Biochemical Genetics, Vol. 23, Nos. 1/2, 1985

Effects of Environmental Temperatures on Alcohol Dehydrogenase Activity Levels in *Drosophila melanogaster*

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Received 1 June 1984—Final 17 Aug. 1984

INTRODUCTION

In an effort to identify environmental factors which might influence alcohol dehydrogenase (ADH) activity in *Drosophila,* we have conducted a number of studies using variations in rearing temperature. Because the ADH allozymes that have been identified differ in their thermostabilities (Gibson, 1970; Thorig *et al.,* 1976; Milkman, 1976; Sampsell, 1977; Wilks *et al.,* 1980) and because the geographic distribution of the various *Adh* alleles has suggested that temperature might be a factor in maintaining the polymorphism (Vigue and Johnson, 1973; Oakeshott *et al.,* 1982), this attention to the effects of temperature on enzyme activity levels seemed appropriate. We were particularly interested to see if flies with the more heat-sensitive allozymes would display lower activity levels when reared at temperatures near the maximum at which they could breed. The results of the studies show exactly the opposite: regardless of their genotype and the relative lability of their enzyme, flies aged at 29°C have higher ADH activity levels than their counterparts aged at 20°C. The basis for the higher activity is probably the presence of more enzyme in the flies aged at the higher temperature.

This research was supported by NSF Grant DEB 79-11538 and NIH Grant RR-08043-10. Some equipment was purchased through NIH Training Grant T32-GM07091 to the University of Iowa.

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0006-2928/85/0200-0053$04.50/0 © 1985 Plenum Publishing Corporation

MATERIALS AND METHODS

Five strains of flies, each homozygous for a different *Adh* allele, were employed in this study. The strains were Sf1 (*Adh-Fr*), Ore (*Adh-Fm*), NCA23 (*Adh-Fs*), SSS (*Adh-Sm*), and GF31 (*Adh-Ss*). See Sampsell and Steward (1983) for further details on these strains. Flies were reared at 22°C until dark pupae were observed, then were placed in temperature-controlled incubators set at 20 or 29°C. Emerging adults were collected daily, placed into fresh food vials, and returned to the high- or low-temperature chambers to age. Aging flies were transferred to fresh food every few days.

ADH activity was measured using standard procedures (Sampsell and Sims, 1982) at 25°C and *p*H 8.6 using 170 mM ethanol and 2 mM NAD^+ as substrates in the reaction mixture.

A series of experiments designed to analyze the basis for the activity differences observed in flies of similar ages aged at the high and low temperatures was conducted on six groups of flies homozygous for *Adh-Fr* which were 9 days old. Crude extract from each group of flies was prepared by homogenizing 45 males in 1.5 ml 100 mM Tris buffer (*p*H 8.6). After centrifuging the extracts for 45 min at 12,000 *g*, the supernatant was tested for its ADH activity (using 25 mM isopropanol as the substrate) and for its protein content by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard and reading the optical density at 660 nm. Samples of each extract were also electrophoresed on acrylamide disc gels to separate the NAD-bound and unbound forms of ADH so that the relative quantities of each could be quantified by densitometry. Disc-gel electrophoresis followed the methods described by Cooper (1977) using 7% gels, with a stacking gel, and the upper reservoir buffer adjusted to a *p*H of 9.0. Afterward the gels were stained for ADH (Sampsell, 1977) for 2 hr and scanned with a densitometer. This period of staining gave intensities that were proportional to the quantity of enzyme extract applied. Quantification of ADH enzyme in the samples was performed by radial immunodiffusion (Sampsell and Steward, 1983). The immunodiffusion gel contained ADH antiserum prepared against ADH purified from strain 168 (homozygous for *Adh-Fm*).

RESULTS AND DISCUSSION

The ADH activities of flies of various strains, ages, and aging temperatures are plotted in Fig. 1. All five strains displayed a similar pattern: ADH activity increased with age, rapidly during the first 3–5 days and more gradually thereafter. Flies reared at 29 rather than 20°C displayed a more rapid increase in ADH activity and reached a higher activity level, which was not approached by flies aged at the lower temperature even after 10 days. An analysis of variance indicated that ADH activity was affected in a highly significant manner by strain (that is, *Adh* genotype), by age, and by aging

temperature ($P < 0.001$ for each variable). There were also significant two-way interactions among all three pairs of variables ($P \leq 0.01$).

The basis for the measured differences in ADH activity was investigated by looking at 9-day-old flies of the ADH-Fr strain which had been aged at 29 or 20°C. A number of variables which might relate to ADH activity levels were considered. The weight of flies was compared, since ADH activity is expressed as units per live weight. If flies aged at either the higher or the lower temperature were larger, this could alter the ADH activity determination. Similarly total protein in the crude extract was determined to see if aging temperature affected body composition. Since conversion of ADH molecules to their NAD-bound form has been shown to lower their catalytic capacity (Anderson and McDonald, 1981), the relative proportion of ADH enzyme in the bound and unbound forms was determined. Finally, the amount of ADH enzyme in the crude extracts was determined by radial immunodiffusion. The results of these tests are given in Table I. There was no significant difference between the flies reared at high and those reared at low temperatures in terms of body weight or total protein. The ADH activity of the flies aged at 29°C was greater than in flies that were aged at 20°C, although the difference was not quite significant at the 5% level ($t = -2.75$, $P = 0.06$). The quantity of ADH in the flies raised at 29°C was also higher ($t = -2.35$, $P = 0.08$).

There was a significant difference in the proportion of ADH dimer in the unbound (ADH-5) versus NAD-bound (ADH-3) form in the two groups of flies, however, the difference was not in the direction which could explain the difference in ADH activity. Rather, the flies aged at 29°C had *more* NAD-bound ADH molecules than those flies aged at 20°C. This should reduce the measured ADH activity level if flies from both temperatures had equivalent amounts of ADH. Thus it seems probable that flies aged at the higher temperature did have more ADH.

Although environmental temperature has been identified by a number of investigators as being a potential agent of natural selection, few experiments

Table I. Comparison of Males Homozygous for *Adh-Fr* Aged at High and Low Temperatures

Aging temperature	Group	Weight of 45 flies (mg)	ADH activity Δ00/min/mg	Protein μg/mg body wt	Relative amount of ADH	Proportion of ADH as FF-5
20°C	a	37.8	46.93	92.98	0.685	0.810
	b	39.0	44.18	94.06	0.698	0.805
	c	37.0	44.80	99.32	0.649	0.830
29°C	a	38.7	47.48	97.91	0.712	0.660
	b	38.7	52.71	94.10	0.862	0.695
	c	40.0	54.59	94.95	0.933	0.665
t		−1.66	−2.75	−0.09	−2.35	10.62
P		ns	0.06	ns	0.08	<0.001

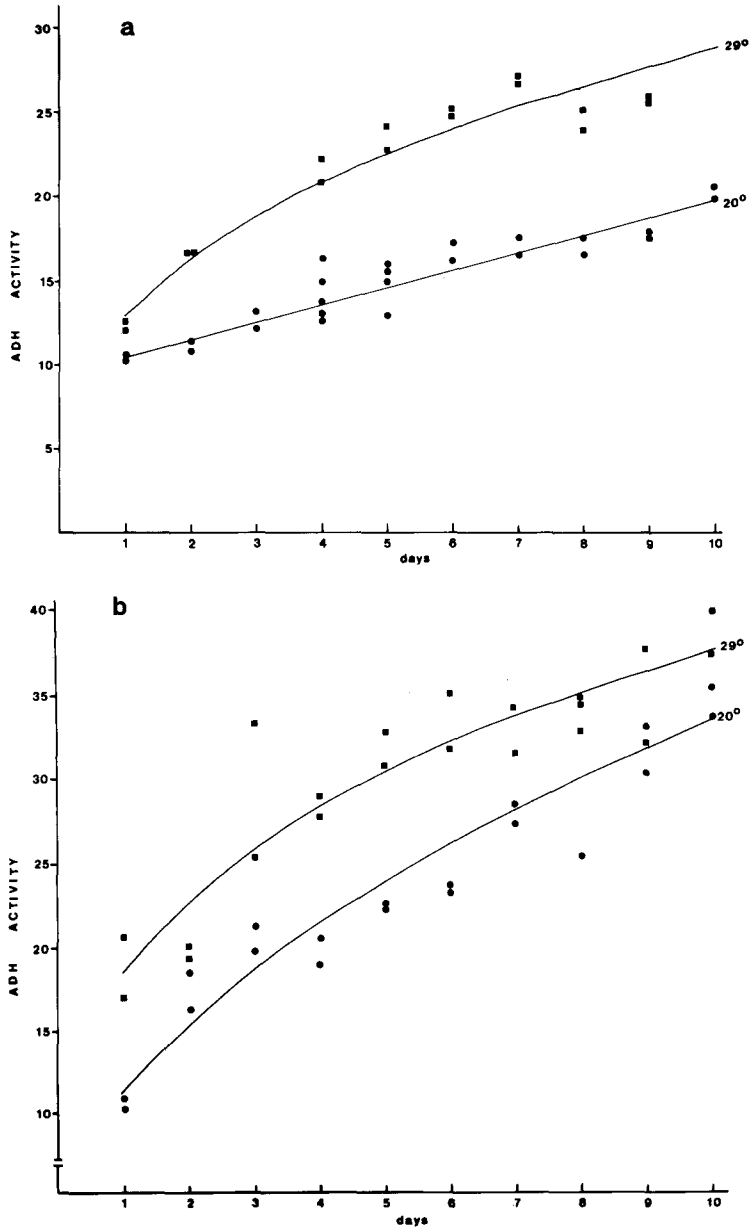

Fig. 1. Alcohol dehydrogenase activity in males homozygous for different *Adh* alleles aged at high and low temperatures. (a) *Adh-Fr*, (b) *Adh-Fm*, (c) *Adh-Fs*, (d) *Adh-Sm*, and (e) *Adh-Ss*. ADH activity is expressed as units per milligram of body weight, where a unit represents a change in absorbance at 340 nm of 0.001 per min. Each point is the activity in crude extract from a group of 30 flies.

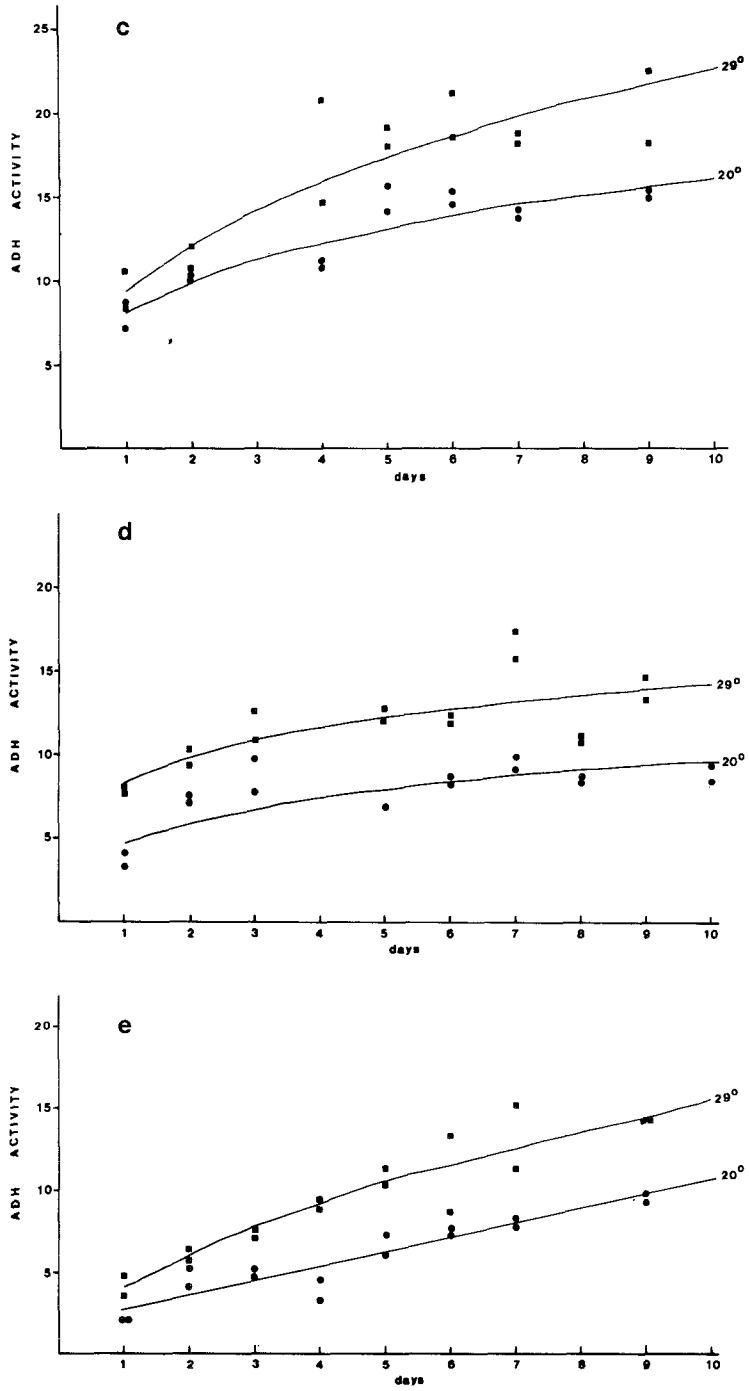

Fig. 1. Continued.

have focused on its direct or indirect role in maintaining the polymorphism at the *Adh* locus. The selective action of temperature has been of particular interest to us ever since the identification of an ADH allozyme with greater than normal thermostability as well as two forms that are unusually heat labile (Sampsell, 1977). Studies to test survival of flies with different *Adh* genotypes under different combination of heat and alcohol stress have been underway for some time. We have previously reported that when flies with ADH allozymes characterized as heat sensitive *in vitro* were subjected to a nonlethal heat treatment of 13 min at 40°C, they showed nearly a complete loss of alcohol tolerance due to the inactivation of their ADH enzyme (Sampsell and Sims, 1982). Since it was presumed that most adult flies would avoid such temperature extremes if possible, it was of interest to determine the effects of more moderate temperatures over a longer period of time. The temperature of 29°C was chosen for this study, since other work in progress involving population cages and short-term survival tests employed this temperature. Although flies can withstand temperatures above this for varying periods of time, physiological effects such as infertility have been observed, and we wished to use conditions that were less severe and in which flies would suffer the minimum stress.

As Fig. 1 clearly shows, flies aged at the higher temperature displayed higher ADH activity. The effect does not appear to be the result of larger body size or greater overall protein content. Since no other proteins were specifically assayed, it is not known if any other enzyme might show a similar increase. Additional studies in which the activities of enzymes are investigated could provide information on the mechanism by which the effect on ADH is achieved.

Recently McElfresh and McDonald (1984) have reported on the effects of aging temperature on several biochemical and molecular properties of alcohol dehydrogenase. They measured ADH activity levels, amount of cross-reacting material, and relative proportions of ADH-5 isozymes among four samples of flies homozygous for *Adh-Fm* or *Adh-Sm* raised at 20, 25, or 30°C. It should be noted that they compared flies of a single age and that while flies raised at 30°C had somewhat higher levels of ADH activity and CRM than those raised at 20°C, the differences were not significant. This suggests that the effect of temperature on ADH activity, while real, is small enough to be obscured by the variability among groups of flies and that only much larger samples or a longitudinal study, such as ours, can demonstrate it.

ACKNOWLEDGMENTS

We wish to thank Dr. John McDonald for the ADH antiserum and interesting discussions. Dr. Richard Beil advised us on protein determinations and

polyacrylamide gel electrophoresis. Able technical assistance was provided by Sharon Sims, Evelene Steward, and Carol Griffin.

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