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Alcohol dehydrogenase of *Drosophila melanogaster*: metabolic differences mediated through cryptic allozymes

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Acetone formation from propan-2-ol, a saturated secondary alcohol, has been analysed in flies of three different *Adh*-genotypes of *D. melanogaster*. The *in vivo* oxidation of propan-2-ol was mainly mediated through ADH activity. It could be demonstrated that flies homozygous for the Adh^{71k} allele produced more acetone than flies homozygous for Adh^{F}. This difference in metabolic flux mediated through the cryptic allozymes under non-saturated ADH-substrate conditions seems to be based on their different kinetic properties *in vivo*. Product inhibition of ADH monitored by means of ADH-isozymes conversion as observed after electrophoresis was similar for both cryptic allozymes.

ADH-71k and ADH-F showed immunological identity, and the *in vivo* protein levels of ADH-71k were 25–30 per cent higher than ADH-F.

The population-genetic implications of our findings have been evaluated.

INTRODUCTION

The alcohol dehydrogenase locus (*Adh*, II-50·1) of *Drosophila melanogaster* is polymorphic in natural populations (van Delden, 1982). Natural populations generally contain three allelic forms, Adh^{F}, Adh^{S}, and Adh^{71k} (probably identical to $Adh^{FCh.D}$ and Adh^{Fr}, see Gibson *et al.*, 1980). Gel electrophoresis has been used to detect their alcohol dehydrogenase protein products (ADH, EC 1.1.1.1.), ADH-F, ADH-S, and ADH-71k, respectively. The ADH-F and ADH-71k allozymes show identical electrophoretic mobility, however, they differ in several fysico-chemical properties (Thörig *et al.*, 1975; Eisses *et al.*, 1985). Moreover, population-genetic studies have shown that a natural substrate of ADH, ethanol, exerts selective pressure on the allele-frequences, favouring Adh^{71k} over Adh^{F} (Scharloo *et al.*, 1977). The functional significance of this variation for individuals still remains an open question when the precise metabolic function of ADH has not been unravelled.

Recent studies have demonstrated a dual, sequential function of ADH in primary alcohol degradation (Heinstra *et al.*, 1983 and 1986*a*; Eisses *et al.*, 1985; Geer and McKechnie, 1984; Moxom *et al.*, 1985). The failure of ADH to oxidise secondary alcohols by means of such a two-step mechanism is caused by the formation of ketone end-products which form inhibitory dead-end ternary complexes with ADH and NAD (Heinstra *et al.*, 1986*a*). This effect can have consequences for variation in ADH activity, because increasing levels of β-keto compounds affect the ratio between the fully-active ADH-5, the half-active ADH-3 and the inactive ADH-1 isozyme *in vivo* (Heinstra *et al.*, 1986*b*).

It has been assumed that the variation in ADH activity causes functional differences in metabolic flux and affects the acute toxic effects of the alcohols present in natural habitats (David and van Herrewege, 1983; McKechnie and Morgan, 1982; van Delden, 1982). However, recent studies of Middleton and Kacser (1983) have shown no close relation between variation in *in vitro* ADH activity caused by different *Adh* genotypes and the *in vivo* flux measured as carbon dioxide and lipid product formation. Such whole flux measurements from ethanol as precursor may provide insight into the ultimate significance of ADH activity variation to control the flux to both end-products. On the other hand, the one-step oxidation of secondary alcohol substrates by ADH allows a direct estimate of action of the enzyme variants (Heinstra *et al.*, 1986*a*).

We report here differences in oxidation rates of the secondary alcohol, propan-2-ol, into acetone between flies homozygous for different *Adh* alleles.

MATERIALS AND METHODS

Strains and rearing of flies

Three strains of *D. melanogaster* were studied. Two strains were homozygous for the world-wide common Adh^F and the less-common Adh^{71k} allele, respectively. The alleles were extracted from two different Notch/delta-49 strains (Adh^F from an N^{faj24a} and Adh^{71k} from an N^8 strain). Both strains were maintained for several years in similar wild-type backgrounds (see Eisses *et al.*, 1985). As a reference, the Adh^{fn23} strain was used which is homozygous for the purple (*pr*) and cinnabar (*cn*) eye-colour mutants. Flies of this strain completely lack ADH activity, but show ADH protein levels, due to a 34 base pairs deletion within exon III of the *Adh* gene (Benyajati *et al.*, 1983). Flies were reared on cornmeal medium (1300 ml water, 10 g agar, 200 g cornmeal, 100 g sugar, 12 g dead yeast, 10 ml 10 per cent nipagine in ethanol) at 25°. Adult males aged for 6·5 days (±12 hr) after emergence were used throughout this study. During ageing flies were transferred to fresh food media after 3·5 and 5·5 days, to remove endogenous ethanol which was often found to be present in the Adh^{fn23} flies.

In vivo studies

Fifteen males were exposed to vapour of propan-2-ol in a closed glass-vial at 25° and 60 per cent R.H. Flies were prevented from sucking the propan-2-ol by separation of the flies from the solution present in cotton-wool (see details, Heinstra *et al.*, 1986*a*), by means of a small strip of cotton-wool which was permeable to vapours. When needed, flies were removed from the vials and immobilised immediately by a cold-treatment of 5 sec at 0°. Two series of five males were homogenised at 100 µl ice-cold pure water. Homogenates were kept cold and within 10 min, 1 µl aliquots were analysed by means of gas chromatography (for details, see Heinstra *et al.*, 1983 and 1986*a*). Extracts of control flies contained neither propan-2-ol nor acetone. Homogenization of control flies of each *Adh* genotype in ice-cold 10 mM propan-2-ol did not generate acetone. Within the period of *in vivo* metabolism, excretion of propan-2-ol and acetone has not been found. The data given here are representative for various independent analyses performed during two months involving four subsequent generations of flies.

Levels of propan-2-ol and acetone in the fly extracts were calculated by means of peak height × relative retention time. Standards of known concentrations of both compounds served as references for the peak heights. Concentrations of the compounds per fly were obtained after multiplying by the dilution factor (20 times per fly) and the mean fresh body weights (see table 1).

Electrophoresis

The procedures of slab-gel and gel-disc electrophoresis are given by Heinstra *et al.* (1983 and 1986*b*). In all cases, 10 µl of independent fly extracts (5 males homogenised in 100 µl 50 mM phosphate-buffer *p*H 6·7) were applied. The staining solution after slab-gel electrophoresis consisted of 100 mM ethanol, 0.23 mM NAD (Boehringer, Mannheim, FRG, Grade I), 1.5 mM MTT (Serva, Heidelberg, FRG) and 1 µM PMS (Sigma, St. Louis, U.S.A.) in a 50 mM phosphate-buffer *p*H 7·4. Staining lasted for one hr at 25° in the dark. Staining for gel-discs used, 13 mM propan-2-ol and 0·15 mM NAD in a 50 mM phosphate-buffer *p*H 7·4 for 30 min at 25° in the dark. Activity of the ADH-isozymes was visualised under ultraviolet illumination ($\lambda = 366$ nm).

Antibody production

Purified ADH-F protein (for the purification procedure, see Eisses *et al.*, 1985) containing approximately 1 mg ADH per ml was mixed with Freund's complete adjuvant in a 1:1 ratio, and 4 ml were injected subcutaneously into New Zealand white

Table 1 Kinetic parameters from the two ADH-allozymes

Genotype	Mean fresh male body weight (mg)	V_{max} (µ*M* product/min/fly)	$[S]_{0.5}$ (µ*M*)	$V_{max}/[S]_{0.5}$
$Adh^{71k/71k}$	0·813 ± 0·049	390·5 ± 24·5	313·1 ± 20·5	1·25
$Adh^{F/F}$	0·755 ± 0·023	168·0 ± 49·0	178·9 ± 55·9	0·94
$Adh^{fn23}prcn$	0·784 ± 0·049	—	—	—

Mean fresh body weights were determined from thirty flies of each genotype and do not show significant differences (Student's *t*-test). $[S]_{0.5}$ values given are those at endogenous substrate levels.

rabbits. After three weeks the rabbits were bled. After centrifugation, the pelleted blood cells were suspended in 4 ml 0·9 per cent NaCl and again centrifuged. After some washes with 0·9 per cent NaCl, two volumes of the final solution were mixed with one volume of purified ADH-F protein. After a two-hour incubation period at room temperature, the rabbits were boosted with 3 ml of this solution. A week later, the animals were bled again and after centrifugation, the antiserum was stored in small aliquots at −25°.

Immunological characterisation

First, characterisation was done by immunodiffusion using the method of Ouchterlony (1953). In order to obtain the relative *in vivo* ADH allozyme quantities, radial immunodiffusion gels were run according to Mancini *et al.* (1965). Homogenates were obtained after homogenization of 4 or 5 males in 100 µl water and centrifugation for 20 min at 6000 rpm and 4°. 5 µl of the resulting supernatant were placed in 2 mm wells in 1 per cent (w/v) agarose − 1 per cent (v/v) antiserum immunodiffusion gels. After developing for 20 hr at 4°, the gels were stained for ADH activity and the ring diameters measured by means of a Biorad-Rad immunodiffusion reader. The amount of ADM is expressed in radical immunodiffusion units relative to the ADH-71k allozyme.

RESULTS

The Adh^{71k} and Adh^{F} alleles were brought into similar wild-type backgrounds (Eisses *et al.*, 1985) which might have affected the original manifestation of their ADH allozymes (*e.g.*, Maroni *et al.*, 1982). However, in complete accordance with previous results (Thörig *et al.*, 1975), crude extracts of ADH-71k showed higher activity on ethanol than ADH-F (fig. 1(a)). Moreover, the difference in temperature-sensitivity has also been sustained. ADH-71k was more active, whereas ADH-F partially lost its activity after a heat-shock *in vitro* (fig. 1(b)).

Exposing flies to increasing levels of propan-2-ol revealed *in vivo* acetone product levels as depicted in the figs. 2(a-c). It is evident that at each substrate level, flies homozygous for Adh^{71k} produced more acetone than those homozygous for Adh^{F}. From measurements of flies without active ADH protein (Adh^{fn23}), it can be deduced that the acetone product formation in both genotypes with ADH activity was mainly mediated by ADH. Therefore, in the initial periods, the formation of

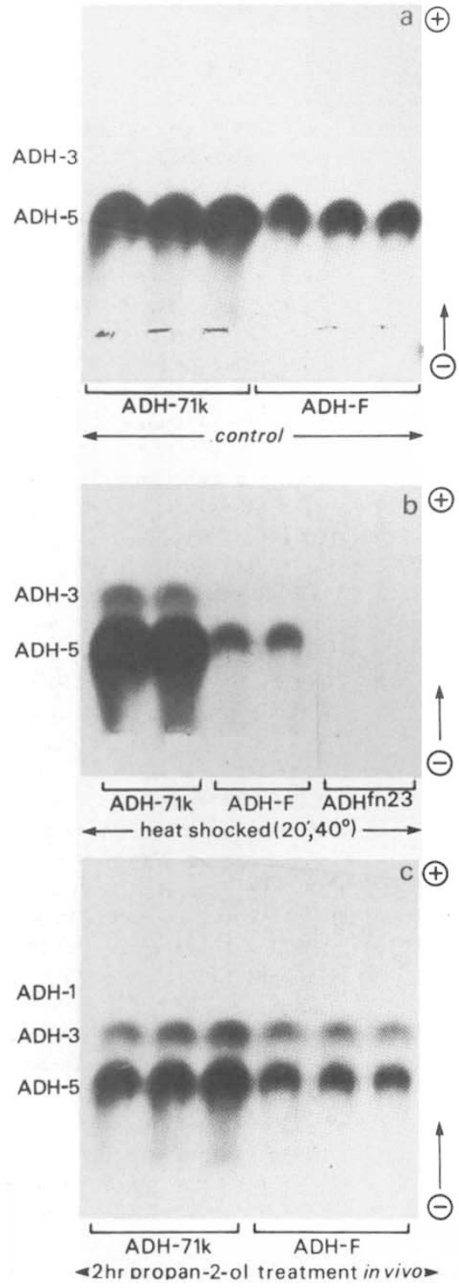

Figure 1 Electrophoretogram stained with the MTT-formazan method. (a), control flies. (b), after application of a heat-shock of the gel. (c), flies exposed to 260 mM (=2 per cent v/v) propan-2-ol.

acetone not mediated by ADH can be neglected in both ADH-active genotypes. The initial rates of product formation (v), as determined from the time zero till 30 min periods (figs. 2(a-c)), have been plotted according to Lineweaver-Burk ($1/v$ vs. $1/[S]$, see fig. 2(d)). From two independent

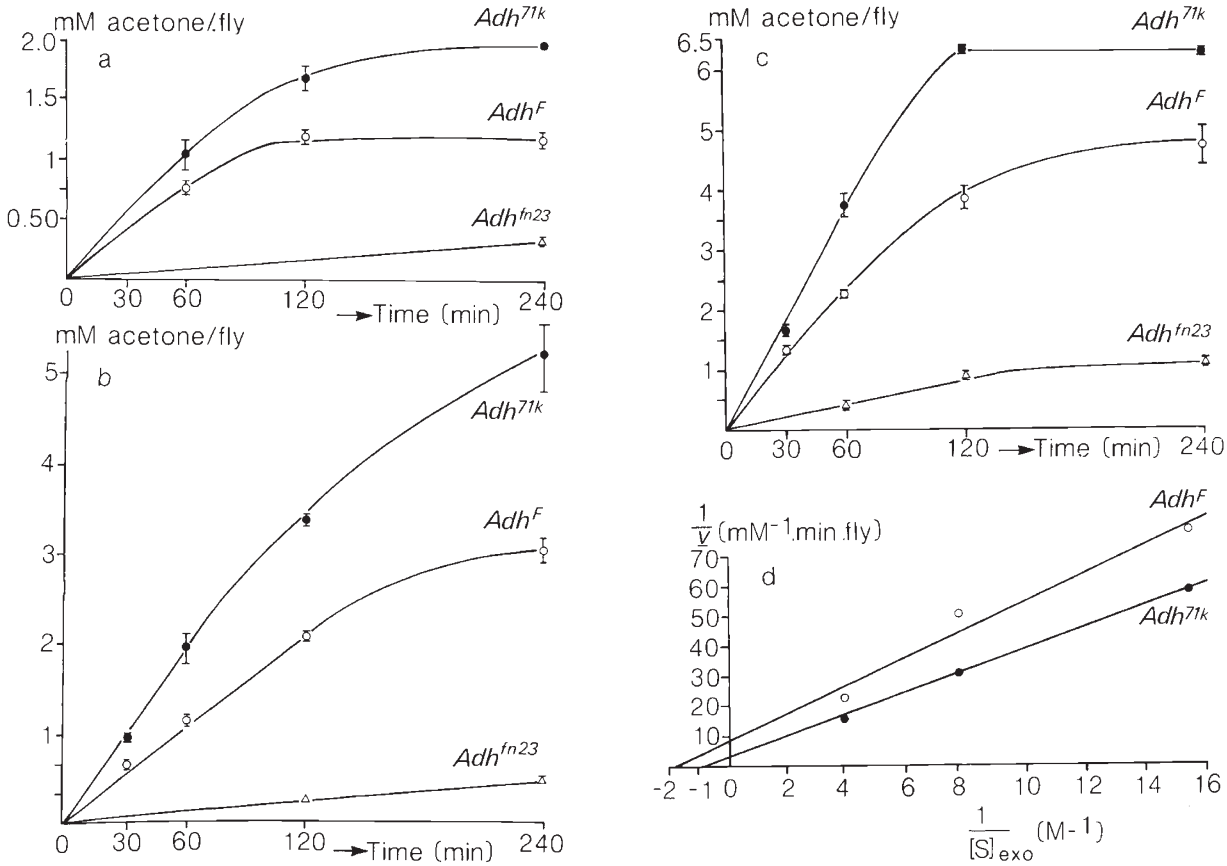

Figure 2 Metabolism of propan-2-ol *in vivo*. (a–c), acetone formation in time at different exogenous liquid-phase propan-2-ol levels. (a), at 65 mM (=0·5 per cent v/v) propan-2-ol. (b), at 130 mM, and (c), at 260 mM. Bars indicate standard errors, n ≧ 4. (d) Lineweaver-Burk plots derived from the curves shown in (a–c) which illustrate the regression of reciprocal values of v against those of exogenous substrate levels.

plots (the other was over the 0–520 mM substrate range), the kinetic parameters, maximum velocity (V_{max}) and the substrate concentration that yields half the maximum velocity ($[S]_{0.5}$), were determined (table 1). It turns out that the ADH-71k allozyme shows on the average higher values of V_{max} as well as of $[S]_{0.5}$ when compared with ADH-F.

The *in vivo* data also allow an analysis of the rates of total propan-2-ol uptake (sum of endogenous propan-2-ol and acetone, table 2). It is found that both ADH-active genotypes had uptake rates of vapours which are nearly identical (non-significant differences in the Student's t-test). Comparable pictures have been obtained at the other substrate levels. Moreover, these data also indicate that per min time interval about 0·02 per cent of the propan-2-ol liquid had entered the fly as vapour. This has been used to transform the $[S]_{0.5}$ values at exogenous levels into their respective endogenous substrate levels (see table 1 for final data).

The administration of propan-2-ol to flies and larvae results in a conversion of the electrophoretic ADH-isozymes pattern (e.g., Schwartz and Sofer, 1976; McKechnie and Geer, 1984). This effect is visualized in the figs. 1(c) vs. 1(a) and fig. 3, based

Table 2 Uptake rates of propan-2-ol vapours

	Genotype	
Time (min)	$Adh^{F/F}$	$Adh^{71k/71k}$
30	1·71 ± 0·05	1·66 ± 0·09
60	3·63 ± 0·25	3·74 ± 0·22
120	5·74 ± 0·35	6·32 ± 0·08
240	7·10 ± 0·05	7·28 ± 0·08

The data represent the sum of endogenous propan-2-ol and acetone (mM/fly) as determined at 260 mM exogenous levels of propan-2-ol.

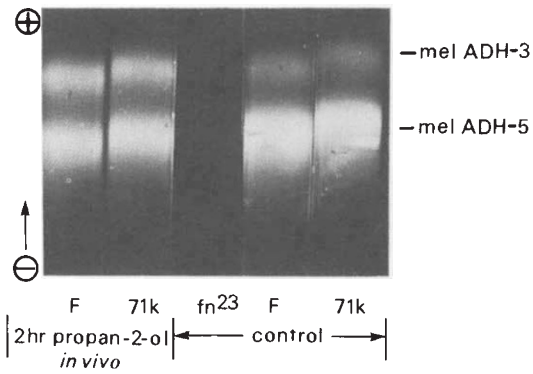

Figure 3 Electrophoretogram visualised by means of NADH fluorescence. For details, see fig. 1(a) and 1(c).

on two different methods of staining (Heinstra *et al.*, 1985*b*). The conversion patterns show that the ADH-5/ADH-3 isozyme activity ratio remained similar for both cryptic allozymes.

Immunological characterisation according to Ouchterlony (1953) indicated that crude extracts of Adh^F and Adh^{71k} flies and an aliquot of ADH-F and ADH-71k purified protein gave single and continuous precipitin lines without spurs with anti-ADH-FF serum (data will be published in detail elsewhere). This provided evidence for immunological identity between the two ADH-allozymes. It also suggested that the antigen in the fly-extract was the same as the purified ADH-protein. Further immunological studies according to the radial immunodiffusion method showed the linear relationship between the ring diameter and the log of the relative enzyme concentration ($r = 0{\cdot}997$) from serial dilutions of an extract of Adh^{71k} flies (fig. 4(a)). Furthermore, it turned out that *in vivo* levels of ADH-FF protein were 25–30 per cent lower than the ADH-71k71k in their respective homozygous male flies (fig. 4(b)), table 3).

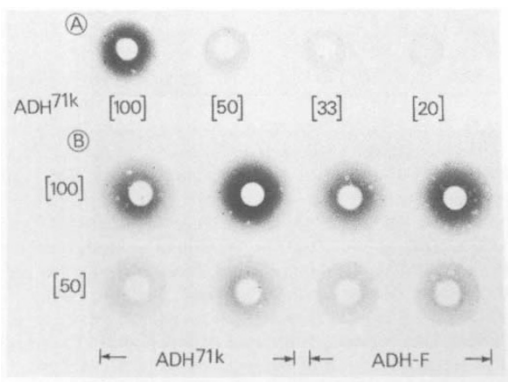

Figure 4 Immunological characterization. Panel A. Serial dilution of crude Adh^{71k} extract. Panel B. Differences in ADH allozyme protein levels from undiluted and diluted extracts.

Table 3 Immunological characterization

Allozyme	Relative *in vivo* protein levels
ADH-FF	73 ± 0·7
ADH-71k71k	100 ± 1·9

* Significantly different from ADH-71k71k, $P < 0{\cdot}01$ ($n = 8$).

DISCUSSION

Catabolism of propan-2-ol *in vivo* has been found to be mediated mainly through ADH activity (see also Heinstra *et al.*, 1986*a*). The two genotypes with ADH activity, and with similar genetic backgrounds, had nearly identical uptake rates of substrate vapours. Nevertheless, flies homozygous for Adh^{71k} produced more acetone than those homozygous for Adh^F. Therefore, it can be concluded that under comparable *in vivo* conditions, the ADH-71k allozyme determines a higher metabolic flux than its cryptic ADH-F counterpart.

Metabolic flux mediated by an enzyme is the net result of several parameters *e.g.*, enzyme quantity, kinetic mechanism and properties, and substrate, coenzyme and product levels. Increase of substrate levels resulted in a proportional increase of product formation (fig. 2(a–c)), which implies first-order conditions. This indicates that the ADH molecules *in vivo* were not saturated with substrate. Under such conditions, the *in vivo* differences between the ADH-allozyme levels would be a minor factor to determine the differential flux (Segel, 1975, p. 41). When it is assumed that NAD^+-coenzyme levels were non-limiting (Heinstra *et al.*, 1986*a*), these findings allow the determination of some kinetic properties. However, it should be mentioned that the *in vivo* system deviates from the usual *in vitro* assays (see Segel, 1975, pp. 46–48 and p. 83). The V_{max} and $[S]_{0{\cdot}5}$ values determined as such, do show considerable differences between the two ADH-allozymes (table 1). This suggests that substitution of one amino acid in the primary sequence affects kinetic properties of an allozyme (see Eisses *et al.*, 1985 for further discussion). Further, it suggests that the flux differences have been generated by the net differences in kinetic properties.

The calculated V_{max} values, though-ADH-71k shows 25–30 per cent more protein *in vivo* than

ADH-F, remain tentative, because product inhibition must also be considered. *Drosophila*-ADH forms strong inhibitory dead-end ternary complexes with NAD and ketones during the oxidation of secondary alcohols (Heinstra *et al.*, 1985*a* and references therein). Moreover, this type of product inhibition, which occurs despite a Theorell–Chance mechanism (Winberg *et al.*, 1982; Hovik *et al.*, 1984), might be followed in time as the concomitant conversion of the electrophoretic ADH-isozymes pattern (Heinstra *et al.*, 1985*b*). The conversion patterns for the two cryptic ADH-allozymes suggested similar relative rates of product inhibition.

Differences in fitness can be anticipated when different activities of the ADH-allozymes cause different accumulation patterns of alcohols in the body. Our *in vivo* findings (see also, Heinstra *et al.*, 1985*a*) combined with those of David *et al.* (1981) indicate that propan-2-ol is *more* toxic than acetone which contrasts previously held opinions. In mammals, propan-2-ol exerts effects on the acid-base status, glucose homeostasis, and lipid metabolism in the cells (Kricka and Clark, 1979). Moreover, alcohols are neurotoxic which might account for the loss of locomotor ability of genotypes without ADH activity during exposure to ethanol (Grell *et al.*, 1968) and to propan-2-ol (figs. 2(a–c); P. Heinstra, unpublished observations). The relationship between the metabolic stress and fitness depends on the effective levels of alcohols entering the individuals and the metabolic transformation of these alcohols and their direct products. We have shown that only a fraction of the propan-2-ol liquid enters the fly as vapour. Despite the different volatilities of alcohols in general, it can be expected that the potential selective action of alcohols present in natural habitats (McKechnie and Morgan, 1982; Briscoe *et al.*, 1975) is rather small in adults. Larvae, however, crawl in fermenting liquids and ingest the alcohols. Under such conditions, already low exogenous substrate levels may reach endogenous levels far above concentrations which saturate ADH. This suggests that the relation between functional differences of ADH allozymes in alcohol metabolism and metabolic stress, and ultimate differences in fitness is more important in larvae than in adults.

In conclusion, an one-step catabolism has been employed to determine metabolic flux mediated through ADH allozymes. It remains to be shown whether the observed differences are also representative for the putative two-step function of ADH allozymes in degradation of primary alcohols. Insight into this issue must await the results of further *in vivo* studies which are in progress.

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REFERENCES

BENYAJATI, C., PLACE, A. AND SOFER, W. 1983. Formaldehyde mutagenesis in *Drosophila*: Molecular analysis of ADH-negative mutants. *Mutation Res.*, *111*, 1-7.

BRISCOE, D. A., MALPICA, J. M. AND ROBERTSON, A. 1975. Dominance at the *Adh* locus in response of adult *Drosophila melanogaster* to environmental alcohol. *Nature*, *255*, 148-149.

DAVID, J. R., VAN HERREWEGE, J., SCHEEMAEKER-LOUIS, M. AND PLA, E. 1981. *Drosophila* alcohol dehydrogenase: Detoxification of isopropanol and acetone, substances not used in energy metabolism. *Heredity*, *47*, 263-268.

DAVID, J. R. AND VAN HERREWEGE, J. 1983. Adaptation to alcoholic fermentation in *Drosophila* species: Relationship between alcohol tolerance and larval habitat. *Comp. Biochem. Physiol.*, *74A*, 283-288.

EISSES, K. TH. SCHOONEN, W. G. E. J., ABEN, W., SCHARLOO, W. AND THÖRIG, G. E. W. 1985. Dual function of the alcohol dehydrogenase of *Drosophila melanogaster*: Ethanol and acetaldehyde oxidation by two allozymes ADH-71k and ADH-F. *Mol. Gen. Genet.*, *199*, 76-81.

GEER, B. W. AND MCKECHNIE, S. W. 1984. Dietary ethanol and lipid synthesis in *Drosophila melanogaster*. *Genetics*, *107*, *s*36.

GIBSON, J. B., CHAMBERS, G. K., WILKS, A. V. AND OAKESHOTT, J. G. 1980. An electrophoretically cryptic alcohol dehydrogenase variant in *Drosophila melanogaster*. I. Activity ratios, thermostability, genetic localization and comparison with two other thermostable variants. *Aust. J. Biol. Sci.*, *33*, 387-398.

GRELL, E. H., JACOBSON, K. B. AND MURPHY, J. B. 1968. Alterations of genetic material for analysis of alcohol dehydrogenase isozymes of *Drosophila melanogaster*. *Ann. N.Y. Acad. Sci.*, *151*, 441-455.

HEINSTRA, P. W. H., EISSES, K. TH., SCHOONEN, W. G. E. J., ABEN, W., DE WINTER, A. J., VAN DER HORST, D. J., VAN MARREWIJK, W. J. A., BEENAKKERS, A. M. TH., SCHARLOO, W. AND THÖRIG, G. E. W. 1983. A dual function of alcohol dehydrogenase in *Drosophila*. *Genetica 60*, 129-137.

HEINSTRA, P. W. H., EISSES, K. TH., SCHARLOO, W. AND THÖRIG, G. E. W. 1986*a*. Metabolism of secondary alcohols in *Drosophila melanogaster*: Effects on alcohol dehydrogenase. *Comp. Biochem. Physiol.*, *83B*, 403-408.

HEINSTRA, P. W. H., SCHARLOO, W. AND THÖRIG, G. E. W. 1986*b*. Alcohol dehydrogenase of *Drosophila*: Conversion and retroconversion of isozyme patterns. *Comp. Biochem. Physiol.*, *83B*, 409-414.

HOVIK, R., WINBERG, J-O. AND MCKINLEY-MCKEE, J. S. 1984. *Drosophila melanogaster* alcohol dehydrogenase: Substrate stereospecificity of the Adh^F alleloenzyme. *Insect. Biochem.*, *14*, 345-351.

KRICKA, L. J. AND CLARK, P. M. S. 1979. *Biochemistry of Alcohol and Alcoholism.* John Wiley & Sons. New York.

MANCINI, G., CARBONARA, G. O. AND HEREMANS, J. F. 1965. Immunochemical quantification of antigens by single radial immunodiffusion. *Immunochemistry, 2,* 235-254.

MARONI, G., LAURIE-AHLBERG, C. C., ADAMS, D. A. AND WILTON, A. N. 1982. Genetic variation in the expression of ADH in *Drosophila melanogaster. Genetics, 101,* 431-436.

MCKECHNIE, S. W. AND MORGAN, P. 1982. Alcohol dehydrogenase polymorphism of *Drosophila melanogaster*: Aspects of alcohol and temperature variation in the larval environment. *Aust. J. Biol. Sci., 35,* 85-93.

MCKECHNIE, S. W. AND GEER, B. W. 1984. Regulation of alcohol dehydrogenase in *Drosophila melanogaster* by dietary alcohol and carbohydrate. *Insect. Biochem., 14,* 231-242.

MIDDLETON, R. J. AND KACSER, H. 1983. Enzyme variation, metabolic flux and fitness: alcohol dehydrogenase in *Drosophila melanogaster. Genetics, 105,* 633-650.

MOXOM, L. N., HOLMES, R. S., PARSONS, P. A., IRVING, M. G. AND DODDRELL, D. M. 1985. Purification and molecular properties of alcohol dehydrogenase from *Drosophila melanogaster*: Evidence from NMR and kinetic studies for function as an aldehyde dehydrogenase. *Comp. Biochem. Physiol., 80B,* 525-535.

OUCHTERLONY, O. 1953. Antigen-antibody reactions in gels. IV Types of reactions in coordinated systems of diffusion. *Acta Pathol. Microbiol. Scand., 22,* 231-240.

SCHARLOO, W., VAN DIJKEN, F. R., HOORN, A. J. W., DE JONG, G. AND THÖRIG, G. E. W. 1977. Functional aspects of genetic variation Christiansen, F. B. and Fenchel, T. M. (eds.) In *Measuring Selection in Natural Populations.* Springer. Berlin.

SCHWARTZ, M. AND SOFER, W. 1976. Diet-induced alterations in distribution of multiple forms of alcohol dehydrogenase in *Drosophila. Nature, 263,* 129-131.

SEGEL, I. H. 1975. *Enzyme kinetics.* John Wiley & Sons. New York.

THÖRIG, G. E. W., SCHOONE, A. A. AND SCHARLOO W. 1975. Variation between electrophoretically identical alleles at the alcohol dehydrogenase locus in *Drosophila melanogaster. Biochem. Genet., 13,* 721-731.

VAN DELDEN, W. 1982. The alcohol dehydrogenase polymorphism in *Drosophila melanogaster*: Selection at an enzyme locus. *Evol. Biol., 15,* 187-222.

WINBERG, J-O., THATCHER, D. R. AND MCKINLEY-MCKEE, J. S. 1982. Alcohol dehydrogenase from the fruitfly, *Drosophila melanogaster*: Substrate specificities of the alleloenzymes Adh^S and Adh^{UF}. *Biochem. Biophys. Acta., 704,* 7-16.