferred | # of *Cy* Adults Eclosed | # of *Cy*+ Adults Eclosed | Development Delay of *Cy*+ Adults |
|---|---|---|---|---|---|---|
| 0 | 1 | 150 | --- | 49 | 28 | 5 |
| | 2 | 150 | --- | 37 | 36 | 5 |
| | 3 | 150 | --- | 46 | 30 | 4 |
| 24 | 1 | 143 | 103 | 15 | 13 | 3 |
| | 2 | 150 | 111 | 26 | 18 | 5 |
| | 3 | 150 | 108 | 17 | 23 | 4 |
| 48 | 1 | 150 | 73 | 34 | 21 | 2 |
| | 2 | 150 | 113 | 30 | 26 | 4 |
| | 3 | 150 | 114 | 37 | 19 | 3 |
| 72 | 1 | 150 | 126 | 38 | 33 | 4 |
| | 2 | 150 | 118 | 45 | 29 | 3 |
| | 3 | 150 | 101 | 29 | 22 | 3 |
| 96 | 1 | 150 | 105 | 38 | 32 | 2 |
| | 2 | 150 | 112 | 38 | 33 | 3 |
| | 3 | 150 | 111 | 39 | 20 | 3 |
| 120 | 1 | 170 | 134 | 48 | 35 | 1 |
| | 2 | 150 | 143 | 66 | 50 | 0 |
| | 3 | 150 | 122 | 39 | 44 | 1 |

APPENDIX 2 (continued): Nutritional Shift Data

II *ade3-1*

B. Cultures initiated on yeast-sucrose and transferred to Sang's medium

| Time of Transfer | Replicate | # of Eggs Transferred | # of Larvae Transferred | # of *Cy* Adults Eclosed | # of *Cy*+ Adults Eclosed | Development Delay of *Cy*+ Adults |
|---|---|---|---|---|---|---|
| 144 | 1 | 180 | 172 | 59 | 33 | 1 |
| | 2 | 150 | 126 | 52 | 42 | 1 |
| | 3 | 150 | 125 | 50 | 45 | 0 |
| 168 | 1 | 150 | 113 | 45 | 50 | 0 |
| | 2 | 150 | 125 | 60 | 37 | 0 |
| | 3 | N.D. | | | | |

APPENDIX 2 (continued): Nutritional Shift Data

III *bur$^{gua2-1}$*

A. Cultures initiated on Sang's and transferred to yeast-sucrose medium

| Time of Transfer | Replicate | # of Eggs Transferred | # of Larvae Transferred | # of *Cy* Adults Eclosed | # of *Cy*$^+$ Adults Eclosed | Development Delay of *Cy*$^+$ Adults |
|---|---|---|---|---|---|---|
| 0 | 1 | 150 | --- | 33 | 19 | 0 |
| | 2 | 150 | --- | 39 | 14 | 0 |
| | 3 | 150 | --- | 31 | 25 | 0 |
| 24 | 1 | 150 | 100 | 24 | 14 | 0 |
| | 2 | 170 | 94 | 19 | 8 | 0 |
| | 3 | 150 | 97 | 14 | 13 | 0 |
| 48 | 1 | 150 | 81 | 27 | 10 | 0 |
| | 2 | 150 | 86 | 19 | 1 | 1 |
| | 3 | 150 | 111 | 31 | 10 | 1 |
| 72 | 1 | 150 | 88 | 34 | 0 | |
| | 2 | 150 | 95 | 36 | 0 | |
| | 3 | 150 | 92 | 34 | 0 | |
| 96 | 1 | 150 | 77 | 25 | 0 | |
| | 2 | 150 | 72 | 19 | 0 | |
| | 3 | 150 | 74 | 25 | 0 | |
| 120 | 1 | 150 | 77 | 22 | 0 | |
| | 2 | 150 | 69 | 30 | 0 | |
| | 3 | 150 | 73 | 19 | 0 | |
| 144 | 1 | 150 | 55 | 17 | 0 | |
| | 2* | | | | | |
| | 3 | 150 | 59 | 19 | 0 | |

*Culture discarded due to microbial contamination

APPENDIX 2 (continued): Nutritional Shift Data

III *bur*$^{gua2-1}$

Cultures initiated on eggs

| B. Transfer | Replicate | # of Larvae Transferred | # of Larvae Transferred | # of *Cy* Adults Eclosed | # of *Cy*$^{+}$ Adults Eclosed | Development Delay of *Cy*$^{+}$ Adults |
|---|---|---|---|---|---|---|
| 0 | 1 | 150 | --- | 25 | 0 | |
| | 2 | 150 | --- | 24 | 0 | |
| | 3 | 150 | --- | 9 | 0 | |
| 24 | 1 | 150 | 58 | 5 | 0 | |
| | 2 | 175 | 57 | 20 | 0 | |
| | 3 | 150 | 52 | 7 | 0 | |
| 48 | 1 | 145 | 56 | 9 | 0 | |
| | 2 | 150 | 72 | 25 | 0 | |
| | 3 | 150 | 32 | 4 | 0 | |
| 72 | 1 | 150 | 53 | 18 | 1 | 4 |
| | 2 | 150 | 74 | 27 | 6 | 3 |
| | 3 | 150 | 67 | 20 | 1 | 5 |
| 96 | 1 | 150 | 45 | 10 | 1 | 5 |
| | 2 | 150 | 68 | 24 | 1 | 5 |
| | 3 | 150 | 66 | 18 | 1 | 6 |
| 120 | 1 | 150 | 68 | 39 | 10 | 0 |
| | 2 | 150 | 78 | 32 | 11 | 0 |
| | 3 | 150 | 83 | 29 | 23 | 0 |
| 144 | 1 | 150 | 75 | 38 | 26 | 0 |
| | 2 | 150 | 70 | 29 | 24 | 0 |
| | 3 | 150 | 55 | 25 | 19 | 0 |