LETHAL EFFECTS OF LOW AND "NULL" ACTIVITY ALLELES OF 6-PHOSPHOGLUCONATE DEHYDROGENASE IN *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

EMS-induced "null" and low activity alleles for 6-phosphogluconate dehydrogenase were characterized with respect to enzymatic activity, relative viability, fertility, and the effective lethal phase. It was determined that flies hemizygous and homozygous for the low activity allele, *Pgd*$^-$, possessed a depressed developmental rate, diminished viability, and loss of female fertility. Heterozygotes for *Pgd*$^-$ and a deficiency for *Pgd*+ were lethal. The "null" activity allele demonstrated a lethal phenotype in both the hemizygous and homozygous condition. The effective lethal phase for the "null" allele occurs during late embryonic development (20–22 hr).

THE "null" alleles of enzyme structural genes in *Drosophila melanogaster* recovered to date exhibit a wide range of viability effects but in general do not meet the criterion of lethality (O'BRIEN 1973). This could be due to the screening procedures employed to recover such alleles. When histochemical staining in spot tests or electrophoresis of hybrid flies are used, low activity alleles with less than 5–6% of normal enzyme levels may be recorded as "null" alleles. In only a few instances have the mutant enzyme levels been measured by analytical enzyme assays or by determining the amount of cross reacting material (GLASSMAN 1965; GRELL, JACOBSON and MURPHY 1968; DICKINSON 1970; O'BRIEN and MACINTYRE 1972; BELL and MACINTYRE 1973). In addition, most of the Drosophila enzymes for which "null" alleles exist appear to serve either a dispensable metabolic role (i.e., α-glycerophosphate dehydrogenase, alcohol dehydrogenase, xanthine dehydrogenase, tryptophan pyrrolase) or else are enzymes whose physiological roles are not well understood (i.e., acid phosphatase, alkaline phosphatase, esterase-C, esterase-6). Finally, the function performed by many of these enzymes can be achieved through alternate metabolic pathways.

Because of its central role in the pentose phosphate pathway, 6-phosphogluconate dehydrogenase would appear to be critical to the normal development and

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viability of Drosophila. This enzyme is a dimer of M. W. 79,000 daltons with a subunit weight of 40,000 daltons (KAZAZIAN, YOUNG and CHILDS 1965; KAZAZIAN 1966). Electrophoretic variants are known and the structural gene locus, *Pgd*$^+$, has been mapped to the *X* chromosome (YOUNG 1966). The 6-PGD activity is not subject to feedback inhibition and the enzyme activities in males and females are dosage-compensated (SEECOF, KAPLAN and FUTCH 1969). In this paper we report on the debilitating effects of an EMS-induced low activity allele isolated by DR. W. J. YOUNG and on the lethal effects of an EMS-induced allele which produces no measurable 6-PGD activity ("null" allele).

MATERIALS AND METHODS

Drosophila melanogaster cultures were maintained in half-pint glass bottles, on standard cornmeal-molasses-yeast medium containing propionic acid and tegosept-M as mold inhibitors. Cultures were supplemented with live yeast and were maintained at 25° unless otherwise stated. Adult samples for enzyme assays consisted of flies collected within 4 days of emergence and aged 2 additional days on standard medium without live yeast.

Larvae were collected from vials by rinsing with tap water. They were cleaned by repeated rinsing and decanting with tap water and examined under a dissecting microscope. Eggs were collected from cross 3 in Table 2. Fifty pairs of adults were placed in egg-laying vials and allowed to deposit eggs for one-hour intervals on blotting paper soaked in 10% commercial vinegar. The eggs were incubated at 25° and embryos were counted and examined under a dissecting microscope.

In this study the following special chromosomes were used. *Pgd*^{A} *w*^{a}: an *X* chromosome bearing a wild-type allele of 6-PGD (electrophoretic variant A) and marked with the visual recessive marker *w*^{a}; *Df(1) w*^{vco}, *v f*: an *X* chromosome with a small deficiency (2B17–C1;3C4–5) which includes the structural gene for 6-PGD; *C(1)RM*, *y pn* and *C(1)DX*, *y f*: compound *X* chromosomes with both *X*'s attached to a single kinetochore *w*+·*Y*: a *Y* chromosome carrying a small insertion of the *X* chromosome (2D1–3D6) which includes the structural gene for 6-PGD. All genetic symbols are described in LINDSLEY and GRELL (1968). The *Pgd*$^-$ (low activity) allele was kindly made available to us by DR. T. R. F. WRIGHT.

EMS mutagenesis of Drosophila males was conducted according to the procedure of LEWIS and BACHER (1968). Two-day-old *Pgd*^{A} *w*^{a}/*Y* males were fed an EMS-sucrose solution for 24 hours and were immediately mated to virgin *C(1)DX*, *y f*/*w*+·*Y* females at 25°. Single *Pgd*^{A} *w*^{a}/*w*+·*Y* male progeny were pair-mated to virgin *Pgd*$^-$/*M-5* females and the cultures were examined for the presence or absence of *Pgd*^{A} *w*^{a}/*Pgd*$^-$ females. The induction of a "null" or "zero" activity allele with a lethal phenotype would necessitate the absence of these females from the progeny.

Flies were homogenized in 0.1 M potassium phosphate buffer (pH 6.8) containing 5 mM ethylenediaminetetraacetic acid, disodium salt (EDTA), and 0.5 mM dithiothreitol (DTT) at a concentration of 20 mg live weight per ml. The homogenates were centrifuged at 12,000 × g (2°) and the supernatant fluid was used for the enzyme assays. Enzyme activities were monitored using a Beckman DB-G spectrophotometer with the sample compartment maintained at 29°. Changes in A_{340} were observed from 30–90 seconds after the addition of the extract. Assays for 6-PGD were conducted in a final cuvette volume of 2.5 ml of tris-HCl (pH 7.5) containing 10 mM $MgCl_2$, 3.0 mM 6-phosphogluconate (sodium salt), and 1.3 mM NADP+. Glucose-6-phosphate dehydrogenase (G-6-PD) assays were conducted in 0.1 M tris-HCl (pH 8.6) buffer containing 10 mM $MgCl_2$, 6.6 mM glucose-6-phosphate (disodium salt), 0.52 mM NADP+ in a final volume of 2.5 ml. A unit of enzyme activity is defined as one micro-mole of NADP+ reduced per minute at 29°.

Protein was measured by the method of LOWRY *et al.* (1951) using bovine serum albumin as a standard.

TABLE 1

Enzyme activities in adult flies bearing wild and mutant Pgd *alleles*

Genetic constitution†	6-PGD	*N*	G-6-PD	*N*
Males:				
+/Y	.131 ± .006	7	.167 ± .008	6
Pgd⁻/Y	.009 ± .003*	4	.122 ± .007*	4
l(1)Pgd-A^n/Y	.000	3	.103 ± .005*	3
Females:				
M-5/M-5‡	.063 ± .007	4	.085 ± .011	4
M-5/l(1)Pgd-A^n	.029 ± .000*	2	.065 ± .005*	2

Enzyme activities are expressed as mean units per mg protein ± the standard error. *N* is equal to the number of determinations.

* Differing from the wild-type allele value at the 1% level of significance.

† The wild-type allele is derived from a wild-type Samarkand strain (electrophoretic variant B) and an M-5 (electrophoretic variant A).

‡ The low enzyme activity levels exhibited by M-5 flies are consistent with our experience using this particular balancer.

Vertical starch gel electrophoresis of single flies was carried out at 4° according to YOUNG (1966) in a 0.5 M EDTA-borate-tris bridge buffer (pH 8.0) while the gel buffer was a 1/10 dilution of the same. The gels were run for 4 to 5 hours at 6.6 V/cm and 20–25 mA current. Gels were sliced and stained histochemically for 6-PGD using phenazine methosulfate 0.025 mg/ml, nitro blue tetrazolium 0.1 mg/ml, 6-phosphogluconate 3.0 mM, and NADP+ 1.3 mM in a tris-HCl buffer (pH 7.5) containing 10 mM $MgCl_2$. The staining reaction was stopped by the addition of 6% acetic acid.

RESULTS

Pgd⁻ *Allele Characterization*

Analytical enzyme assays were conducted on males bearing the *Pgd⁻* allele and the activity was compared to that in males of a wild-type Samarkand strain (Table 1). The specific activity of 6-PGD was reduced to a level of 6.9% of the wild-type activity, and *Pgd⁻* was designated as a low activity allele. The specific activity of G-6-PD, a control enzyme, was observed to be 73% that of wild-type activity. The reduction in G-6-PD activity may be related to the fact that both enzymes are sequentially linked in the pentose phosphate pathway. Fertility studies indicated that hemizygous *Pgd⁻/Y* males are fertile while homozygous *Pgd⁻* females were observed to be sterile in 27 separate single-fly crosses to wild-type males at 25°. In addition, the developmental rate for hemizygous and homozygous *Pgd⁻* flies, the progeny of a cross illustrated in Table 2, exp't. 1, is depressed at 25° and the relative viability is diminished to the semi-lethal range, which is in general agreement with WRIGHT (1973). When an appropriate cross is made (Table 2, exp't. 2) such that the *Pgd⁻* allele is heterozygous with a deficiency for *Pgd⁺*, half of the expected female progeny should consist of this genotype when in fact they constituted only 1.95% of expected females. Since the *Pgd⁺* locus is dosage-compensated, we believe that the lethal effect could be the result of a reduction in 6-PGD activity from a level

TABLE 2

Viability of flies bearing Pgd$^-$ *and/or* l(1)Pgd-A^n *mutant alleles**

Expt. no.	Parental genotype	Relative viability of *Pgd* mutant alleles†		progeny scored
1.	$Pgd^-/Y \times Pgd^-/M\text{-}5$	$\frac{Pgd^-/Y + Pgd^-/Pgd^-}{M\text{-}5/Y + Pgd^-/M\text{-}5}$	$\times 100 = 39$	725
2.	$Pgd^-/Y \times Df(1)w^{vco}/M\text{-}5$	$\frac{Pgd^-/Df(1)w^{vco}}{Pgd^-/M\text{-}5}$	$\times 100 = 3.9$	2795
3.	$l(1)Pgd\text{-}A^n/w^+ \cdot Y \times C(1)RM/Y$	$\frac{l(1)Pgd\text{-}A^n/Y}{C(1)RM/w^+ \cdot Y}$	$\times 100 = 1.5$	4458
4.	$l(1)Pgd\text{-}A^n/w^+ \cdot Y \times Df(1)w^{vco}/M\text{-}5$	$\frac{l(1)Pgd\text{-}A^n/Df(1)w^{vco}}{l(1)Pgd\text{-}A^n/M\text{-}5}$	$\times 100 = 7.1$	497
5.	$l(1)Pgd\text{-}A^n/w^+ \cdot Y \times Pgd^-/M\text{-}5$	$\frac{Pgd^-/l(1)Pgd\text{-}A^n}{l(1)Pgd\text{-}A^n/M\text{-}5}$	$\times 100 = 13$	360

* All data for relative viability were pooled from three or more half-pint culture bottles with 15 female parents per bottle.

† Relative viability is expressed as % expected relative to the number of siblings emerging concomitantly at 25°.

of 6.9% to 3–4% in $Pgd^-/Df(1)w^{vco}$ females. Apparently the level of enzyme activity in hemizygous and homozygous Pgd^- flies approaches the critical level that is compatible with life as evidenced by the diminished viability and loss of female fertility.

EMS Screen

The Pgd^- allele was subsequently used in a screen to recover putative EMS-induced *Pgd* lethal alleles. Inherent to the experimental design is the assumption that the Pgd^- allele would demonstrate the same lethal phenotype with an induced "null" activity allele for 6-PGD as was found when in heterozygous combination with the deficiency chromosome. Of the 1280 treated chromosomes which were examined, one was found bearing a putative lethal allele of Pgd^+ and designated as $l(1)Pgd\text{-}A^n$. Stock cultures were kept at 22° as $l(1)Pgd\text{-}A^n\ w^a/C(1)DX,y\ f/w^+ \cdot Y$ and as $l(1)Pgd\text{-}A^n\ w^a/M\text{-}5$ balanced females. Since the screen was based on lethality, the efficiency, 0.07%, is most likely a reflection of failure to detect putative low activity alleles (greater than 5% wild-type activity).

l(1)Pgd-A^n *Allele Characterization*

The specific activities of 6-PGD and G-6-PD were determined for $l(1)Pgd\text{-}A^n\ w^a/M\text{-}5$ and for $M\text{-}5/M\text{-}5$ females (Table 1). The flies represented as heterozygotes exhibited dosage dependence in that the 6-PGD activity is approximately 45% that of the *M-5* homozygotes. Single-fly electrophoresis on starch gels is represented in Figure 1. It is evident that $Pgd^A\ w^a/Pgd^B$ females (slot 3) exhibit the typical three-band hybrid pattern for 6-PGD, while $l(1)Pgd\text{-}A^n\ w^a/Pgd^B$

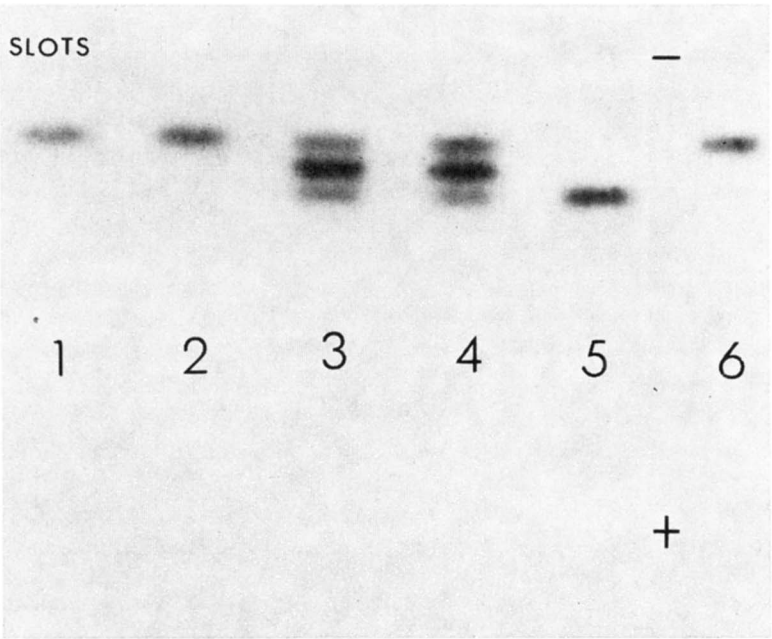

FIGURE 1.—Starch gel electrophoresis illustrating the relative staining intensity of 6-PGD in single flies. Genotypes are as follows:
slots 1–2 = *l(1)Pgd-A^n w^a/Pgd^B* female
slot 3 = *Pgd^{A}w^a/Pgd^B* female
slot 4 = *Pgd^B/w$^+$·Y* male
slot 5 = *Pgd^{A}w^a* male
slot 6 = *Pgd^B* female.
The *Pgd^B* allele is derived from a wild-type Samarkand stock.

females (slots 1 and 2) lack the hybrid and fast bands. Visual estimation of the staining intensity indicates that the single slow band present in *l(1)Pgd-A^n w^a/M-5* females has approximately twice the 6-PGD activity as the slow band for the normal hybrid pattern. This reflects a lack of intragenic complementation which could result from either a lack of polypeptide synthesized from the *l(1)Pgd-A^n* allele or defective subunit association such that the only dimeric enzyme molecules formed are from polypeptides of the *Pgd^B* allele.

The viabilities of hemizygous *l(1)Pgd-A^n w^a* and heterozygous *l(1)Pgd-A^n w^a/Df(1)w^{vco}* flies are represented by experiments 3 and 4 in Table 2. The viabilities of flies in both experiments parallel closely the results obtained for *Pgd$^-$/Df(1)w^{vco}* flies in experiment 2. The few *l(1)Pgd-A^n w^a* male escapers of experiment 3 exhibit several mutant phenes including a dark thoracic trident, small bristles, sterility, and general weakness. Most of these flies die within 48–72 hours post-emergence. By setting up large-scale crosses of the type in Table 2, exp't. 3, we were able to collect a sufficient number of male escapers to conduct analytical enzyme assays (Table 1). It is evident that there is no detectible 6-PGD activity in these assays conducted for up to 10 minutes. The viability

value of 13% for heterozygous *Pgd⁻/l(1)Pgd-A^n* flies (Table 2, experiment 5) may reflect a level of complementation between the two mutant alleles.

Determination of the effective lethal phase was conducted by counting and examining late-stage embryos and third instar larvae, produced by crossing *l(1)Pgd-A^n/w$^+$·Y* males to *C(1)RM,y pn/Y* females (Table 2, exp't. 3), for the presence of male progeny. Of 296 eggs collected, 124 hatched. First instar larvae were counted, placed in vials, and scored at the third instar for the presence of male progeny. In no instance were male larvae present. The unhatched eggs were dechorionated with a Na hypochlorite solution (commercial chlorox diluted 1:1 with insect ringer's) and examined by light microscopy. Fifty-eight per cent had not developed past the blastula stage and most likely correspond to nullo-*X* zygotes and unfertilized eggs. The remaining unhatched embryos appeared to be morphologically normal and to have developed up to the time of hatching (20–22 hours), as evidenced by body segmentation and pigmentation of the mouth parts. These embryos most likely correspond to some metafemales and to the lethal *l(1)Pgd-A^n/Y* class. In accord with this conjecture, it is of interest to note that the time of gene expression for the *Pgd⁺* locus during embryogenesis is at approximately 20 hours of development and up to this time there is a steady decrease in maternal 6-PGD activity (WRIGHT and SHAW 1970). Since the lethal embryos appear to be morphologically normal, *l(1)Pgd-A^n* may be considered as an example of a mutation which interrupts a metabolic pathway not directly related to cellular differentiation or morphogenesis (WRIGHT 1970).

DISCUSSION

The possibility that lethality is only coincidental to "null" 6-PGD activity and that it results from the induction of a lethal allele at a locus adjacent to the *Pgd⁺* gene cannot be completely ruled out. However, the probability of two adjacent but distinctive loci being mutated simultaneously in two separate experiments yielding low 6-PGD activity and reduced viability in one case and "null" 6-PGD activity and lethality in the other case is rather remote. The same qualifications would apply to the occurrence of a deficiency involving the putative adjacent loci. Furthermore, the occurrence of a few *l(1)Pgd-A^n w^a* male escapers would argue against a deficiency. In addition, we have been able to correlate 6-PGD enzyme activity changes with viability and fertility changes in flies bearing the *Pgd⁻* allele. The *Pgd⁻* flies must be periodically selected since enzyme assays have revealed a progressive increase in 6-PGD activity in flies collected from cultures kept over a 6–9 month period. Several substrains were started from single male *Pgd⁻* flies. Enzyme assays on subsequent generations from each substrain revealed a progressive increase in 6-PGD activity from an initial level of less than 10% to 40–50% of wild type. Concomitant with enzyme activity changes, homozygous *Pgd⁻* females became fertile and the relative viability of *Pgd⁻/Df(1)w^{vco}* females increased as much as 50%.

There is an increasing list of Drosophila enzymes which have been mapped genetically and characterized, to various extents, biochemically. "Null" alleles

for these enzyme structural genes provide the means to assess the relative importance of the enzymes in metabolism. For example, by the production of "null" allelic mutants for soluble α-GPDH (O'BRIEN and MACINTYRE 1972) the importance of the α-glycerophosphate cycle in the production of rapid energy for sustained flight has been demonstrated. This enzyme has an obvious selective advantage in the wild, but laboratory populations of Drosophila survive quite well in its absence. In contrast, the operation of the pentose phosphate pathway appears to be essential for life.

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