THE $\alpha$-GLYCEROPHOSPHATE IN DROSOPHILA MELANOGASTER

II. GENETIC ASPECTS

STEPHEN J. O'BRIEN AND ROSS J. MACINTYRE

Section of Genetics, Development and Physiology, Cornell University, Ithaca, New York 14850

Manuscript received October 22, 1971
Revised copy received January 10, 1972

ABSTRACT

Seven alleles of the $\alpha$-Glycerophosphate dehydrogenase-1 ($\alpha$Gpdh-1) locus of Drosophila melanogaster have been described. These include two naturally occurring electrophoretic variants, one EMS-induced electrophoretic variant, and four EMS-induced "null" or "zero" mutants. With the electrophoretic variants, the locus was mapped to II-20.5 ± 2.5. A complementation matrix was prepared utilizing the null mutants. Three of the four mutants and a deletion of the locus (Grell 1967) exhibit dosage dependency. The dosage independent mutant exhibits complementation with two of the other null alleles. Flies genetically deficient in $\alpha$-glycerophosphate dehydrogenase are fertile, but their relative viability is severely diminished. Such flies also lose the ability to sustain flight, an observation consistent with the enzyme's function in energy production. The levels of mitochondrial $\alpha$-glycerophosphate oxidase, measured in flies genetically deficient in the cytoplasmic enzyme, were normal.

THE combination of gel electrophoresis, naturally occurring allelic isozyme (allozyme) variation, and certain special genetic techniques, has permitted the genetic localization of over twenty Drosophila loci whose gene products can be measured on the molecular level (O'Brien and MacIntyre 1971; Fox, Abacheli and Ursprung 1971). The use of ethyl methane sulphate (EMS) and colorimetric spot tests has led to the induction and characterization of "null" or "zero" point mutations in several of these loci (Grell, Jacobson and Murphy 1968; Bell and MacIntyre 1972; W. J. Young, personal communication). In addition, naturally occurring null alleles at other loci have been found (Johnson, Wallis and Denniston 1966; Dickinson 1970; Glassman 1965). Complementation matrices have been presented for ma-1 null alleles with respect to xanthine dehydrogenase (Chovnick et al. 1969) and Acp-1 null alleles for acid phosphatase (Bell and MacIntyre 1972). The usefulness of these null alleles is apparent for a number of experimental situations such as: (1) the determination of time and mode of initiation of embryonic gene action (Yasbin 1971), (2) the measurement of dosage compensation of X-linked loci (Chovnick et al. 1969) and dosage dependency of autosomal loci (Grell 1962) and (3) the tracing of developmental patterns through experimentally induced mosaicism for null and active alleles of histochemically demonstrable gene enzyme systems.

$^a$ Supported by Genetics Training Grant T1 GM 1035 from the National Institute of General Medical Sciences.

$^b$ Present address: Gerontology Research Center, NIH, Baltimore City Hospitals, Baltimore, Maryland.

The enzymes participating in the α-glycerophosphate cycle of insect flight muscle are particularly attractive candidates for genetic analysis because of the relatively thorough understanding of the cycle's physiological character in several orders of insects (Sacktor 1965, Hansford and Sacktor 1971). The cycle is composed of two distinct α-glycerophosphate dehydrogenases, a soluble NAD-linked enzyme and a mitochondrial flavoprotein. The soluble dehydrogenase reduces the dihydroxyacetone phosphate produced during glycolysis to α-glycerophosphate, and in the process regenerates oxidized NAD in the cytoplasm. The α-glycerophosphate diffuses into the mitochondria where the particulate enzyme oxidizes the metabolite back to dihydroxyacetone phosphate and donates the electron to the respiratory chain for oxidative phosphorylation. The dihydroxyacetone phosphate then becomes available for further reduction in the cytoplasm. The whole process constitutes a shuttle which allows ATP production and cytoplasmic NAD regeneration despite the permeability barrier of the mitochondrion to NADH itself (Sacktor and Dick 1962).

The cycle's enzymes have also been linked to lipid anabolism by providing α-glycerophosphate, a precursor of phosphatidic acid (Kennedy 1957).

The level of biochemical differentiation in Drosophila appears to be even more complex than was expected. Three isozymes of the soluble dehydrogenase and two isozymes of the mitochondrial oxidase have been described, and each has a characteristic spatial and developmental distribution (Wright and Shaw 1969; O'Brien and MacIntyre 1972).

We shall describe in this report the isolation (or induction) and characterization of seven alleles of the aGpdh-1 (α-glycerophosphate dehydrogenase-1) locus in Drosophila melanogaster (Grell 1967; O'Brien and MacIntyre 1968). A complementation matrix of these alleles, their effect upon viability, flying ability, and the amount of mitochondrial α-glycerophosphate oxidase (αGPO) (O'Brien and MacIntyre 1972) will also be presented.

MATERIALS AND METHODS

Stocks: The wild stock utilized in these experiments is an inbred stock from Riverside, California which has been described previously (O'Brien and MacIntyre 1969). The visible mutants and marker chromosomes are designated according to the conventions in Lindsley and Grell (1967). Df(2L)GdhA is a deficiency of cl which includes the aGpdh-1 locus with break points at 25F1 and 26B-C1. This deficiency was generously provided by Dr. E. H. Grell. Culturing conditions are described elsewhere (O'Brien and MacIntyre 1972).

Enzyme preparation and assay: Quantitative estimation of the soluble NAD-linked α-glycerophosphate dehydrogenase (αGPDH; L-glycerol-3-phosphate : NAD oxidoreductase E. C. 1.1.1.8) involved spectrophotometric monitoring of NAD reduction at 340 nm. Enzyme preparations were crude supernatant fractions. Specific details of these procedures are described elsewhere (O'Brien and MacIntyre 1972). The mitochondrial NAD-independent α-glycerophosphate oxidase (αGPO; L-glycerol-3-phosphate : cytochrome c oxidoreductase E. C. 1.1.99.5) was extracted from Drosophila mitochondria preparations with 1% Triton X-100. Enzyme activity was estimated with a phenazine methosulfate-gelatin-INT tetrazolium mixture which absorbs at 490 nm (O'Brien and MacIntyre 1972).

Starch gel electrophoresis of αGPDH was in a Tris HCl continuous buffer system (O'Brien and MacIntyre 1969).
Mutagenesis: EMS mutagenesis of Drosophila males was according to the procedure of Lewis and Bacher (1968).

RESULTS

A number of wild-type stocks were screened for electrophoretic variation of aGPDH. Three populations were polymorphic for a fast and slow electrophoretic variant (Ceres, N.Y.; Painesville, O.; and Oxford, N.C.), while all other stocks (wild and laboratory mutant stocks) were monomorphic for the fast allele. We have designated the common, "fast," i.e. the more electronegative enzyme, as aGPDH-1AA (AA), the product of the aGpdh-1A (A) allele; and the rare "slow" or more electropositive enzyme as aGPDH-1BB (BB), the product of the aGpdh-1B (B) allele (Figure 1).

A monomorphic B stock was constructed and crossed to an A stock. F1 heterozygotes exhibited the three-band pattern shown in Figure 1. Sib mating of F1 resulted in 1:2:1 segregation of the AA:AB:BB phenotypes respectively in the F2, suggesting single gene inheritance.

"Hemizygotes" of A or B over Df(2L)GdhA exhibit only a single zone of activity in the region of the active enzyme.

Genetic Mapping: The electrophoretic analysis of 65 backcross progeny from the parental cross of Cy/Pm; Sb/Ubx (A/A) × B/B demonstrated segregation of the B allele from the second chromosome markers, and independent assortment with the X and chromosome III. This result indicated that the aGpdh-1 locus lies on chromosome II. In order to map the locus precisely, virgin females of the B/B

![Figure 1](image-url)

Figure 1.—Starch gel zymogram of various aGpdh-1 genotypes developed for aGPDH. Whole flies of the indicated genotype were homogenized with a teflon motor-driven homogenizer in ten volumes of 0.05 M Tris HCl pH 8.7 and centrifuged for 15 min at 25,000 × g. 0.2 ml of the supernatant was applied to a strip of Whatman No. 3 filter paper and placed in a horizontal starch gel. Samples were subjected to electrophoresis in a continuous Tris-HCl buffer system for 6 hr at 6 v/cm. Histochemical enzyme visualization was identical to the procedure for single flies (O'Brien and MacIntyre 1969). Scale units are centimeters and the origin is at the bottom of the figure.
<table>
<thead>
<tr>
<th>Female parent</th>
<th>Male parent</th>
<th>Offspring maternal chromosome</th>
<th>aGPDH-1 A/B</th>
<th>A/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ + + + + + + +</td>
<td>al dp b pr cn c px sp (A)</td>
<td>al + + + + + + + +</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp b pr cn c px sp (A)</td>
<td>+ dp b pr cn c px sp</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp b pr cn c px sp (A)</td>
<td>+ + + b pr cn c px sp</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp + + + + + + + + + +</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp + + + + + + + + +</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp + + + + + + + + + +</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp + + + + + + + + + +</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp + + + + + + + + + +</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp + + + + + + + + + +</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp + + + + + + + + + +</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp + + + + + + + + + +</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp + + + + + + + + + +</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp b pr cn c px sp (A)</td>
<td>al dp + + + + + + + + + +</td>
<td>48</td>
<td>176</td>
</tr>
<tr>
<td>+ + + +</td>
<td>al dp b pr</td>
<td>al dp + b</td>
<td>157</td>
<td>41</td>
</tr>
</tbody>
</table>

**TABLE 1**

*Localization of aGpdh-1 locus*
genotype were crossed to an A/A stock which contained eight recessive chromosome II mutations (al, dp, b, cn, pr, c, px, and sp). F1 virgin females were backcrossed to this mutant stock to expose single crossovers in the regions between the mutant genes. The data which are summarized in Table 1 indicate a map position of II-20.5 ± 2.5.

**Induction of aGpdh-1 alleles:** Two-three day old al aGpdh-1B males, originally free from 2nd chromosome lethals, were fed on the EMS-sucrose solution for 24 hr and mated immediately to virgin SMI(A), alCy cn sp aGpdh-1A/Df(2L)-GdhA females as outlined in Figure 2. Single F1 SM1(A)/al aGpdh-1B males

![Diagram of crossing scheme](image)

Subjected to electrophoresis.

If the expected AB pattern was altered,

sibs were intercrossed to maintain the stock.

\[ B^* \quad \text{mutagenized B allele} \]

Figure 2.—Crossing scheme for induction and isolation of aGpdh-1BO mutants.
were backcrossed to SM1(A)/Df(2L)GdhA, and single SM1(A)/aGpdh-l(B) offspring from each mating were subjected to starch gel electrophoresis. Second generation offspring were examined instead of F1 in order to avoid mosaic mutants which are common in EMS mutagenesis (Jenkins 1967). Scoring of F1 flies might result in missing many induced mutants in our scheme. Since one must cross the F1 individuals in any event, it seemed a priori to be more efficient to analyze backcross progeny (whole body mutants) and thus eliminate mosaic putatives. Of course, if an F1 fly had a mosaic gonad, its offspring will be polymorphic for the normal and mutant alleles. Deviation from the 3-band (AB) pattern in Figure 1 was indicative of a mutation event. SM1A/al aGpdh-lB* siblings were mated to each other for maintenance of the mutant chromosome over SM1. It should be pointed out that EMS in our mutagenic procedure invariably induced lethals at other loci on the recovered second chromosome. “Null” mutants were confirmed by making these hemizygous over Df(2L)GdhA and quantitating aGPDH activity spectrophotometrically (see below).

Of approximately 1000 examined chromosomes, five new alleles of aGpdh-1 were obtained. One electrophoretic variant, aGpdh-1B0, migrated more slowly than the naturally occurring B variant (Figure 1). The other four mutations significantly diminished the amount of aGPDH measurable in the fly. Zymograms of individuals heterozygous for the aGpdh-1A allele and each of the 4 mutant (designated aGpdh-1B0 or BO) alleles are given in Figure 1. Two lack activity in the BB position but retain hybrid or AB activity indicating that these contain an active heteromultimer (AB) but an inactive homomultimer (BB). Flies containing aGpdh-1B0-1-4 make hybrid enzyme, but its position is intermediate between the AA and AB position, suggesting that the electrophoretic mobility of the BO-1-4 subunit is altered. The gene product of aGpdh-1B0-1-5 also has an altered mobility so that the hybrid band is in the BB position. aGpdh-1B0-1-5 apparently is a leaky allele. If many aGpdh-1B0-1-4 flies, hemizygous with Df(2L)GdhA, are concentrated in the sample mixture and subjected to electrophoresis, a faint zone of activity can be seen in the CC position.

Quantitative analysis of aGpdh-1 heterozygotes: As mentioned above, the four mutants of aGpdh-1 derived by EMS mutagenesis also contained a number of residual lethal alleles at different loci on the treated chromosome. Since removal of these lethals is tedious indeed (see Bell and MacIntyre 1972), we did not attempt to make the BO mutants homozygous. However, inter se crosses between the four mutants, Df(2L)GdhA, and aGpdh-1A were made so that the various heterozygous combinations could be analyzed. The 1-5/5-4 combination was not obtained, presumably because the independently isolated aGpdh-1 mutant strains shared allelic chromosome II lethal mutations.

The specific activities of aGPDH were determined for each of the heterozygotes and are included in Table 2. Several important results emerge from the data in Table 2: (1) Three of the four null alleles and the deletion of aGpdh-1 exhibit dosage dependency; i.e. flies heterozygous for one of these null alleles and a wild chromosome exhibit approximately 50% of the activity of flies containing two doses of an active allele. This dosage dependency is characteristic of auto-
**TABLE 2**

*aGPDH activity in flies heterozygous for different aGpdh-1 alleles*  

<table>
<thead>
<tr>
<th>Maternal chromosome</th>
<th>Df(2L)GdhA</th>
<th>BO-0</th>
<th>BO-1-4</th>
<th>BO-5-4</th>
<th>BO-1-5</th>
<th>++‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Df(2L)</td>
<td>—</td>
<td>0.0</td>
<td>4.0</td>
<td>3.4</td>
<td>12.7</td>
<td>46.3</td>
</tr>
<tr>
<td>BO-0</td>
<td>0.1</td>
<td>—</td>
<td>0.0</td>
<td>0.0</td>
<td>22.2</td>
<td>41.1</td>
</tr>
<tr>
<td>BO-1-4</td>
<td>0.0</td>
<td>0.0</td>
<td>—</td>
<td>2.8</td>
<td>15.8</td>
<td>51.6</td>
</tr>
<tr>
<td>BO-5-4</td>
<td>5.2</td>
<td>0</td>
<td>0.7</td>
<td>—</td>
<td>—‡</td>
<td>49.5</td>
</tr>
<tr>
<td>BO-1-5</td>
<td>11.8</td>
<td>29.8</td>
<td>18.5</td>
<td>—‡</td>
<td>—‡</td>
<td>49.5</td>
</tr>
<tr>
<td>++‡</td>
<td>46.3</td>
<td>44.1</td>
<td>51.6</td>
<td>49.5</td>
<td>98.4</td>
<td>100</td>
</tr>
</tbody>
</table>

*Expressed as percent wild-type activity. 100% = 0.37 μmoles NAD/min/mg protein. Flies were 3–5 days old when assayed. Values presented represent averages of at least four measurements from independent crosses and isolations. 0% represents less than 0.1% wild-type activity. Standard error of values greater than 25% was ± 10%. Lower aGPDH values had variances no greater than ± 3%. That is, the coefficients of variation do not appear to differ from each other at different measured enzyme levels. Values of aGPDH activity of 0–5% could be due to enzyme activity from yeast.

‡ Offspring of reciprocal crosses were pooled and measured together.  
‡‡ BO-1-5 and BO-5-4 share background lethals.

somal structural genes in eukaryotes (Glassman 1965). (2) Single doses of the four mutants over the deletion result in flies with less than 4–5% of wild-type enzyme levels in all but BO-1-5 hemizygotes which have 12% of normal enzyme levels. (3) BO-1-5 is a complementing allele, in that in both BO-0/BO-1-5 and BO-1-5/BO-1-4 heterozygotes, aGPDH activity levels are significantly higher than those of deficiency hemizygotes. In fact, the BO-1-5/BO-0 heterozygotes’ activity is nearly two times greater than the predicted diploid sum of two hemizygote activities. BO-1-5 also does not exhibit dosage dependency, rather, flies heterozygous with it and a wild-type allele have normal levels of activity.

**aGPO activity in aGpdh-1bo mutants:** The Drosophila mitochondrial enzyme, α-glycerophosphate oxidase (aGPO), catalyzes the same reaction as aGPDH and cooperates with aGPDH in the α-glycerophosphate cycle (Sacktor 1965; O'Brien and MacIntyre 1972). It was of particular interest to examine aGPO activity in the aGpdh-1 null mutants. Utilizing the same flies as were examined in Table 2, we assayed the various aGpdh-1bo heterozygotes for aGPO activity. The results of this analysis, in Table 3, clearly demonstrate that the aGPDH level, reduced by structural gene mutations, has no effect on the level of aGPO present in Drosophila mitochondria. This observation strongly suggests that the soluble and mitochondrial enzymes are coded by different structural genes.

**Competitive viability of aGpdh-1bo heterozygotes:** The construction of the heterozygote combinations discussed above involved the crossing of one SM-1/aGpdh-1bo hemizygote with a different SM-1/aGpdh-1bo hemizygote and recovering the non-Curly (Cy+) offspring. The expected frequency of Cy+ flies is 33%. However, we noticed during the process of collection that only some of the crosses were producing expected frequencies of Cy+. If the diminished frequency in some of the crosses were due to selective pressure against Cy+ homozygotes
deficient in αGPDH activity, we surmised that it might be possible to directly associate viability with αGPDH levels.

Five or more replicates of each of the mutant crosses were constructed and the numbers of Cy vs. Cy+ offspring were determined. Approximately 200–300 flies per bottle were counted. The frequency of Cy+ offspring in each bottle was then plotted vs. the αGPDH level detected in that class of heterozygote. The results appear in Figure 3. The mutant hemizygotes were not included in this analysis because the deletion would also permit expression of any of EMS-induced mutant alleles on the mutagenized chromosome within the limits of the deletion. Hence, deviation from the 33% Cy+ might not be the result of selection on the αGpdh-l locus.

Figure 3 demonstrates that a clear depression of viability is associated with the low levels of αGPDH. Alternatively, the complementing heterozygotes which have over 15% wild αGPDH levels are clearly more viable and reach normal viability as the αGPDH activity rises to 25% of normal enzyme levels. The correlation in these crosses strongly suggest a selective pressure associated with very low levels of αGPDH but near normal viability with as little as 25% of wild-type levels.

The flight of an αGpdh-l1Bo mutant fly: There is no visible morphological phenotype associated with the αGpdh-l1Bo mutants. However, a major function of the α-glycerophosphate cycle is the generation of the energy necessary for flight in
the insect flight muscles. One might expect that flies lacking αGPDH, and hence unable to provide α-glycerophosphate as a substrate for mitochondrial oxidative phosphorylation, might be deficient in flying ability. This indeed is the case for those heterozygotes and hemizygotes discussed above which have less than 5% αGPDH activity. This "phenotype" is difficult to quantitate but can be qualitatively determined in two ways. The first method involves releasing unetherized flies on a large table. Normal flies begin flight and are airborne indefinitely.
\( \alpha \text{Gpdh-1}^{bo} \) mutants initiate flight, rise from 1–3 feet and fall to the table within a radius of 2–3 feet from the initial release point. Two or three less successful flight attempts usually follow the first, after which even poking with a brush cannot force the fly to again take off. The second test merely involved dropping unetherized flies 6 feet above the floor. Normal flies fly away and mutant flies fall to the floor, beating their wings vigorously but unsuccessfully resisting the fall. Those flies with less than 5% \( \alpha \text{GPDH} \) activity are unable to sustain flight by these two criteria; those with at least 10% wild-type activity fly normally.

**DISCUSSION**

Two lines of evidence suggest that \( \alpha \text{Gpdh-1} \) is the structural gene for the soluble NAD-linked \( \alpha \)-glycerophosphate dehydrogenase. These are: (1) The Mendelian segregation of the electrophoretic variants, (2) The dosage dependency of the deletion and the three non- or only slightly leaky \( \text{BO} \) mutants. Although GRELL’S (1967) estimate of the locus position, II-17.8, appears to fall outside the 95% confidence limits of our estimate, II-20 ± 2.5, the apparent difference is probably not significant because of the associated errors of both GRELL’S and our own estimates.

**Complementation:** \( \text{BO-1-5} \) exhibits interallelic complementation with all other point mutants tested. It is leaky in that \( \text{BO-1-5}/\text{Df(2L)GdhA} \) has 12% normal activity. This allele also fails to exhibit dosage dependency in combination with a wild chromosome; i.e. normal \( \alpha \text{GPDH} \) levels are observed in this \( \text{A/BO-1-5} \) heterozygote. These observations are consistent with the model of interallelic complementation proposed by FINCHAM (1966). Interallelic complementation in this model results from the combination of two or more differently defective polypeptide subunits to form a “pseudowild” multimeric enzyme. The three band \( \text{A/B} \) heterozygote pattern (Figure 1) is consistent if \( \alpha \text{GPDH} \) is a dimer. If \( \text{BO-1-5} \) is a point mutant which severely restricts homologous multimeric formation, but not the heterologous combination of \( \text{BO-1-5} \) subunits and normal A or B subunits, then \( \text{BO-1-5} \) hemizygotes (or homozygotes) would have low activity levels, and \( \text{BO-1-5}/\text{B} \) or \( \text{A} \) would have normal enzyme levels, as we have observed. A corollary to such an hypothesis in light of our data is that \( \text{BO-0} \) and \( \text{BO-1-5} \) make an inactive protein which is capable of binding with the \( \text{BO-1-5} \) product and restoring the activity of the heterodimer. Again, the appearance of the \( \text{AB} \) hybrid band in the \( \text{A/BO} \) heterozygotes of both alleles supports this corollary (Figure 1). Hence, these two are probably missense mutations or perhaps distal nonsense mutants that do not alter the binding ability of the protein subunit.

**Mitochondrial \( \alpha \)-glycerophosphate oxidation in \( \alpha \text{GPDH} \) deficient flies:** Normal levels of mitochondrial \( \alpha \)-glycerophosphate oxidase (\( \alpha \text{GPO} \)) in \( \alpha \text{Gpdh-1}^{bo} \) flies demonstrate that the two enzymes are transcribed by distinct structural genes. Since the direction of the cycle is such that \( \alpha \text{GPDH} \) provides the substrate for \( \alpha \text{GPO} \) in a normal system, the presence of \( \alpha \text{GPO} \) in the \( \alpha \text{Gpdh-1}^{bo} \) flies is probably of little use in the operation of the cycle.
A preliminary search for the aGPO locus which included a survey of over thirty wild stocks and a large mutagenesis experiment (S. J. O'Brien unpublished) failed to produce either Mendelian allozymes detected by isoelectric focusing in polyacrylamide gels (O'Brien and MacIntyre 1972), or "null" mutants detected by a colorimetric assays in single flies. A more extensive search for this locus must be undertaken so that the genetics of the cycle can be better characterized.

The effect of aGpdh-1Bo on the healthy fly: The enzymes of the α-glycerophosphate cycle perform a three-fold function in insects: (1) maintenance of an NAD-NADH equilibrium in the cytoplasm (Sacktor and Dick 1962), (2) energy production (Sacktor and Dick 1962; Lennie and Birt 1967), (3) providing of α-glycerophosphate as a substrate for lipid synthesis (Kennedy 1957). Since the cycle cannot operate without the cooperation of the soluble and mitochondrial enzymes, neither the decrease in viability nor the impediment of flight when the cycle is disrupted is unexpected. Perhaps the viability effect is due to a lack of sufficient α-glycerophosphate for phospholipid synthesis during larval and pupal development, while the flight effect results from insufficient substrate concentrations for mitochondrial oxidation and energy production in the adult flight muscle.

A final aspect of these observations is the somewhat surprising fact that the BO/BO fly survives at all in light of this seemingly drastic disruption of intermediary metabolism. Such survival of "null" mutants in other systems, however, is not uncommon; in fact, it seems to be the rule. The survival and continued reproduction of these mutants suggests that D. melanogaster has a tremendous physiological and/or genetic plasticity. That is, it has the ability to physiologically or genetically compensate for lesions of certain seemingly important functions. Such compensation of the α-glycerophosphate cycle could be provided by the malate dehydrogenase cycle for NADH production (Sacktor 1965), the Krebs cycle for energy production, and certain lipid degradative enzymes for generation of α-glycerophosphate for lipid anabolism.

LITERATURE CITED


Grell, E. H., 1962 The dose effect of ma-1 and ry on xanthine dehydrogenase in Drosophila


