THE CHARACTERIZATION OF α-GLYCEROPHOSPHATE DEHYDROGENASE MUTANTS IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Thirty mutants of α-glycerophosphate dehydrogenase (αGPDH, EC 1.1.1.8) from *Drosophila melanogaster* were produced with the chemical mutagen ethyl methanesulfonate (EMS). These mutants and nine others previously obtained have been characterized with respect to level of enzymatic activity, viability, flight ability, and presence of cross-reacting material (CRM). The presence of αGPDH mRNA in several of the mutants has been tested by *in vitro* translation. There are strong correlations between the level of enzyme activity, viability and flight ability. Thirteen of the mutants are CRM^- by solution immunoprecipitation experiments, but of these, only three are CRM^- by a more sensitive ^{125}I-protein A-based radioimmune gel assay. The viability of the three CRM^- mutants suggests that the absence of αGPDH protein is not a lethal condition. The immunoprecipitated protein of the low activity mutant, $\alpha Gpdh^{nGL3}$, has a smaller apparent molecular weight on polyacrylamide-SDS gels than does the protein from wild type. Criteria for the identification of nonsense mutations in Drosophila are discussed.

CONSIDERABLE effort has been directed toward the identification of genetic elements that control gene expression in higher eukaryotes. The large body of information on the genetics and biochemistry of Drosophila make it an organism well suited to such investigations. Two well-characterized gene-enzyme systems in Drosophila are alcohol dehydrogenase (WOODRUFF and ASHBURNER 1979a,b; SCHWARTZ and SOFER 1976; SCHWARTZ and JORNVALL 1976; O'DONNELL *et al.* 1977) and xanthine dehydrogenase (see CHOVNICK, GELBART and MCCARRON 1977 for a review; GIRTON, LO and BELL 1979; HILLIKER, CLARK and CHOVNICK 1980). However, only for the *Xdh* locus have both structural and regulatory elements been mapped.

We have chosen to study the structural gene encoding α-glycerophosphate dehydrogenase (*αGpdh*) of *Drosophila melanogaster* and its presumed associated regulatory elements for several reasons. First, a considerable amount is known about its role in metabolism. αGPDH and a related but genetically and biochemically distinct enzyme, α-glycerophosphate oxidase (αGPO), constitute the α-glycerophosphate cycle in the thoracic flight muscle (MACINTYRE and

O'BRIEN 1976). The cycle maintains NAD-NADH equilibrium in the cytosol resulting in a very rapid production of ATP (SACKTOR 1965, SACKTOR 1970; SACKTOR and DICK 1962). The cycle also provides a precursor (α-glycerophosphate) for phospholipid biosynthesis throughout development (KORNBERG and PRICER 1953; KENNEDY 1957).

Second, the level of αGPDH activity is modulated during the course of development of the insect. Enzymatic activity increases throughout larval development, peaking in the third instar and then declining to near zero in the pupae. At eclosion, the activity level increases dramatically to sixfold that observed in the third instar larvae (O'BRIEN and MACINTYRE 1972a).

Third, αGPDH is abundant in the fly, making up 2% of the protein synthesized in newly eclosed adults (D. T. SULLIVAN, personal communication; M. KOTARSKI, unpublished observations). It has been purified to homogeneity by COLLIER, SULLIVAN and MACINTYRE (1976) and antisera specific to αGPDH have been produced (COLLIER and MACINTYRE 1977a; D. T. SULLIVAN, personal communication). This makes αGPDH an attractive enzyme for biochemical studies.

The genetic and biochemical analysis of any gene is facilitated by a large number of mutants. In this paper, we describe the production of 30 new null and low activity mutants of *αGpdh*. These mutants, as well as nine others, were characterized with respect to levels of enzymatic activity, viability, hybrid enzyme formation and the presence of cross-reacting material (CRM) by several sensitive immunological assays. The presence of αGPDH mRNA was also tested in several mutant extracts by *in vitro* translation.

MATERIALS AND METHODS

Cultures and conditions: All Drosophila stocks were maintained at 25° on a standard medium of cornmeal, molasses, yeast and agar to which tegosept was added as a mold inhibitor. When necessary, crosses were performed at 27° to enhance the expression of the dominant mutant Curly.

Stocks: The following chromosomes were used in this study. All visible mutations were described by LINDSLEY and GRELL (1968).

(1) Balancer chromosomes: Second chromosomes were balanced with either Inversion (*2LR*) of Oster, *In(2LR)O, $Cydp^{lvl}$ pr cn^2* or *In(2LR)SM1, al^2 $Cydp^{lvl}$ pr Bl cn^2 L^4*. Both of these balancer chromosomes carry the *$\alpha Gpdh^A$* allele, which specifies the "fast" electrophoretic variant (M. KOTARSKI, unpublished observation). For brevity, the *In(2LR)O* chromosome will be abbreviated as "*CyO*" or "*CyO, pr*" and the *In(2LR)SM1* chromosome as "*SM1, Bl L*" throughout this paper.

(2) Deficiencies: *Df(2L)GdhA* is an X-ray-induced deficiency of both clot and *$\alpha Gdph$* (GRELL, 1967).

Df(2L)cl-7, b pr cn bw is an X-ray-induced deficiency of clot (VELISSARIOU and ASHBURNER 1980) which was subsequently found to be deficient for *$\alpha Gdph$* (KOTARSKI, PICKERT and MACINTYRE 1983).

Df(2L)50078a was kindly provided by ROBERT VOELKER. It is a γ-ray-induced deficiency of *$\alpha Gpdh$* (RACINE, LANGLEY and VOELKER 1980; KOTARSKI, PICKERT and MACINTYRE 1983).

(3) *$\alpha Gpdh$* mutants: *$\alpha Gpdh^{n0}$*, *$\alpha Gpdh^{n1\text{-}4}$*, *$\alpha Gpdh^{n1\text{-}5}$* and *$\alpha Gpdh^{n5\text{-}4}$* were produced in our laboratory by O'BRIEN and MACINTYRE (1972b) and are maintained as balanced stocks over *CyO*. Stocks homozygous for *$\alpha Gpdh^{nNC5}$*, *$\alpha Gpdh^{nNC6}$* and *$\alpha Gpdh^{nNC7}$* were kindly supplied by GLEN BEWLEY. These mutants were isolated from natural populations near Raleigh, North Carolina (personal communication, R. VOELKER). *$\alpha Gpdh^{nAW338}$* and *$\alpha Gpdh^{nJH231}$* are spontaneous mutants on the *In(2LR)SM1, al^2 Cy cn^2 sp^2* chromosome (BEWLEY and LUCCHESI 1977; YAMAGUCHI and MUKAI 1974). These

mutants were also supplied by GLEN BEWLEY. $\alpha Gpdh^{nRZ1}$ was induced by EMS on an $\alpha Gpdh^{B}$ spd^{fg} *pr* chromosome and provided by ROBERT ZIELINSKI from our laboratory.

(4) Other chromosomes: Chromosomes carrying the $\alpha Gpdh^{B}$ (slow) electrophoretic allele include: *cl l(2)gdh-1* $\alpha Gpdh^{B}$ *pr*; and $\alpha Gpdh^{B}$ *l(2)gdh-2* spd^{fg} *pr*. The nonallelic recessive lethal mutations, *l(2)gdh-1* and *l(2)gdh-2*, are very close to the right of the *αGpdh* gene. They have been positioned 1.59 cm to the left and 0.048 cm to the right of the *αGpdh* locus, respectively (KOTARSKI, PICKERT and MACINTYRE 1983). The *cl* $\alpha Gpdh^{n0}$ chromosome was recovered as a recombinant from *cl* $\alpha Gpdh^{A}$/ cl^{+} $\alpha Gpdh^{n0}$ females.

Detection of enzymatically active αGPDH:

(1) Electrophoresis: αGPDH was visualized after electrophoresis on cellulose acetate membranes according to the procedure of COLLIER and MACINTYRE (1972).

(2) Spectrophotometric assay: Crude homogenates (100 mg wet weight/ml) were assayed for αGPDH activity by monitoring the reduction of NAD at 340 nm as described by O'BRIEN and MACINTYRE (1972a). Protein was assayed using the method of LOWRY *et al.* (1951).

(3) Spot test: Flies were spot tested for αGPDH activity as described (KOTARSKI, MACINTYRE and PICKERT 1982).

Production of αGpdh *mutants: αGpdh* null and low activity mutants were produced in two different chromosomal backgrounds, the *cl l(2)gdh-1* $\alpha Gpdh^{B}$ *pr* chromosome and the $\alpha Gpdh^{B}$ *l(2)gdh-2* spg^{fg} *pr* chromosome. Males of the genotype *CyO/cl l(2)gdh-1* $\alpha Gpdh^{B}$ *pr* were starved on agar for 24 hr and then fed 0.03 M ethyl methanesulfonate (EMS) according to the procedure of LEWIS and BACHER (1968). The males were mated *en masse* to *CyO pr/Df(2L)GdhA* females, and individual F_1 *Cy pr* males were mated to *CyO pr/cl* $\alpha Gpdh^{n0}$ females. The *cl* $\alpha Gpdh^{n0}$/*cl l(2)gdh-1* $\alpha Gpdh^{B}$ *pr* progeny were spot tested for αGPDH enzymatic activity. If less than wild-type αGPDH levels were indicated, stocks were constructed from the *CyO pr/cl l(2)gdh-1* $\alpha Gpdh^{n}$ *pr* sibs.

Males of the genotype, *CyO pr/*$\alpha Gpdh^{B}$ *l(2)gdh-2* spd^{fg} *pr*, were starved and fed EMS as described before and then mass mated to *CyO pr/Df(2L)cl-7, b pr cn bw* females. Single F_1 *Cy pr* males were then crossed to *SM1 Bl L/Df(2L)cl-7, b pr cn bw* females. The $\alpha Gpdh^{B}$ *l(2)gdh-2* spd^{fg} *pr/Df(2L)cl-7, b pr cn bw* progeny were spot tested for αGPDH activity. If less than wild-type levels of enzymatic activity were present, stocks were made by mating *SM1, Bl L* male sibs to *CyO pr/Df(2L)clot-7, b pr cn bw* females and intercrossing the *CyO pr/*$\alpha Gpdh^{n}$ *l(2)gdh-2* spd^{fg} *pr* progeny. Matings that did not produce deficiency heterozygotes in the F_2 generation were scored as newly induced recessive lethal mutations within the *cl-7* deficiency. In these cases, the *SM1, Bl L/*$\alpha Gpdh^{?}$ *l(2)gdh-2* spd^{fg} *pr* male sibs were subjected to electrophoresis to ascertain whether a mutation of *αGpdh* had been produced. The chromosomes containing the new recessive lethal mutations were recovered and balanced by *CyO pr* in the same manner as the *αGpdh* mutants. Flies from all of the recessive lethal stocks were crossed once again to *CyO pr/Df(2L)cl-7, b pr cn bw* females to verify the lethality [see KOTARSKI, PICKERT and MACINTYRE (1983) for details].

Antisera: Antisera elicited in rabbits to purified, native αGPDH were provided by GLEN COLLIER and DAVID SULLIVAN. Goat anti-αGPDH serum was provided by DAVID SULLIVAN.

Polyacrylamide-SDS gel electrophoresis: Polyacrylamide-SDS slab gels were prepared according to the procedure of LAEMMLI (1970) or MAIZEL (1971). The running gel was 15% acrylamide and 1.3% bis-acrylamide. Molecular weight standards were BDH molecular weight mixtures (BDH Chemicals, Ltd; M_r: 14,300, 28,600, 42,900 and 57,200), bovine serum albumin (M_r: 63,800), purified αGPDH [M_r: 32,000 (COLLIER, SULLIVAN and MACINTYRE 1976)] and lysozyme (M_r: 13,930). Gels were stained with 0.3% Coomassie brilliant blue-R in 50% methanol, 10% acetic acid.

Immunoprecipitation of αGPDH from crude extracts: Flies were homogenized (133 mg of wet weight/ml) in 1.0 M sodium phosphate buffer, pH 7.1, 10 mM α-glycerophosphate 10^{-6} M NAD, 0.5 mM dithiothreitol, 1.0 mM EDTA. Homogenates were centrifuged at 15,600 × *g* for 15 min to remove debris. The immunoprecipitation reaction mixtures consisted of 100 μl of the crude homogenate, 50–100 μl of antiserum (goat or rabbit) and 50 μl of 0.05 M NaCl. The reaction was allowed to continue at 4° for 3–12 hr. The immunoprecipitate was recovered by centrifugation for 10 min at 13,000 × *g*. The pellet was washed four times with 0.2 M Tris-HCl, pH 7.5, 0.2 M NaCl, 2% Triton-X 100, 2 M urea and 2% glycerol and once with 0.05 M Tris-HCl, pH 7.5, 0.15 M NaCl and 1% glycerol. The pellets were prepared for polyacrylamide-SDS gel electrophoresis

by dissolving them in 70 μl of electrophoresis sample buffer (0.5 M Tris-HCl, pH 6.8, 1% β-mercaptoethanol, 1% SDS, 25% glycerol, 0.1% bromophenol blue), heated to 95° for 2 min and stored frozen at −70°; 20-μl samples (20 μg of protein in the case of wild-type αGPDH) were applied to the polyacrylamide-SDS gels.

Visualization of αGPDH with ^{125}I-protein A: Flies were homogenized in 0.05 M Tris-HCl, pH 7.5, 1.0 M $(NH_4)SO_4$, 30% glycerol at 200 mg wet weight/ml. Debris was removed by centrifugation for 15 min at 15,600 × *g*. Crude extract was mixed with an equal volume of electrophoresis sample buffer, heated to 95° for 2 min and stored frozen at −70°. Approximately 75 μg of protein were applied to polyacrylamide-SDS gels. Antigenic αGPDH was visualized according to the method of BIGELIS and BURRIDGE (1978) with the following modifications: Gels were overlain for 24 hr with antiserum (rabbit) that had been diluted to 6 mg/ml of total protein. The uncomplexed antibody was washed from the gel by repeated buffer changes during 70–75 hr. The gels were then overlain for 24 hr with ^{125}I-protein A (Amersham, specific activity 55–65 mCi/mg) at 4.0 μCi/ml. Unreacted ^{125}I-protein A was washed from the gels by repeated buffer changes during 48 hr. Gels were dried and exposed to Kodak XR-5 film for 12–48 hr to visualize the bound ^{125}Iprotein A.

RNA extraction: Zero- to 12-hr-old adults were collected, frozen and stored in liquid nitrogen until use. RNA was extracted using a procedure from MICHAEL ASHBURNER'S laboratory (personal communication). All solutions were treated with diethyl pyrocarbonate and autoclaved before use. Flies (100–200) were ground to a powder in liquid nitrogen. RNA was extracted with phenol and 2× NETS buffer (100 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl, pH 7.5, 0.5% SDS). The phenol phase was extracted a second time with fresh 2× NETS, and the combined aqueous phases were extracted twice with fresh water-saturated phenol. RNA was precipitated by the addition of 2 volumes of 95% ethanol. The precipitate was dried and resuspended in distilled water. An equal volume of 5 M LiCl was added, and RNA was precipitated on ice for at least 1 hr. The LiCl pellet was resuspended in distilled water and the RNA concentration determined using the formula: an A_{260} of 23 = 1 mg/ml. RNA was precipitated by addition of 0.1 volume of 2 M ammonium acetate and 2 volumes of 95% ethanol and stored as a precipitate at −70° until use.

In vitro *translation:* RNA was translated in a rabbit reticulocyte messenger-dependent lysate system (Amersham Corporation). Final concentrations of K^+ and Mg^{+2} were adjusted to 200 and 2 mM, respectively (D. SULLIVAN, personal communication). RNA was dissolved in distilled water at 10 mg/ml and added at a final concentration of 0.78 mg/ml. A high specific activity ^{3}H-amino acid mixture (TRK 0.550 Amersham Corporation) was used as radiolabeled precursor. Reactions were incubated at 30° for 60 min. Incorporation of acid-precipitable radioactivity was determined as follows: 1 μl of the translation reaction mix was added to 25 μl of 1 N NaOH, 0.5 N H_2O_2 and incubated at 37° for 10 min. Samples were spotted on 3MM (Whatman) filters, and the filters were batch processed for three washes with a 10% trichloroacetic acid solution (10 min each wash), one wash with 95% ethanol (5 min) and one wash with absolute ether (5 min). Filters were air dried and counted. Aliquots were prepared for electrophoresis by the addition of 2 volumes of 2× SDS-sample buffer (LAEMMLI 1970), heated to 95° for 2 min and stored at −70°.

Immunoprecipitation of in vitro *translation products:* Following the translation reaction, 30 μl of the reaction mixture were placed in a tube containing 70 μl of NET buffer and 30 μl of goat anti-αGPDH antiserum and incubated overnight at room temperature. A suspension of formalin-fixed *S. aureus* cells (30 μl) was added and the reaction incubated on a rotary shaker at room temperature for 1–2 hr. The Staphylococcus cells were used as an adsorbent for the complexed immunoglobulin (KESSLER 1976). The reaction mix was centrifuged, and the pellets were resuspended in fresh NET, transferred to a clean centrifuge tube and washed three times with NET. The pellet was resuspended in 20 μl of SDS-sample buffer (LAEMMLI 1970), and the sample was heated to 90° for 5 min with occasional vortexing to elute the antigen from the Staphylococcus cells. The *S. aureus* cells were removed by centrifugation, and the supernatant was frozen at −70° until use.

Partial proteolysis and cyanogen bromide cleavage of αGPDH: Purified αGPDH (provided by D. SULLIVAN) was subjected to partial proteolysis according to the method of CLEVELAND *et al.* (1977). Substrate at 0.5 mg/ml was incubated with *S. aureus* V8 protease (10 or 100 μg/ml) at 37° for 30 min. The reaction was stopped by the addition of β-mercaptoethanol and SDS and heating at 95° for 2 min. Samples were frozen at −70° until use.

Cyanogen bromide cleavage of purified αGPDH was carried out in concentrated formic acid at final concentrations of 0.5 mg/ml αGPDH and 50 mg/ml cyanogen bromide. The samples were incubated at room temperature for 24 to 72 hr. They were then lyophilized, resuspended in SDS-sample buffer, neutralized by addition of 6 N NaOH and frozen at −70° until use.

Fluorography: Gels containing ^{3}H-labeled proteins were fluorographed using sodium salicylate as described by CHAMBERLAIN (1979). Dried gels were exposed to preflashed Kodak XR-5 X-ray film at −70° (LASKEY and MILLS 1975).

RESULTS

Mutagenesis: The mutagenesis of the *cl l(2)gdh-1 αGpdh^B pr* chromosome produced six low and three null αGPDH activty mutants among the 11,191 mutagenized chromosomes tested. A null mutation is operationally defined here as one that possesses no detectable αGPDH enzymatic activity in the spectrophotometric assay or upon electrophoresis and histochemical staining.

The mutagenesis of the *αGpdh^B l(2)gdh-2 spd^{fg} pr* chromosome resulted in the recovery of 14 low and six null αGPDH activity mutants among the 11,182 chromosomes tested. We also recovered 27 recessive lethal mutations from the mutagenesis of the *αGpdh^B l(2) gdh-2 spd^{fg} pr* chromosome. These lethals map within the *cl-7* deficiency (KOTARSKI, PICKERT and MACINTYRE 1983). Although, in theory, this mutagenesis scheme makes possible the recovery of recessive lethal mutations at the *αGpdh* locus, none was obtained, *i.e.*, all of the 27 chromosomes carrying newly induced recessive lethals also carried normal *αGpdh^B* alleles.

Enzymatic activity, flight ability and viability of Gpdh *mutants:* Of the 39 *αGpdh^n* mutants characterized in this study, 25 possess some αGPDH enzymatic activity (Table 1), whereas 14 are devoid of enzymatic activity by our tests. The αGPDH activity of the mutants ranges from 0 to 78.5% of the activity of a fly heterozygous for a deficiency of αGpdh, *e.g.*, *αGpdh^B/Df(2L)cl-7*. The relative viability of the mutants was determined by crossing *CyO/Df* with *CyO/αGdph^n* heterozygotes. The deficiencies used in these crosses were *Df(2L)50078a* for the *αGpdh^n* mutants *nS1* through *nS10* and *Df(2L)cl-7* for the others. The relative viability of the *αGpdh^n/Df* (Curly$^+$) heterozygotes is calculated as the proportion of expected heterozygous progeny produced by the cross. The expected proportion of *αGpdh^n/Df* progeny is 33 ⅓%. Flight ability was tested using the method described by O'BRIEN and MACINTYRE (1972b). Unetherized flies were dropped from a height of 6 feet above the floor. Wild-type flies initiate flight immediately when they begin to drop. Flies unable to fly fall to the floor.

When the viability and enzymatic activity data from Table 1 are compared (Figure 1), a positive correlation is apparent. The correlation coefficient for these two parameters is 0.71 (P = 0.001). Crosses that produce heterozygotes with 0% αGPDH activity generally yielded less than 60% of the expected number of Curly$^+$ progeny (*nS3* and *nS8* are exceptions to this). Mutants that have between 0 and 15% of wild-type levels of enzymatic activity have viabilities ranging from 0.35 to 1.1. *Df/αGdph^n* heterozygotes possessing greater than 15% of αGPDH activity have viabilities between 0.75 and 1.3. The reduced viability of *αGpdh^n/Df* heterozygotes was also observed by O'BRIEN, WALLACE and MACINTYRE (1972). *αGpdh^{nS3}* and *αGpdh^{nS8}* represent conspicu-

TABLE 1

Characteristics of the αGPDH^n *mutants in this study*

Mutant	Chromosome[a]	% Wild-type activity[b]	*αGpdh^n/Df* relative viability (*n*)[c]	Flight ability[d]	Hybrid enzyme class[e]	CRM[f]
n1-4	*a1*	0.0	0.20 (268)	−	1	+
n5-4	*a1*	0.0	0.28 (251)	−	2	+
n1-5	*a1*	26.1	1.10 (355)	+	1	+
n0	*a1*	0.0	0.20 (228)	−	1	+
nRZ1	*spd^{fg} pr*	0.0	0.20 (147)	−	3	+ (I^{125})
nAW338	*SM1 Cy*	11.3	NT[g]	NT	2	+ (I^{125})
nJH231	*SM1 Cy*	0.0	NT	NT	2	−
nNC5	+	6.0	NT	+	NT	+ (I^{125})
nNC6	+	6.0	NT	+	NT	+ (I^{125})
nNC7	+	7.5	NT	+	NT	+ (I^{125})
nSP1	*l(2)gdh-2 spd^{fg} pr*	6.5	0.82 (451)	+	1	+
nSP2	*l(2)gdh-2 spd^{fg} pr*	4.0	0.99 (636)	+	1	+ (I^{125})
nSP3	*l(2)gdh-2 spd^{fg} pr*	0.0	0.23 (330)	−	1	+
nSP4	*l(2)gdh-2 spd^{fg} pr*	0.0	0.06 (361)	−	3	+ (I^{125})
nSP5	*l(2)gdh-2 spd^{fg} pr*	71.4	0.68 (252)	+	1	+
nSP6	*l(2)gdh-2 spd^{fg} pr*	8.3	0.35 (632)	+	2	+
nSP7	*l(2)gdh-2 spd^{fg} pr*	28.0	1.00 (442)	+	1	+
nSP8	*l(2)gdh-2 spd^{fg} pr*	9.2	0.95 (372)	+	1	+
nSP10	*l(2)gdh-2 spd^{fg} pr*	0.0	0.25 (1016)	−	3	−
nSP11	*l(2)gdh-2 spd^{fg} pr*	30.4	0.98 (247)	+	2	+
nSP12	*l(2)gdh-2 spd^{fg} pr*	0.0	0.42 (451)	−	3	−
nSP14	*l(2)gdh-2 spd^{fg} pr*	0.0	0.27 (853)	−	3	+ (I^{125})
nSP15	*l(2)gdh-2 spd^{fg} pr*	11.1	0.54 (436)	±	2	+ (I^{125})
nSP16	*l(2)gdh-2 spd^{fg} pr*	0.0	0.55 (375)	±	1	+
nSP17	*l(2)gdh-2 spd^{fg} pr*	1.8	0.74 (498)	+	2	+
nSP18	*l(2)gdh-2 spd^{fg} pr*	74.5	0.99 (522)	+	1	+
nSP19	*l(2)gdh-2 spd^{fg} pr*	18.8	0.76 (447)	+	1	+
nGL1	*l(2)gdh-2 spd^{fg} pr*	4.4	0.70 (280)	+	1	+
nGL2	*l(2)gdh-2 spd^{fg} pr*	9.5	1.1 (335)	+	2	+
nGL3	*l(2)gdh-2 spd^{fg} pr*	22.2	1.3 (468)	+	1	+
nS1	*c1 l(2)gdh-1 pr*	25.4	0.78 (192)	+	1	+
nS2	*c1 l(2)gdh-1 pr*	0.0	0.33 (144)	−	3	+ (I^{125})
nS3	*c1 l(2)gdh-1 pr*	0.0	0.85 (123)	+	2	+
nS4	*c1 l(2)gdh-1 pr*	78.5	1.0 (124)	+	2	+
nS5	*c1 l(2)gdh-1 pr*	56.6	0.88 (212)	+	2	+
nS6	*c1 l(2)gdh-1 pr*	4.6	0.93 (152)	+	1	+
nS7	*c1 l(2)gdh-1 pr*	52.4	1.30 (149)	+	1	+
nS8	*c1 l(2)gdh-1 pr*	0.0	0.81 (115)	+	2	+
nS10	*c1 l(2)gdh-1 pr*	74.8	0.82 (113)	+	1	+

[a] All mutant alleles were induced in chromosomes originally containing *αGPDH^B* alleles, except *nAW338* and *nJH231*, which were obtained in chromosomes carrying *αGPDH^A*. It is not known from what allele or alleles the naturally occurring nulls *nNC5*, *nNC6* and *nNC7* were derived.

[b] Expressed as percent wild-type specific activity. Wild type = *αGpdh^B/Df(2L)cl-7* heterozygotes. 100% = 0.156 μmol NAD reduced/min/mg protein. Values presented are averages of two measurements of independent homogenates. 0% represents less than 0.2% wild-type activity. Mutants were assayed as *αGpdh^n/Df* heterozygotes.

[c] Df = *Df(2L)cl-7* for *n1-4* to *nGL3* and *Df(2L)50078a* for *nS1* to *nS10*. Relative viability is determined as described in the text. Numbers in parentheses are the total numbers of progeny in the cross used to determine relative viability.

TABLE 1

Continued

[d] − indicates not able to fly; + indicates flight ability comparable to wild type; ± indicates intermediate flight ability as described in the text.

[e] Class 1: hybrid enzyme is present in appropriate *n*/+ electropherograms, but its mobility is not the same as an $\alpha GPDH^A \cdot \alpha GPDH^B$ heterodimer. Class 2: hybrid enzyme is present in appropriate *n*/+ electropherograms and has the same mobility as an $\alpha GPDH^A \cdot \alpha GPDH^B$ heterodimer. Class 3: no hybrid enzyme is detected in either $n/\alpha GPDH^A$ or $n/\alpha GPDH^B$ electropherograms.

[f] + indicates CRM can be detected by Coomassie blue staining of immunoprecipitates separated in SDS gels; + (I^{125}) indicates CRM can only be detected by the I^{125}-based method described in the text.

[g] NT, not tested.

ous exceptions to the correlation between enzyme activity levels and flight ability. These mutants have high viabilities but no detectable enzymatic activity.

Figure 1 also shows that the flying ability of the *αGpdh*^{n} mutants correlates well with their viability. The mutants that have viabilities from 0–0.35 do not fly. Some, but not all, of the mutants in the viability range from 0.35–0.60 can fly. For example, *αGpdh*^{nSP6} is able to fly normally, *αGpdh*^{nSP12} cannot fly at all, while *αGpdh*^{nSP15} and *αGpdh*^{nSP16} have intermediate flying abilities. *αGpdh*^{nSP15}*/Df* and *αGpdh*^{nSP16}*/Df* heterozygotes are able to fly only for very short periods. They can be prodded into initiating flight but are only able to fly 2–3 feet at a time. This is somewhat more than can be achieved by non-flying mutants, but certainly cannot be considered as normal flight. All *αGpdh*^{n} mutants that are within the range of 0.60–1.3 viability fly normally.

Hybrid enzyme formation: It is important when characterizing a group of mutants to determine as far as possible whether they are structural gene mutations or mutations outside the structural gene that modify the level of enzyme activity. The detection of an inactive αGPDH protein is *prima facie* evidence of a structural gene mutation. The demonstration that the mutant polypeptide also has an altered electrophoretic mobility adds strong support to this contention (SCHWARTZ and SOFER 1976; GIRTON, LO and BELL 1979). In the present study, therefore, the *αGpdh* mutants were examined for the presence of hybrid dimeric enzymes when they were heterozygous with *αGpdh* alleles specifying wild-type enzymes with different electrophoretic mobilities.

The active αGPDH enzyme has been shown to be a dimer of identical subunits of molecular weight 32,000 daltons (COLLIER, SULLIVAN and MACINTYRE 1976). Thus, electrophoresis of extracts of *αGpdh*^{A}*/αGpdh*^{B} heterozygotes produce a three-band pattern typical of a dimeric enzyme (O'BRIEN and MACINTYRE 1972b; COLLIER and MACINTYRE 1977b). The heterodimeric or "hybrid" enzyme band is approximately twice the intensity of either homodimeric band (*e.g.*, see Figure 3).

Figure 2 represents, diagrammatically, the electropherograms of all the *αGpdh* mutants, as either *αGpdh*^{n}*/αGpdh*^{A} or *αGpdh*^{n}*/αGpdh*^{B} heterozygotes. *αGpdh* mutants that failed to produce a detectable hybrid enzyme band were also tested as heterozygotes with an *αGpdh*$^+$ allele specifying a different electrophoretic variant. This second test excludes the possibility that a hybrid enzyme was not detected because it comigrated with the homodimeric enzyme.

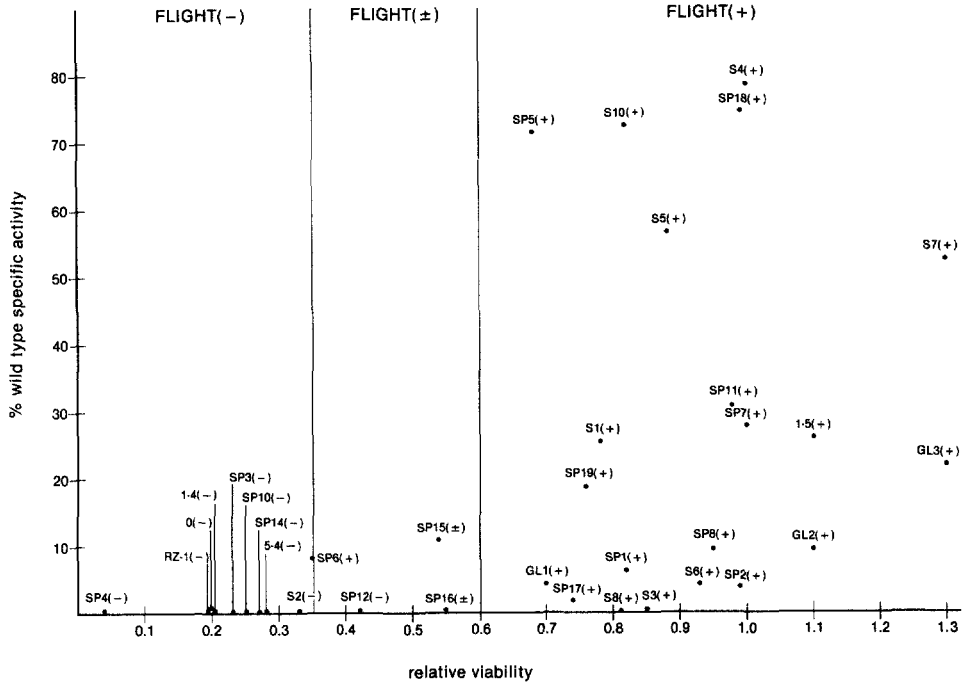

FIGURE 1.—The viability, enzymatic activity and flight ability of $\alpha Gpdh^{n}$ mutants. − indicates not able to fly, + indicates flight comparable to wild type, and ± indicates intermediate flight ability as described in the text.

For example, $\alpha Gpdh^{nRZ1}$, $\alpha Gpdh^{nSP4}$, $\alpha Gpdh^{nSP10}$, $\alpha Gpdh^{nSP12}$, $\alpha Gpdh^{nSP14}$ and $\alpha Gpdh^{nS2}$ showed no hybrid enzyme as $\alpha Gpdh^{n}/\alpha Gpdh^{A}$ heterozygotes. Extracts of these mutants as $\alpha Gpdh^{n}/\alpha Gpdh^{B}$ heterozygotes were then subjected to electrophoresis. None of these mutants exhibited a hybrid enzyme in this additional test. The null mutant $\alpha Gpdh^{nJH231}$, originally induced in a $\alpha Gpdh^{A}$ allele, was reexamined by subjecting extracts of $\alpha Gpdh^{nJH231}/\alpha Gpdh^{A}$ heterozygotes to electrophoresis, but again there was no indication of a hybrid enzyme. The *αGpdh* mutants shown in Figure 3 can be divided into three classes: (1) mutants that produce both an enzymatically active hybrid enzyme and a subunit that has an altered electrophoretic mobility, as judged by the position of the $\alpha GPDH^{n}\cdot\alpha GPDH^{n}$ homodimer (in the low activity mutants) or by the position of the $\alpha GPDH^{n}\cdot\alpha GPDH^{+}$ hybrid enzyme; (2) mutants that produce a hybrid enzyme whose subunits still have the electrophoretic mobility characteristic of the "premutagenized" allele; and (3) mutants that do not form a hybrid enzyme. The mutants in each of the three classes are listed in Table 1.

Figure 2 contains one unexpected result. $\alpha Gpdh^{nSP15}$ is the only mutation that appears to affect the expression of the *trans* $\alpha GPDH^{+}$ allele in an $\alpha Gpdh^{n}/\alpha Gpdh^{+}$ heterozygote. Electrophoresis of extracts made from $\alpha Gpdh^{nSP15}/\alpha Gpdh^{A}$ heterozygotes show a lowered activity of the $\alpha GPDH^{A}\cdot\alpha GPDH^{A}$ homodimer in the electropherogram. This is also observed in $\alpha Gpdh^{nSP15}/\alpha Gpdh^{B}$

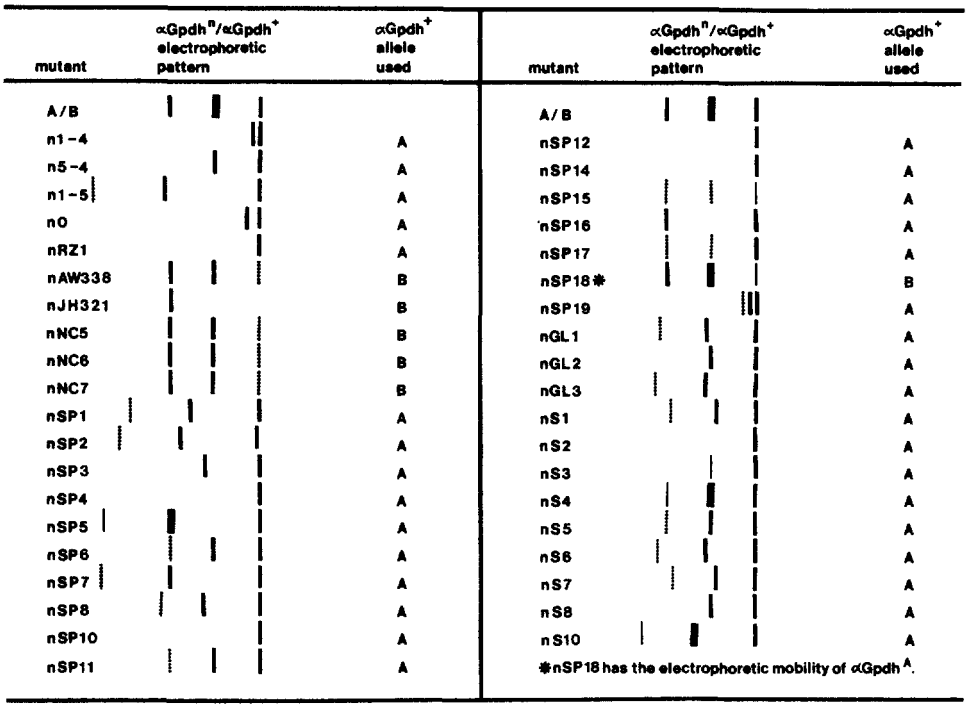

FIGURE 2.—Diagrammatic representation of cellulose acetate electropherograms of extracts of *αGpdh*^{n}/*αGpdh*$^+$ heterozygotes. Direction of migration is from left to right. A = *αGpdh* fast electrophoretic allele; B = *αGpdh* slow electrophoretic allele. Relative amounts of enzyme activities in the electropherograms are indicated by band intensities, *e.g.*, ▬ indicates wild type activity and .... indicates a very low level of enzyme activity.

heterozygotes (Figure 4). To rule out the possibility that flies containing the *αGpdh*^{nSP15} chromosome produce an inhibitor of αGPDH enzyme activity, *αGpdh*^{nSP15}*/Df(2L) cl-7* and *αGpdh*^{A}*/Df(2L)cl-7* heterozygotes were homogenized together, and the extract was subjected to electrophoresis. The level of activity of the αGPDH^A·αGPDH^A homodimer was compared with that in extracts of *αGpdh*^{A}*/Df(2L)cl-7* flies with the same wet weight per volume ratio. The results are shown in Figure 4. The presence of a fully active αGPDH^A·αGPDH^A enzyme in the mixture of αGPDH^{nSP15} + αGPDH^A suggests there is no diffusible inhibitor of αGPDH activity in *αGpdh*^{nSP15}*/αGpdh*^{A} heterozygotes. One explanation is that the nSP15 subunit binds with exceptionally high affinity to the αGPDH^A monomer and, in doing so, forms a heterodimer with virtually no enzymatic activity. Further experiments are necessary to resolve the exact nature of the mutant nSP15 subunit.

Characterization of αGPDH antisera: We next examined *αGPDH*^{n}*/Df* heterozygotes for the presence of αGPDH CRM. Since we subjected mutant αGPDH proteins to polyacrylamide-SDS gel electrophoresis in order to visualize the CRM in antigen-antibody complexes, the apparent molecular weights of the

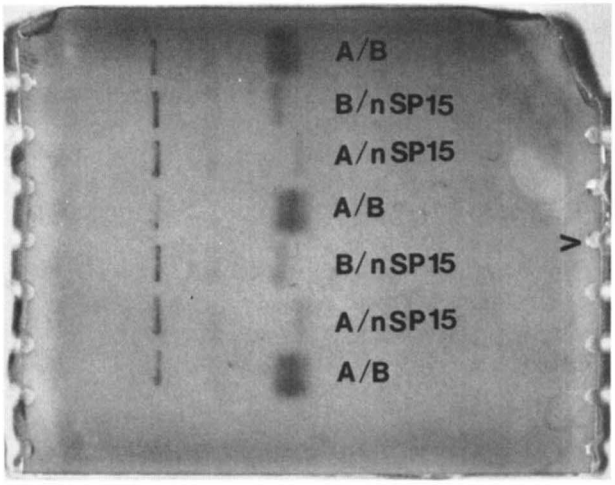

FIGURE 3.—Cellulose acetate electrophoresis of extracts of $\alpha Gpdh^{A}/\alpha Gpdh^{B}$(A/B), $\alpha Gpdh^{A}/\alpha Gpdh^{nSP15}$ and $\alpha Gpdh^{B}/\alpha Gpdh^{nSP15}$ heterozygotes. The membrane was stained for αGPDH activity. The effect of the $\alpha Gpdh^{nSP15}$ allele on the activity of a normal $\alpha GPDH^{A}\cdot\alpha GPDH^{A}$ homodimer can best be seen by comparing the electropherograms of the $\alpha Gpdh^{nSP15}/\alpha Gpdh^{A}$ heterozygotes in this figure with those of the $\alpha Gpdh^{A}/Deficiency$ heterozygotes in Figure 4.

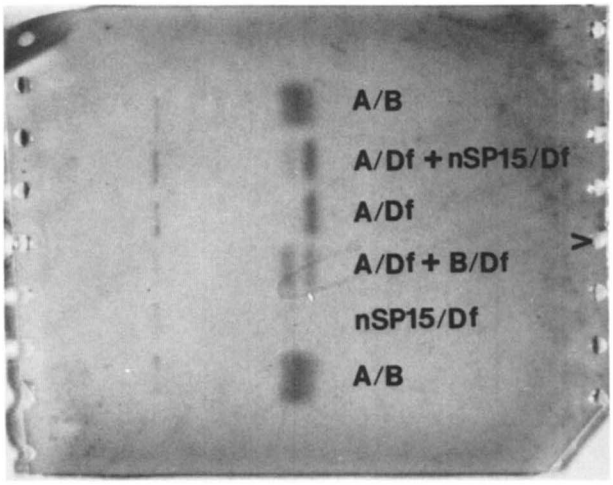

FIGURE 4.—Cellulose acetate electrophoresis of extracts of $\alpha Gpdh^{A}/\alpha Gpdh^{B}$(A/B), $\alpha Gpdh^{nSP15}/Df(2L)cl\text{-}7$(nSP15/Df) and $\alpha Gpdh^{A}/Df(2L)cl\text{-}7$ (A/Df) heterozygotes. Also subjected to electrophoresis was an extract of $\alpha Gpdh^{nSP15}/Df(2L)cl\text{-}7$ heterozygotes mixed with $\alpha GPDH^{A}/Df(2L)cl\text{-}7$ heterozygotes (A/Df + nSP15/Df) and a mixture of $\alpha Gpdh^{B}/Df(2L)cl\text{-}7$ and $\alpha Gpdh^{A}/Df(2L)cl\text{-}7$ heterozygotes (A/Df + B/Df). The membrane was stained for αGPDH activity. The results show that an extract of flies carrying an $\alpha Gpdh^{nSP15}$ allele does not contain a diffusible inhibitor of $\alpha GPDH^{A}\cdot\alpha GPDH^{A}$ homodimers.

mutant polypeptides are, in fact, determined at the same time. A partial in-frame deletion within the *αGpdh* gene or a nonsense mutant of *αGpdh* that produces an antigenic polypeptide fragment could be identified by this procedure. Before these tests for αGPDH, CRM were carried out, however, the specificity and reactivity of the antisera were examined.

Crude wild-type adult extracts were incubated with various amounts of antiserum in order to immunoprecipitate the αGPDH protein. The reaction mixtures were centrifuged, and the supernatants were assayed for αGPDH activity, whereas the pellets were subjected to SDS gel electrophoresis. Figure 5 shows that, as more antiserum is added to the reaction, more αGPDH protein is complexed by the antiserum and is removed by centrifugation. This correlates with the loss of αGPDH activity in the supernatants. The presence of a single Drosophila protein in the immunoprecipitates and the correlation between the appearance of this protein in the SDS gels and the loss of αGPDH activity from the supernatant indicate that the antisera are specific for αGPDH. The molecular weight of the precipitated protein is 32,000, the same as the molecular weight of the subunit from purified αGPDH.

Since the anti-αGPDH serum was to be used to analyze mutant αGPDH peptides, it was important to determine whether any antibodies in this serum could complex with any short αGPDH peptides. This was determined by testing the antigenicity of cyanogen bromide fragments and partial proteolytic products of purified αGPDH. Purified αGPDH was incubated with either cyanogen bromide or *S. aureus* V8 protease as described in MATERIALS AD METHODS. The resulting peptides were separated on a polyacrylamide-SDS gel. The gel was cut in half and one-half stained with Coomassie brilliant blue-R to visualize the peptides. The remaining half of the gel was overlain first with antiserum, then with ^{125}I-protein A, and autoradiographed to detect any antigenic peptides. An incomplete cyanogen bromide cleavage of αGPDH produced eight peptides that were detectable by staining with Coomassie blue. The molecular weights of the peptides ranged from 9,800 to 32,000 daltons (uncleaved). The V8 protease digestion produced seven peptides with molecular weights ranging from 10,800 to 32,000 daltons (undigested). All of the peptides that were visualized with Coomassie blue were also detected with the ^{125}I-protein A method and, therefore, are antigenic (see KOTARSKI 1982 for details). Since all of the peptides are recognized by the antiserum, the population of antibodies in the anti-αGPDH serum should be useful for the analysis of presumptive missense and nonsense mutants of the *αGpdh* structural gene.

Detection of αGPDH CRM in αGpdh *mutants*: Two different experimental procedures were used to detect CRM; immunoprecipitation in solution and ^{125}I-protein A labeling of αGPDH-antibody complexes in polyacrylamide-SDS gels. In the first method, crude homogenates of *αGpdh^n/Df* heterozygotes or *αGpdh^n/αGpdh^n* homozygotes were immunoprecipitated with anti-αGPDH sera, and the immunoprecipitates were subjected to electrophoresis on polyacrylamide-SDS gels as described in MATERIALS AND METHODS. These gels were then stained for protein. This method allows one to determine whether a

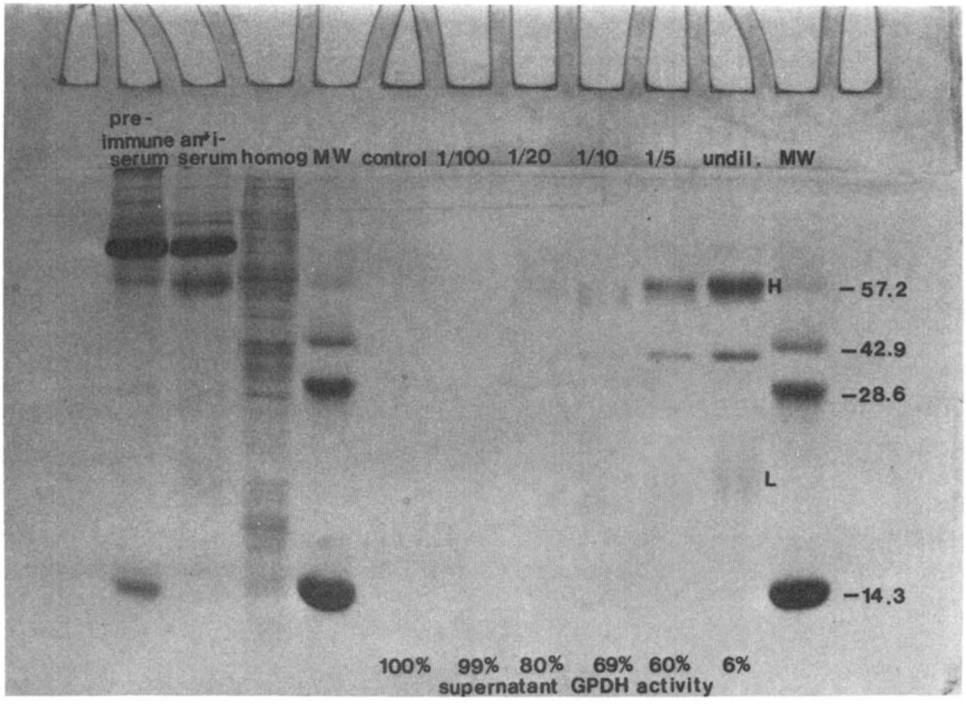

FIGURE 5.--Polyacrylamide-SDS gel electrophoresis of immunoprecipitable proteins from homogenates of wild-type flies ($\alpha Gpdh^B/\alpha Gpdh^B$) using an antiserum against purified αGPDH. The first three wells from left to right contained, trichloroacetic acid precipitable proteins from preimmune serum, antiserum and crude fly homogenates. Molecular weight markers are in wells 4 and 11, with the kilodaltons of each marker indicated on the right. Wells 5–10 contained precipitates from fly homogenates mixed with no serum (well 5), four dilutions of antiserum (wells 6–9) and undiluted antiserum (well 10). The amount of αGPDH activity remaining in the supernatant after removal of the precipitates is indicated at the bottom of the gel below wells 5–10. H and L indicate heavy and light rabbit immunoglobulin chains, respectively. The gel was stained for protein with Coomassie brilliant blue-R.

particular mutant is CRM^+ or CRM^- and, if it is CRM^+, to estimate the apparent molecular weight of any immunoprecipitated αGPDH protein. Table 1 presents the results of experiments on all of the $\alpha Gpdh^n$ mutants examined in this study. Of the 39 $\alpha Gpdh^n$ mutants, 25 are fully CRM^+ by this method, and the mutant proteins are indistinguishable from immunoprecipiated wild-type αGPDH with regard to molecular weight.

Thirteen of the 39 *αGpdh* mutants are CRM^- following immunoprecipitation from solution. In these mutants, some of the mutant αGPDH proteins may have a conformation drastically different from native αGPDH and are, therefore, not complexed by the antibody. This seems unlikely, however, since all of the peptides of αGPDH generated by cyanogen bromide and protease digestion are recognized by the antiserum used in these experiments. On the other hand, either the mutants may be producing very low levels of αGPDH protein or the mutant proteins are so rapidly degraded that there are not sufficient

steady state amounts to be seen with Coomassie blue staining of proteins. Also, of the 13 CRM$^-$ mutants, five have measurable levels of αGPDH activity (*nSP2, nSP15, nNC5, nNC6* and *nNC7*) in spectrophotometric or gel assays. One might expect that, if a mutant polypeptide is similar enough to wild type to possess some enzymatic activity, it is also likely to be antigenic.

These 13 CRM$^-$ mutants were tested for CRM using the ^{125}I-protein A method to visualize antigenic αGPDH. This method does not depend on the presence of an immunoprecipitable antigen-antibody complex for the detection of CRM. The results of testing some of the *αGpdh^n* mutants for CRM with this method are shown in Figure 6, and a summary of the results with the 13 CRM$^-$ mutants is given in Table 1 where they are indicated as +(^{125}I). The level of CRM varies within the mutants, but all of the mutants that have some αGPDH activity are also CRM$^+$ by this test. Three mutants, *nJH231, nSP10* and *nSP12*, do not produce any detectable αGPDH protein by this method. We have determined in other experiments (see KOTARSKI 1982) that the ^{125}I-protein A method is capable of detecting 0.5% of wild-type levels of αGPDH protein (wild type = *αGpdh^B/Df*). Further, we estimate that it could detect as little as 0.1% of wild-type αGPDH if autoradiograms are exposed for longer periods of time. If the amount of αGPDH is taken to be 2% of the total soluble protein of the fly, then 0.1% of wild-type αGPDH represents 1 ng of αGPDH protein.

In summary, the two methods for detecting CRM, *viz.*, immunoprecipitation of αGPDH and the I^{125}-based radioimmune assay, show that of the 39 mutants tested, all but three are CRM$^+$. In addition, the molecular weight of the CRM from 35 of the 36 CRM$^+$ mutants is indistinguishable from that of wild-type αGPDH. The one exception is *αGpdh^{nGL3}*. *αGpdh^{nGL3}* is CRM$^+$, but the antigenic polypeptide appears to be slightly smaller than wild-type αGPDH. This is shown in Figure 7. Three interpretations of these results are (1) *αGpdh^{nGL3}* is a nonsense mutation, and the protein produced reflects a premature termination of translation of *αGpdh*; (2) this mutant is a partial deletion of the *αGpdh* gene resulting in translation of a smaller than wild-type protein; and (3) the *αGpdh^{nGL3}* mutation changes the primary structure of the αGPDH protein such that a precise proteolysis of a completely translated polypeptide produces an antigenic fragment of slightly smaller molecular weight. The question of proteolysis of the nGL3 protein will be addressed in more detail later in this paper.

Analysis of αGpdh^n mutants by in vitro *translation*: The procedures for detecting CRM depend upon the detection of steady state levels of antigenic αGPDH. The three CRM$^-$ mutants, *αGpdh^{nJH231}*, *αGpdh^{nSP10}* and *αGpdh^{nSP12}*, may synthesize a defective αGPDH protein that is rapidly degraded *in vivo*. To address this possibility, we have studied the ability of RNA extracted from these mutants to direct the synthesis of Drosophila αGPDH in a message-dependent, rabbit reticulocyte *in vitro* translation system. Since endogenous proteolytic activity is low, this system may allow the detection of an unstable mutant αGPDH protein.

Total RNA was phenol extracted from 0–12-hr-old *αGpdh^n/Df(2L) cl-7* adults

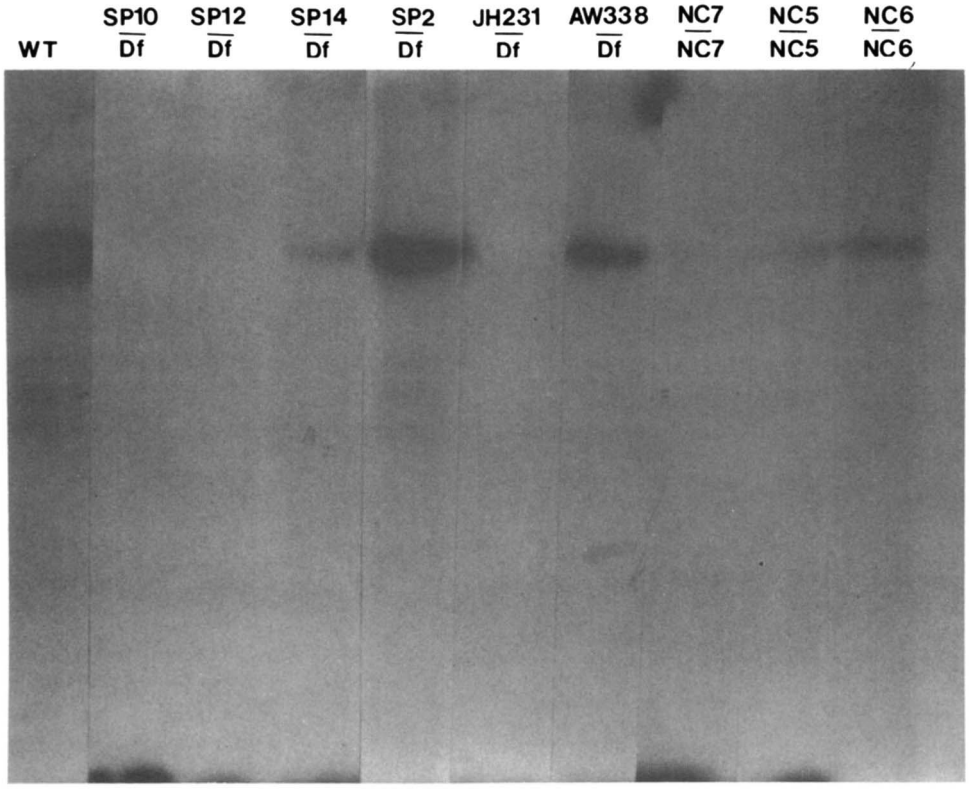

FIGURE 6.—Results of the ^{125}I-protein A method for the detection of αGPDH CRM in several *αGpdh*^{n} / *Deficiency* heterozygotes as described in MATERIALS AND METHODS. WT = *αGpdh*^{B} / *αGpdh*^{B}; Df = *Df(2L)cl-7*. Exposure of autoradiogram was for 24 hr. The results are not qualitatively different if the autoradiogram is exposed for longer than 24 hr, but the background becomes more intense, in some cases obscuring the bands containing the αGPDH-antibody-*S. aureus* protein complexes.

and translated in the reticulocyte lysate system as described in MATERIALS AND METHODS. The addition of Drosophila RNA to the reaction mixture routinely produced a five- to tenfold stimulation of the incorporation of ^{3}H-amino acids into protein compared with endogenous levels. The products of the *in vitro* translation reactions were analyzed by immunoprecipitating αGPDH protein with anti-αGPDH serum in the presence of formalin fixed, *S. aureus* cells. The immunoprecipitates were then subjected to electrophoresis on polyacrylamide-SDS gels and the tritiated proteins detected by fluorography. Immunoprecipitation of the products of the *in vitro* translation of wild-type mRNA produces one major band. This band comigrates with pure αGPDH. There is no detectable protein at the αGPDH position following the translation of RNA from the *αGpdh*^{nJH231}, *αGpdh*^{nSP10} or *αGpdh*^{nSP12} mutants. In addition, there are no detectable αGPDH polypeptide fragments made in the *in vitro* translation of these mutants. These results suggested that, within the limits of detection of αGPDH protein by this method, these mutants do not produce any antigenic αGPDH protein.

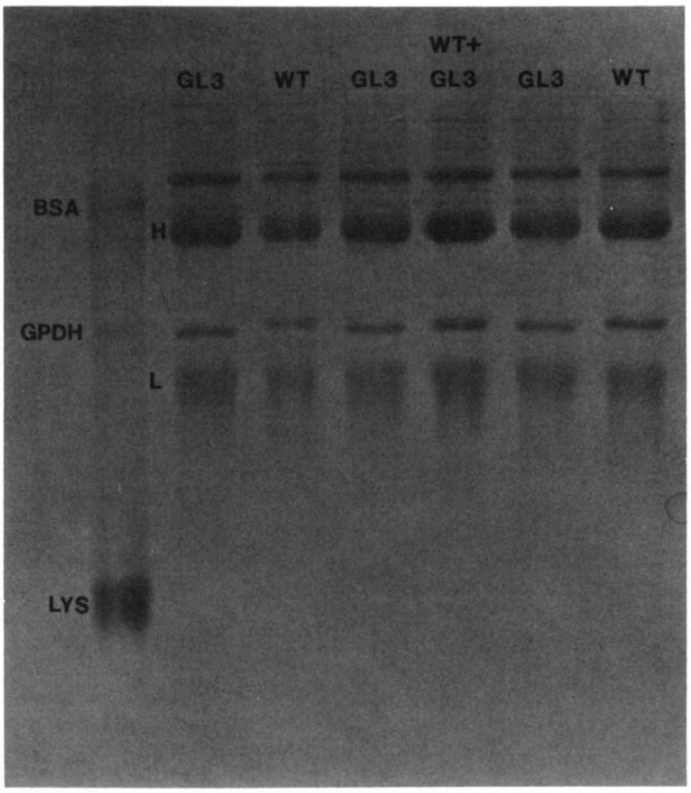

FIGURE 7.—Polyacrylamide-SDS gel electrophoresis of immunoprecipitated proteins from wild-type and $\alpha Gpdh^{nGL3}$ mutant. GL3 = immunoprecipitable proteins from homogenates of $\alpha Gpdh^{nGL3}/Df(2L)cl\text{-}7$ heterozygotes; WT = immunoprecipitable proteins from homogenates of $\alpha Gpdh^{B}/Df(2L)cl\text{-}7$ heterozygotes, WT + GL3 = mixture of immunoprecipitable proteins from homogenates of $\alpha Gpdh^{B}/Df(2L)cl\text{-}7$ and $\alpha Gpdh^{nGL3}/Df(2L)cl\text{-}7$ heterozygotes. H = goat immunoglobulin heavy chain; L = goat immunoglobulin light chain, BSA = bovine serum albumin (M_r = 63,800), αGPDH = purified αGPDH, LYS = lysozyme (M_r = 13,930). The gel was stained for protein with Coomassie brilliant blue-R.

We should point out that the direct analysis of the *in vitro* translation products of these three mutants *and wild type* by the ^{125}I-protein A method results in no detectable αGPDH protein. Apparently, this method of detection is not sensitive enough to detect the small amounts of αGPDH translated from the *in vitro* system without prior immunoprecipitation to concentrate the αGPDH protein.

The $\alpha Gpdh^{nGL3}$ mutant was also analyzed in the *in vitro* translation system. RNA extracted from $\alpha Gpdh^{nGL3}/Df(2L)cl\text{-}7$ heterozygotes and $\alpha Gpdh^{B}/Df(2L)cl\text{-}7$ homozygotes was translated *in vitro*, and the products were immunoprecipitated and subjected to electrophoresis on gels, as described before. As Figure 8 illustrates, the αGPDH synthesized by $\alpha Gpdh^{nGL3}$ RNA *in vitro* migrates to a more anodal position on the gel, indicating that it is slightly smaller than wild-type αGPDH. This suggests that a posttranslational modification or proteolysis of the protein *in vivo* is probably not the cause of the apparently shorter polypeptide in this mutant.

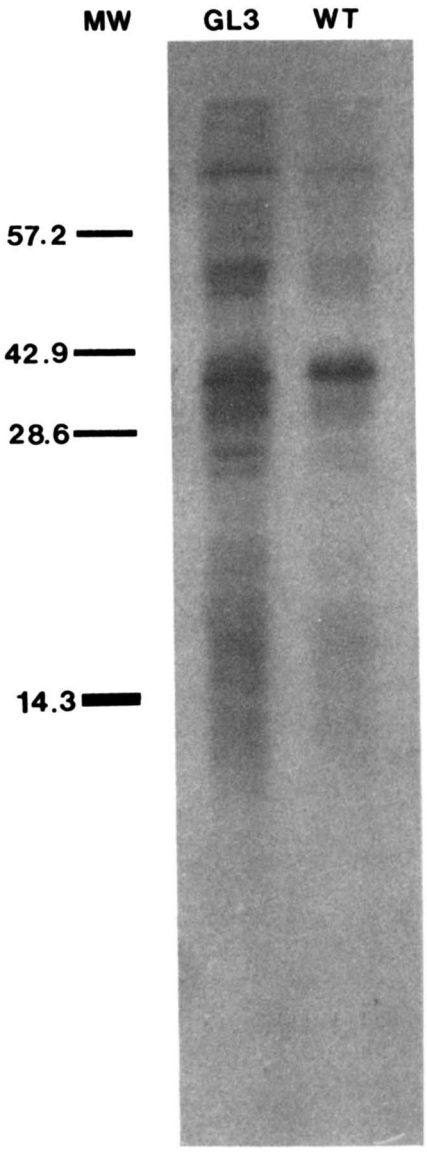

FIGURE 8.—Results of the immunoprecipitation of proteins from *in vitro* translation reactions using RNA extracted from $\alpha Gpdh^{nGL3}/Df(2L)cl\text{-}7$ (GL3) and $\alpha Gpdh^{B}/Df(2L)cl\text{-}7$ (WT) heterozygotes. ^{3}H-labeled proteins were visualized by fluorography. αGPDH is the most intensely labeled protein between the molecular weight markers of 28.6 and 42.9 kilodaltons. MW = schematic of positions of the molecular weight markers (molecular weights in kilodaltons).

DISCUSSION

The α-glycerophosphate cycle performs three functions in Dipteran insects: (1) the maintenance of the NAD-NADH balance in the cytosol (SACKTOR and DICK 1962), (2) the production of ATP in the flight muscle sarcosomes (SACKTOR and DICK 1962), and (3) the production of α-glycerophosphate as a sub-

strate for lipid biosynthesis (KENNEDY 1957). Since the operation of the α-glycerophosphate cycle is dependent upon an enzymatically active αGPDH, the decrease in viability concordant with the lower levels of enzymatically active αGPDH in the mutants of this study is not unexpected. It was demonstrated by O'BRIEN and MACINTYRE (1972b), using the CRM$^+$ mutants *αGpdh*$^{n1\text{-}4}$, *αGpdh*$^{n5\text{-}4}$ and *αGpdh*^{n0}, that the absence of αGPDH enzyme activity is not lethal. These observations are supported by the data presented here. Furthermore, the lack of detectable amounts of αGPDH protein in three CRM$^-$ mutants (*αGpdh*^{nJH321}, *αGpdh*^{nSP10}, *αGpdh*^{nSP12}) suggests that the absence of the αGPDH protein is itself not a lethal condition. The viability of these CRM$^-$ mutants is approximately the same as the viability of some of the CRM$^+$ null mutants, indicating that a lack of αGPDH protein is no more detrimental to the organism than is the lack of enzymatic activity.

It was pointed out (see also Figure 1) that the *αGpdh*^{nS3} and *αGpdh*^{nS8} mutants are able to fly and have a high viability, despite the fact that they have no detectable αGPDH activity. It is possible that the activity of the enzyme in these mutants is unstable *in vitro*, and, in this regard, both *αGpdh*^{nS3} and *αGpdh*^{nS8} are CRM$^+$. On the other hand their behavior can also be explained by the action of a suppressor of the αGPDH null phenotype. Such a suppression has been observed by O'BRIEN and SHIMADA (1974). When flies that are hemizygous or homozygous for some *αGpdh* null mutants are kept in culture for ten generations or more, adults regain the ability to fly normally and lose a premature aging syndrome. These adapted flies, however, still have no detectable αGPDH activity. Since the suppression appears to be dominant, it has been termed "S(G)" to denote the suppression of *αGpdh* (O'BRIEN and SHIMADA 1974). To date, the exact physiological mechanism of the suppression of the *αGpdh* null phenotype has not been elucidated, but it appears to have a simple genetic basis, at least in the case of one particular suppressed null, *αGpdh*$^{n1\text{-}4}$. A gene responsible for suppression has been mapped to a position on the second chromosome approximately 10 cM from the *αGpdh* locus (COLLIER 1979). In light of the observations stated above, it is possible that a mutation at the "suppressor locus" has been produced or at least recovered concurrently with the production of the *αGpdh*^{nS3} and *αGpdh*^{nS8} mutants. This would account for the high viability and the flight ability of these *αGpdh* null mutants.

Five criteria have been used in the past to identify putative nonsense mutations in Drosophila (MACINTYRE and O'BRIEN 1976). With regard to the mutant gene product, these criteria are (1) a lack of enzymatic activity, (2) an inability to participate in the formation of a multimer with a normal subunit, (3) an absence of CRM, (4) a polypeptide of lower molecular weight than the wild type on SDS gels, and (5) a lack of interallelic complementation in heterozygotes with other mutant alleles. We have not addressed the criterion of interallelic complementation with the αGPDH mutants in this study, but the remaining four criteria have been explored in detail. The CRM$^-$ mutants, *αGpdh*^{nJH231}, *αGpdh*^{nSP10} and *αGpdh*^{nSP12}, lack enzymatic activity, do not form hybrid enzymes and produce no detectable αGPDH protein. Thus, they fit most of the criteria for classification as putative nonsense mutations. It is clear, however, that the lesions in one or all of these three mutants may involve a

defect in transcription rather than translations, *i.e.*, they may be mRNA$^-$ mutants. In addition, in our sample of 39, mutant *nGL3* is perhaps the best candidate for a nonsense mutation, yet it fails three of these criteria. The mutant *nGL3* is CRM$^+$, produces a αGPDH polypeptide that dimerizes readily with wild-type subunits, and $\alpha Gpdh^{nGL3}/Df$ heterozygotes exhibit 22% of the wild-type level of αGPDH enzymatic activity. In fact, the only criterion for a nonsense mutation listed here that *nGL3* fits is the production of a smaller molecular weight protein. Nonsense mutants with some of these properties have been reported previously. BIGELIS and BURRIDGE (1978) demonstrated that three yeast *his-4* nonsense mutants used in their study are CRM$^+$ by the ^{125}I-protein A assay. In fact, one of these mutants is immunoprecipitated in large enough quantities so that it can be detected on polyacrylamide-SDS gels stained with Coomassie blue. This particular mutant produces a protein fragment of molecular weight 45,000 daltons (the molecular weight of the wild-type *his-4* protein product is 95,000). The other two CRM$^+$ nonsense mutants in the study produced polypeptides of 84,000–88,000 daltons. Thus, it may well be that a nonsense mutation near the 5′ end of the αGPDH gene may conform to the criteria of nonsense mutations listed here, but there is no *a priori* reason to believe that a nonsense mutation positioned near the 3′ end of the gene should do so. Clearly, the characterization of mutants of Drosophila is badly hampered by the lack of identified nonsense suppressors.

We have not attempted in this study to correlate the amount of αGPDH activity in the 36 CRM$^+$ mutants with their levels of CRM. Since the level of detectable αGPDH CRM is dependent upon both the steady state amounts of the αGPDH protein in the mutant flies and the degree of complexing between the antibody and the mutant polypeptide, statements concerning the absolute amount of αGPDH protein produced by a particular mutant would not be valid. Indeed, some of the CRM$^+$ mutants could be regulatory mutants that cause the underproduction of αGPDH. On the other hand, the 17 mutants that produce αGPDH CRM and have altered electrophoretic mobilities in the hybrid enzyme tests are most likely due to mutations within the αGPDH structural gene.

Twenty-nine of the $\alpha Gpdh^n$ mutations characterized in this study were induced in chromosomes containing recessive visible and lethal mutations that are closely linked to the *αGpdh* locus. Twenty of these mutations are on chromosomes carrying genetic markers that map just to the right of the *αGpdh* locus [*l(2)gdh-2* and spd^{fg}], whereas nine of the $\alpha Gpdh^n$ mutants were made on chromosomes that carry markers that are immediately to the left of αGpdh [*cl* and *l(2)gdh-1*] (KOTARSKI, PICKERT and MACINTYRE 1983). Flies carrying these particular chromosomes were mutagenized so that the induced null and low αGPDH activity mutants can be used immediately in a fine structure genetic analysis of the *αGpdh* locus. By constructing these marked chromosomes prior to the mutagenesis, the laborious task of constucting chromosomes bearing the $\alpha Gpdh^n$ mutations and the flanking genetic markers from independent stocks is avoided. The fine structure analysis of the *αGpdh* locus is presently underway in our laboratory and should provide valuable information concerning the

position within the *αGpdh* locus of many of the mutations described in this paper.

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