Anderson, S., M. Santos*, and J. McDonald. Iowa State University, Ames. Comparative study of the thermostability of crude and purified preparations of alcohol dehydogenase (EC 1.1.1.1) from D. melanogaster.†

Thermostability studies are often employed as a means of uncovering enzyme variation, which may go undetected with routine electrophretic techniques (e.g., Singh et al. 1975; Milkman 1976). In this note, we report a study to evaluate the comparability of the results of thermostability studies carried out on crude vs. purified preparations of Drosophila ADH. Enzymes from three electromorphically identical "fast" strains (F-1, F-2, F-3) and two electromorphically "slow" strains (S-1, S-2) were analyzed.

Crude extracts were prepared by homogenizing 30 mg of 5 ± 1 day old adults in 1.0 ml of Tris-HCl Buffer, pH 8.6. After centrifugation for 20 min at 28,000Xg, a constant amount of supernatant (200 µl) was placed into microtubes for use in the temperature stability studies. ADH was purified according to the techniques of McDonald et al. (1977) and prepared for thermostability studies as described.

Thermostability studies were carried out by placing the microtubes in a 45°C water bath. The temperature in the water bath was kept constant by means of a constant-temperature thermomix pump, model 1440. Preparations were exposed to this temperature for various periods (5-90 min) after which time they were immediately placed on ice until assayed for ADH activity (McDonald and Avise, 1976). The results are presented in Fig. 1. Crude extract ADH for all strains was extremely heat labile. No significant differences are detectable between the strains (Fig. 1a). In contrast, the thermostability of the purified preparations was generally increased. Significant variation both within and between the "fast" and "slow" forms of the enzyme are clearly evident (Fig. 1b).

Two conclusions can be drawn from this study. (1) Thermostability studies are most reliably carried out on pure enzyme. Spurious results may result from the analysis of crude preparations due to the presence and/or temperature activation of other factors affecting enzyme stability (e.g., proteases). (2) Degree of thermostability does not seem to be diagnostic of electrophoretic class; i.e., some slow strains are more heat stable than some fast strains and vice versa.

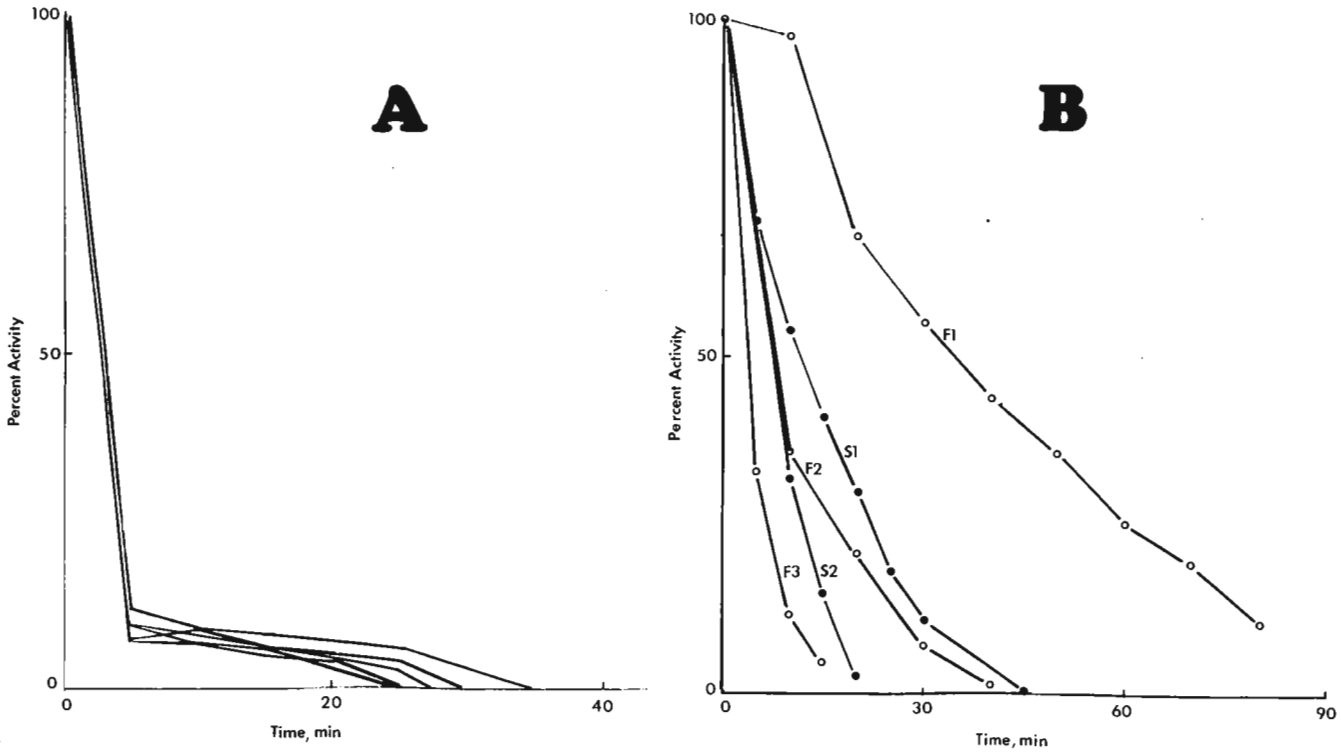

Fig. 1. Percent activity remaining after exposure of (a) crude and (b) purified ADH to 45°C for varying lengths of time in 3 Fast (0) and 2 Slow (●) strains of Drosophila melanogaster. See text for details.

References: McDonald, J.F. and J.C. Avise 1976, Biochem. Genet. 14:347-355; McDonald, J. F., G.K. Chambers, J. David and F.J. Ayala 1977, PNAS 74:4562-4566; Milkman, R. 1976, Biochem. Genet. 14:383-387; Singh, R.C., J.L. Hubby and L.H. Throckmorton 1975, Genetics 80:637-650.

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Toxicity to the dietary administration of hydrogen peroxide in acatalasemic Drosophila.

We have recently demonstrated that the dietary administration of the catalase inhibitor 3-amino-1,2,4-triazole (AT) provides a very sensitive and simple technique for the destruction of existing catalase molecules in vivo (Lubinsky and Bewley, 1979). Adult flies are starved for 24 hours on agar, and then fed on a 5 mM AT-sucrose solution for two hours, which results in a complete destruction of catalase activity with no apparent effect on viability. This technique has provided a mechanism for examining the toxicity of the substrate H_2O_2 in flies with normal catalase activity and flies made acatalesemic by the AT-method.

Adult flies with normal catalase levels appear to be relatively resistant to the dietary administration of the substrate H_2O_2 (Fig. 1). However, these same concentrations of H_2O_2 are extremely toxic to flies that have been made acatalesemic following the administration of 5 mM AT. In fact, as little as 0.05% H_2O_2 in the diet results in 100% mortality within five days of exposure while 0.1% results in 100% mortality within three days of exposure. The threshold for H_2O_2 tolerance in normal flies is apparently close to 1% H_2O_2 since this concentration will eliminate a population with normal catalase levels within three days of exposure. These results indicate that H_2O_2 can serve as a sensitive discriminator between CAT-positive and CAT-negative flies in a similar fashion that the substrate ethanol serves as a discriminator between ADH-negative and ADH-positive flies (Vigue and Sofer, 1976), and as such may prove useful as a positive selection agent in studies focusing on reversion, intracistronic recombination, conversion, and suppression at the Cat locus.

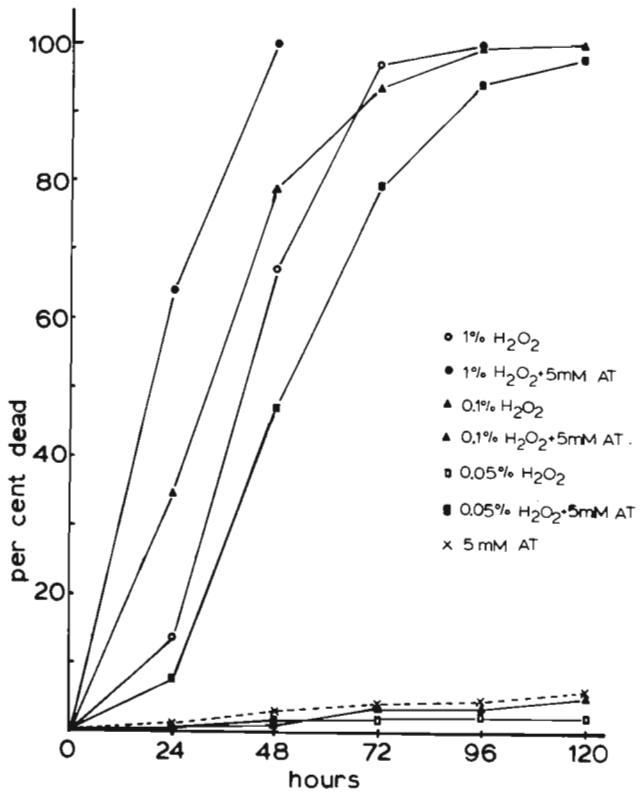

Fig. 1. The mortality rate of adult male Drosophila of an Oregon-R strain when fed on differing concentrations of H_2O_2 with or without 5 mM AT.

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References: Lubinsky, S. and G.C. Bewley 1979, Genetics 91:723-742; Vigue, C. and W. Sofer 1976, Biochem. Genet. 14:127-135.