specific molecule was detected by electrophoresis and immunoprecipitation. The cell lines produced either the myeloma immunoglobulin (in the case of M_{x1}) or nothing (all the other hybrids were obtained with a nonsecreting line).

The hybrid cells were injected repeatedly into BALB/c mice to raise antibodies against frog antigens that could be expressed at the surface of the hybrid cell. In no case did the injection of 2.5×10^6 to 5×10^6 hybrid cells (all lines) cause death; an injection of myeloma cells is fatal within 3 weeks. All the hybrids except for M_{x2} induced the production of antibodies. M_{x2}, after multiple injections over 2 months, finally provoked a slow-growing tumor that killed the mice 1 month later. It is possible that during growth the tumor retained only the *Xenopus* HGPRT and lost all genes expressing *Xenopus* surface antigens.

Antibodies to hybrid M_{x1} have been characterized in some detail. Indirect immunofluorescence showed that, at a final dilution of 1:60, serum samples from mice injected with M_{x1} reacted with antigens on *Xenopus* red cells and leukocytes. These serums did not, at a final dilution of 1:2, react with any antigens on mouse tissue, nor did the supernatant of P3-x63Ag8 myeloma cells. Absorption with red cells eliminated the activity of all but one of the antiserums, which retained some antileukocyte activity. The mouse that produced this leukocyte-specific serum was killed for fusion to produce monoclonal antibodies. From this fusion 11 hybridomas were selected, some of which reacted with both *Xenopus* frog red cells and leukocytes, others only with leukocytes, suggesting that at least two *Xenopus* antigens were expressed against M_{x1}. Xen 10, one of the hybridomas that reacted only with leukocytes, was used to precipitate a frog antigen from the membranes of M_{x1} and *Xenopus* spleen cells. The precipitate, analyzed by electrophoresis, revealed a single band in the region of 85,000 daltons (Fig. 2). *Xenopus* cells yielded a large amount of this material, whereas the M_{x1} hybrid cells yielded only a very narrow band in the corresponding molecular weight region. This may indicate differential expression of the membrane product under natural and hybrid conditions. The red cell extract showed no band, and no material beyond background amounts was precipitated from labeled myeloma cells.

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Purine Resistant Mutants of *Drosophila* Are Adenine Phosphoribosyltransferase Deficient

Abstract. *A deficiency for adenine phosphoribosyltransferase activity is the primary biochemical defect in mutants of* Drosophila *selected for resistance to purine-induced lethality.*

Although many techniques have been used for the direct selection of specific gene mutations in prokaryotes and eukaryotic cells in culture (*1*), only rarely has this been possible in intact higher animals. The absence of selective systems for the recovery of specific gene alterations in whole, developmentally complex organisms has been an obstacle to the study of gene organization and the associated biochemical mechanisms that give rise to the differentiated state. The single exception is the alcohol dehydrogenase (*Adh*) locus of *Drosophila*, where mutations resulting in the absence of alcohol dehydrogenase (ADH) activity rescue flies from an otherwise lethal exposure to certain secondary alcohols (*2*). A large number of point mutations and small deletions in the *Adh* genetic element were rapidly isolated through this specific selection procedure (*3*). In an effort to reveal other genetic loci in whole complex organisms at which mu-

Table 1. Adenine phosphoribosyltransferase (APRT) activity in *Drosophila melanogaster*. Heterozygotes were constructed reciprocally between the purine-resistant mutant, *aprt*^{1}, and the wild type (+) *Ore-R*. Since there was no significant difference in APRT activity between the adult progeny of reciprocal matings, the data for the heterozygote were combined. APRT activity is expressed as the mean number of disintegrations per minute of [8-^{14}C]AMP per minute per microgram of protein. APRT activity was measured at 25°C (*p*H 7.5) by a modification of the procedure of Merril (*13*). Assay mixtures contained: 60 m*M* tris-HCl, 0.1 m*M* Na-EDTA, 26.0 m*M* $MgCl_2 \cdot 7H_2O$, 2.0 m*M* dithiothreitol, 6.25 m*M* Na-PRPP, 7.6×10^{-5} *M* [8-^{14}C]adenine (62 mCi/mmole), and 10 μl of *Drosophila* homogenate in a total volume of 35 μl. All chemicals were purchased from Sigma except [8-^{14}C]adenine, which was obtained from Amersham. Homogenates of *Drosophila* were prepared by extraction of ten newly eclosed adult flies (5♀ and 5♂) in 100 μl of deionized H_2O in a Kontes Duall all-glass tissue grinder at 4°C, or single flies were homogenized in 20 μl of deionized water and assayed as described above. Separate 5 μl portions of reaction mixture were removed at 5, 10, and 15 minutes after initiation of the reaction and placed on thin-layer chromatography (PEI-TLC) plates (20 by 20 cm; Brinkmann) at points previously overlaid with 5 μl of unlabeled 5′ AMP and adenosine (2 mg/ml). The AMP and adenosine markers were located using a shortwave ultraviolet mineral light after development of the TLC plate in 0.1*M* LiCl. The TLC chips corresponding to AMP and adenosine were dried; the radioactivity was determined by liquid scintillation counting. Radioactivity associated with the adenosine marker represented less than 1 percent of that in AMP, and therefore AMP nucleotidase (E.C. 3.1.3.5) activity was ignored in the determination of APRT activity. Protein was measured by the method of Lowry (*14*). The synthesis of [8-^{14}C]AMP from [8-^{14}C]adenine and PRPP by extracts of *Drosophila* is a linear function of time and protein concentration. S.D., standard deviation.

Genotype	APRT activity	S.D.	*N*	Wild-type APRT activity (%)
+/+	97.3	7.0	8	100
aprt^1/*aprt*^{1}	1.8	0.2	7	2
aprt^2/*InP18 kar ry*^{41} *Ubx e*^{4}	13.4	1.4	3	14
aprt^1/ +	60.2	4.9	5	62
aprt^1/*aprt*^{2}	5.5	1.3	4	6
aprt^1 *ry*^{2}/*aprt*^{1} *ry*^{2}	1.8	0.6	7	2
ry^2/*ry*^{2}	40.5	3.7	7	42

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tations might be directly selected, we have investigated the mechanism of purine-induced lethality in the wild type and the resistance to purine-mediated lethality in two mutants of *D. melanogaster*. Our results show that the absence of adenine phosphoribosyltransferase (APRT; E.C. 2.4.2.7) activity in the mutants confers resistance to purine toxicity.

Glassman (*4*) observed that xanthine dehydrogenase–deficient *Drosophila* (*5*) resulting from defects either at the autosomal rosy (*ry*) locus or the sex-linked maroon-like (*ma-l*) locus were killed by 7*H*-purine [Sigma, 7*H*-imidazo(4,5-*d*)pyrimidine] at a concentration to which the wild type is resistant. In separate experiments with purine-supplemented culture media, two mutants showing unexplained exceptional resistance to purine were isolated. Gelbart and Chovnick (*6*) mutagenized wild type, *Ore-R*, with ethyl methanesulfonate and selected for survival on a concentration of purine sufficient to kill the wild type. Duck (*7*) mutagenized *ma-l Drosophila* and recovered a single purine-resistant fly, which remained deficient for xanthine dehydrogenase activity and exhibited a greater resistance to purine killing than the wild type. We undertook to identify the primary biochemical defect which renders these two mutants resistant to purine.

Our investigation focused initially on the enzymes of the purine salvage pathway since variants of cultured animal cells capable of growing in the presence of purine-base analogs such as diaminopurine and 8-azaguanine are typically deficient for adenine phosphoribosyltransferase and hypoxanthine-guanine phosphoribosyltransferase (HGPRT), respectively (*8*). We hypothesized that purine-induced lethality in wild-type *Drosophila* might result from the anabolic metabolism of purine through a salvage pathway leading to the synthesis of a toxic nucleotide, as has been suggested for 6-mercaptopurine in mammalian somatic cells (*9*). If this were true, we would expect to find alterations or deficiencies of one of the enzymes of the purine salvage pathway in purine-resistant mutants of *Drosophila*. We have determined that the purine-resistant mutants of *Drosophila*, which we designate $aprt^1$ and $aprt^2$, are deficient in APRT activity (Table 1); APRT catalyzes the synthesis of adenosine monophosphate (AMP) from adenine and 5-phosphorylribose-1-pyrophosphate (PRPP).

Flies deficient for APRT activity ($aprt^1$ homozygotes) have, throughout the life cycle, no more than 2 percent of the wild-type APRT activity. A high level of APRT activity is therefore not essential for fertility or viability. In the wild type, APRT activity varies only slightly during all stages of development (*10*).

Table 2. Sensitivity of *Drosophila melanogaster* to killing by purine. Twenty-five first instar larvae were added to 10 ml of food in a shell vial (22 by 94 mm) containing various concentrations of 7*H*-purine. Food contained 2200 ml of water, 30 g of carrageenan, 206 ml of unsulfured molasses, 275 g of corn meal, 23.5 g of brewer's yeast, and 16.5 ml of propionic acid. There were eight replicate vials for each of five different purine concentrations for each genotype. Data are means of three independent determinations. LD_{50} is the concentration of purine that allows 50 percent of the larvae to survive to adults. LD_{100} is the minimum concentration of purine that kills all *Drosophila* either as larvae or pupae.

Genotype	Purine (m*M*)	
	LD_{50}	LD_{100}
+/+	0.88	2.1
$aprt^1/aprt^1$	> 13.2	
$aprt^1/+$	1.4	4.2
$aprt^1/aprt^2$	> 13.2	
$aprt^1\ ry^2/aprt^1\ ry^2$	6.6	13.7
ry^2/ry^2	0.08	0.29

The degree of purine resistance is inversely related to the quantity of APRT activity. For instance, the APRT activity of heterozygotes ($aprt^1/+$) is intermediate between that of parental strains (Table 1); these heterozygotes are less resistant to purine killing than the $aprt^1$ homozygote but are more resistant than the wild type (Table 2).

In the *trans* heterozygote, $aprt^1/aprt^2$, the APRT activity is only 6 percent that of the wild type, and thus these two APRT-deficient mutations fail to complement (Table 1). The simplest explanation for the failure to complement is that these two mutations are alleles. Mapping data (*10*) indicate that both mutations, $aprt^1$ and $aprt^2$, which render *Drosophila* resistant to purine and deficient for APRT activity, can be assigned to the third chromosome. The third chromosome carrying $aprt^2$ appears to contain a recessive lethal and, therefore, an $aprt^2$ homozygote has not yet been obtained.

A deficiency for the majority of APRT activity permits *Drosophila* as well as cultured human somatic cells (*11*) to survive high concentrations of purine. It is possible that purine, a competitive inhibitor of mammalian APRT (*12*), is an alternative substrate for APRT and that the product of this reaction is the lethal agent. The simultaneous absence of APRT activity and xanthine dehydrogenase activity in the double mutant $aprt^1\ ry^2$ of *Drosophila* results in approximately an eightfold increase in resistance to purine killing as compared to the wild type (Table 2), whereas a deficiency for xanthine dehydrogenase alone renders *Drosophila* 11-fold more sensitive to purine killing than the wild type. Our data show that the enhanced sensitivity of *ry* to purine is not due to higher than wild-type levels of APRT activity (Table 1). An alternative explanation proposed by Glassman (*4*) for the hypersensitivity of xanthine dehydrogenase–deficient mutants is that purine is also a substrate for xanthine dehydrogenase and the product of this reaction has reduced toxicity.

There is no direct evidence that the *aprt* locus in *Drosophila* is the structural gene for the enzyme APRT. Two mutations independently isolated by selection on purine medium resulted in a loss of APRT activity and increased resistance to purine killing. This suggests that selection of additional purine-resistant *Drosophila* should uncover both structural gene mutations and mutations in regulatory loci necessary for expression of APRT activity.

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