doreduplication, and with the hypothesis that a complete second round of DNA synthesis has occurred in one mitotic cycle of these cells.

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Glucose-6-Phosphate Dehydrogenase in Drosophila: X-Linked Electrophoretic Variants

Abstract. *Three electrophoretic patterns of glucose-6-phosphate dehydrogenase have been found in wild-type strains of* Drosophila melanogaster. *Genetical control of the variants in enzyme mobility (fast or slow) is X-linked; strains possessing both bands (fast and slow) appear to be heterogeneous, having individuals of three types (fast, slow, and double).*

Although an enormous number and variety of mutant phenotypes is known in *Drosophila melanogaster*, there are in this organism only six well-characterized gene-enzyme systems available for analyzing the genetical control of protein structure (*1*). In man, on the other hand, where genetic analysis is much more tedious, there is a broad range of biochemical properties whose variants are under genetical control. The absence of glucose-6-phosphate dehydrogenase in the erythrocytes of primaquine-sensitive human beings is inherited as an X-linked trait (*2*). The qualitative variants of this enzyme, discovered by starch-gel electrophoresis, also segregate with the X chromosome (*3*). It seemed reasonable to suppose that qualitative polymorphism is present and identifiable in *Drosophila*, as well as in human, populations.

A search for such a polymorphism was undertaken by examination of homogenates of 50 to 100 flies, and of single flies of various wild-type strains, by vertical starch-gel electrophoresis. The procedure was modified from those previously reported (*3*).

The flies were ground in 0.5 ml of NADP (*4*) (2mg/ml), and the homogenates were cleared by centrifugation at 0°C. Samples of the supernatants were loaded into ten-slot vertical gels (*5*) and 5 to 6 volts/cm were applied for 12 to 16 hours at 4°C. The gel (Connaught starch, hydrolyzed) was prepared in 0.05*M* tris HCl (*6*) at *p*H 8.6, and the bridge solution was 0.05*M* tris at the same *p*H. Five milliliters of 0.27*M* EDTA and 4 mg of NADP were added to the starch before pouring. Sliced gels were developed in the dark at 25°C for 2 to 12 hours in a solution containing 80 ml of 0.5*M* tris HCl, *p*H 8.6; 10 ml of 0.01*M* $MgCl_2$; 50 mg glucose-6-phosphate; 10 to 20 mg NBT (*7*); 8 mg phenazine methosulfate, and 10 mg NADP. Single flies were ground in microhomogenizers (*8*) and the uncentrifuged homogenates were loaded directly onto the gel.

Three different electrophoretic patterns of the glucose-6-phosphate dehydrogenase occur among various wild-type strains of *D. melanogaster*. We found, using mass homogenates, that of the 19 wild-type stocks from various localities, five show only a single slow-moving band; twelve have only a single fast-moving band; and two strains present both fast and slow bands. The appearance of these bands is illustrated in Fig. 1.

Although the photograph is of a gel in which there were only homogenates of single flies, the patterns are typical of the multiple-fly homogenates. Numbers 2, 3, and 6, are bands of fast-moving dehydrogenase, 4 of slow, and 1, 5, and 7, of double. In the homogenates of flies illustrated here, the leading component of the double band is more intensely stained; in other strains, the slower component stains more brilliantly.

Table 1. Distribution of glucose-6-phosphate dehydrogenase phenotypes in offspring in *D. melanogaster* as a result of "fast × slow" (electrophoretic mobility of dehydrogenase) mating. The numbers in parentheses indicate numbers of individuals examined.

Parents		Offspring	
Male	Female	Male	Female
Fast	Slow	Slow (13)	Double (13)
Slow	Fast	Fast (11)	Double (12)

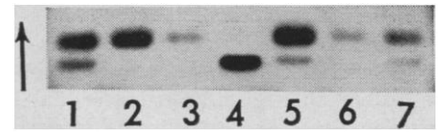

Fig. 1. Glucose-6-phosphate dehydrogenase phenotypes of single flies from mating of slow male (No. 4) by fast female (No. 3). The F_1 males are Nos. 2 and 6; F_1 females are Nos. 1, 5, and 7. The arrow indicates direction of migration.

These observations clearly establish polymorphism for this dehydrogenase in *Drosophila* populations. Since the strains containing the fast and slow enzyme variants each appear to be phenotypically constant, and the "doubles" are apparently heterogeneous, no information on the mode of inheritance is provided, except the inference of a pair of codominant alleles. Examination of single flies from one strain (Woodbury, New Jersey) suggests X linkage as a probable mode of inheritance, since we have observed three phenotypes in females (10 slow, 11 double, 48 fast), and only two phenotypes in males (2 slow, 25 fast).

Two additional lines of evidence, however, establish the X-linked control of this polymorphism:

1) Various combinations of single pair matings were made. In matings of flies containing slow bands, all progeny had slow bands; these matings are uninformative. However, the fast × slow matings provided critical information on the mode of inheritance. The phenotypes of the parents and offspring of such pair matings are given in Table 1 and are illustrated in Fig. 1. The results of the reciprocal matings are entirely consistent with transmission by way of the X chromosome. That is, in both cases all F_1 males have the maternal phenotype, and the F_1 females, whose X chromosomes come from both parents, are invariably double-banded.

2) Single flies from two stocks of attached-X females were examined. In as much as the males in these stocks are patroclinous, then if the character in question is X-linked all males from a given stock should be identical. If the phenotype of the females happens to differ, then the two phenotypes will segregate strictly according to sex. Seventy-four individual flies were examined; all of the 38 males were classed as *slow*; all of the 36 females were classed as *fast*. The only chromosome possessed by all the males but by none

of the females is the X (since the Y chromosome here is passed from father to daughter). We do not yet have information on the map location of the responsible genetic unit.

The length of the human X chromosome is, at metaphase, about 0.06 of the total haploid set (*9*); it is the only chromosome on which there are markers (*10*). Any marker would be expected to have, on the basis of chromosome length alone, about one chance in 16 of being found on the X chromosome. Actually, of course, the recovery is much higher, because of the ease of analysis of X-linked traits. In *D. melanogaster*, the X chromosome represents about 0.19 of the total haploid complement, and hence there is about one chance in five of finding that a given marker is X-linked. The locus controlling the electrophoretic mobility of this enzyme in both man and *Drosophila* would be expected to be found on the sex-associated chromosome about once in 80 samples, by chance alone. Since this particular sample contains such disparate organisms, we wonder whether the association is purely coincidental, or whether selection pressure has maintained the relationship of this structural locus with the sex-determining apparatus for functional reasons which are not evident to us at this time.

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Heat Adaptation and Ion Exchange in Bacillus megaterium Spores

Abstract. *A new spore phenomenon is described in which induction of increased heat resistance occurs during the process of heating. In buffered environments the acid form of dormant mature bacterial spores shows a temperature dependent adaptation to heat which is correlated with the rate of uptake of $Ca(OH)_2$ by titration at constant* p*H in neutral salt solution.*

In a recent investigation (*1*) relating base exchange and heat resistance in bacterial spores, heat-survival curves similar to curve *A*, Fig. 1 were obtained for the acid form of *Bacillus megaterium* spores when heated in calcium buffer solutions. Such curves frequently have been interpreted to indicate innate heterogeneity in the population with respect to heat resistance. An alternate explanation of such decreasing death-rate curves would be the hypothesis that heating slowly evokes a protective response in the spore. This view, that greatly increased heat resistance gradually developed during the heating, is supported by our data. By manipulation of the duration and severity of heating, the spores could be protected substantially against subsequent heat treatments which, otherwise, would have been quickly lethal. Rates of the later stages of calcium hydroxide consumption at constant *p*H by suspensions of the spores in neutral salt solutions were correlated with heat adaptation.

The *B. megaterium* spores, from the preparation previously described (*1*), were converted to the hydrogen form (stripped) at 25°C by holding them at *p*H 4 with nitric acid for 4 to 5 hours in a *p*H-stat. These stripped spores were washed with water and dried from the frozen state. Heating for the resistance tests was carried out in sealed 7-mm glass tubes containing 0.1 to 1.0 ml of spore suspension (2.5 mg of spores/ml). Survivors were measured as previously described (*1*). According to common practice, heat death was defined as failure to grow to visible colonies under these conditions.

Curve *A* of Fig. 1 with decelerating death rate was obtained when a freshly made suspension of stripped spores in 0.02*M* calcium acetate buffer at *p*H 5.7 was subjected to lethal temperatures in the usual way, that is with almost instant equilibration to the heating temperature. That the decelerating death rate was due to an adaptive reaction was indicated by a curve of a type like *B*, which shows high resistance after either of the following treatments of similar spore suspensions: (i) bringing the spore suspension slowly up to the lethal temperature over a 2- to 3-hour period, or (ii) holding the spore suspension at a moderately elevated, but sublethal, temperature such as 50°C overnight. Either pretreatment changed the initial slope of the curve of the log of the number of survivors plotted against time more than 30-fold.

The rate of this adaptation to heat was followed at several sublethal temperatures in calcium acetate buffer (0.02*M* Ca) at *p*H 5.7 and in calcium diethyl barbiturate (0.012*M* Ca) at *p*H 7.9. For assay the spores were brought to 83°C instantly and the number surviving for 10 minutes was used as a measure of the reaction's progress. This number would have been influenced somewhat by any adaptation occurring during the 10-minute test at 83°C. In Fig. 2 the log of survivors per gram of spores is plotted against time. In addition to the moderate p*H* effect the results showed a marked temperature dependence. Days were required at moderately low temperatures such as 18°C to accomplish the protection afforded by 1 or 2 hours at 50° to 65°C. The rate data of Fig. 2 roughly approxi-

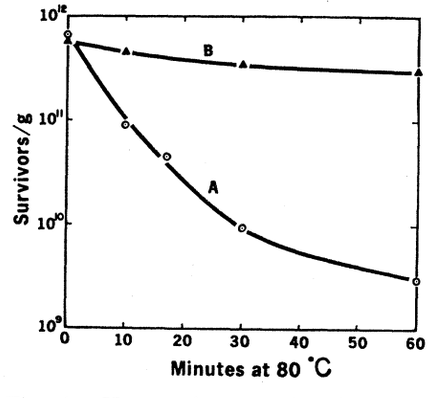

Fig. 1. Heat resistance of stripped *B. megaterium* spores in *p*H 5.7 calcium acetate buffer (0.02*M* Ca). Curve *A*, tubes of freshly made suspension plunged directly into 80°C water bath. Curve *B*, spore suspension conditioned prior to lethal heating (80°C) by either (i) slow ascent (2 to 3 hr) to lethal temperature, or (ii) holding at 50°C overnight.