presence of 0.04 mg% ChE. Maximum effect is obtained with 0.01 M H_2O_2, while further increases in H_2O_2 concentration cause a steady decrease of maximum luminescence intensity. The effects of 3 ChE inhibitors, tetraethylammonium iodide (TEA), physostigmine sulphate and an organophosphorous insecticide, dimethoate, have been studied. The enzyme was incubated with the inhibitor for 5 min prior to addition to the reaction mixture. In the experiments with TEA and physostigmine the concentration of ChE was 0.02 mg% and of H_2O_2 0.007 M. No significant change in light emission was observed with the addition of TEA to ChE. The maximal concentration of TEA investigated was 0.01 M. The addition of physostigmine to ChE markedly inhibited luminescence. The molar concentration of the inhibitor which inhibited the maximum luminescence intensity by 50% (I_{50}) was $4 \cdot 10^{-3}$ mM of physostigmine.

Dimethoate itself activates the oxidation of luminol[5]. Before investigating the interaction of this compound and ChE the effect of H_2O_2 concentration in the presence of dimethoate as the activator was determined. Figure 2 shows that dimethoate requires higher concentrations of H_2O_2 than ChE, to exert its activating action. Experiments with the combination of ChE and dimethoate as the activator were carried out with 2 different concentrations of H_2O_2. With 0.007 M H_2O_2 in the reaction mixture ChE is maximally effective, while dimethoate is completely ineffective as activator. The addition of dimethoate to ChE results in an inhibitory effect of the organophosphorous agent, with $I_{50} = 0.018$ mM. The curve of inhibition is shown in figure 3. With 0.176 M H_2O_2 in the reaction mixture dimethoate is maximally effective, while the action of ChE is negligible. When various concentrations (up to 0.16 mg%) of ChE were added to dimethoate, there was only a slight increase of luminescence intensity, but no inhibition.

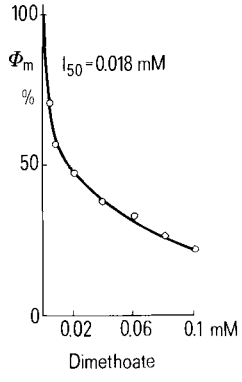

Fig. 3. Inhibition of luminescence by dimethoate. Activator: ChE 0.02 mg%. Conditions: 0.007 M H_2O_2, $4 \cdot 10^{-4}$ M luminol, reaction pH 12.2.

The present results show that ChE activates the oxidation of luminol with H_2O_2. The part of the enzyme molecule involved in this reaction could be deduced by examining the effect of the various inhibitors. It is well known that TEA combines with ChE only at the anionic site of the enzyme, physostigmine is attached to the enzyme at both the anionic and esteratic sites, while the reaction between ChE and the organophosphorous inhibitors which do not possess a quaternary nitrogen, such as dimethoate, occurs only at the esteratic site. Since physostigmine and dimethoate, and not TEA, had inhibitory action on the chemiluminescent activated by ChE, it could be suggested that the esteratic site is involved in the activating action of the enzyme.

1 E.H. White, in: Light and Life, p. 183. Ed. W.D. McElroy and B. Glass. Baltimore 1961.
2 J. Goldenson, Analyt. Chem. *29*, 877 (1957).
3 J. Matković and K. Weber, Arh. Hig. Rada *15*, 141 (1964).
4 K. Weber, Lj. Huić and M. Mrazović, Arh. Hig. Rada *9*, 325 (1958).
5 K. Weber, J. Matković and M. Bušljeta, Acta pharm. jugosl. *19*, 47 (1969).
6 E. Kunec-Vajić and K. Weber, Croat. chem. Acta *37*, 211 (1965).
7 E. Kunec-Vajić and K. Weber, Experientia *23*, 432 (1967).
8 E. Kunec-Vajić, Experientia *29*, 1010 (1973).
9 K. Weber, Dt. Z. ges. gericht. Med. *57*, 410 (1966).

Selection at the Adh locus in *Drosophila melanogaster:* Adult survivorship-mortality in response to ethanol[1]

S. M. Anderson, J. F. McDonald and M. Santos[2]

Department of Genetics, Iowa State University, Ames (Iowa 50011, USA) 4 August 1980

Summary. Functionally significant biochemical properties of the naturally occurring electrophoretic variants at the Adh locus (ADH^{Fast} and ADH^{Slow}) are correlated with the adult flies' ability to utilize and survive in an ethanol environment. The results are consistent with the idea that an environmentally dependent form of balancing selection is responsible, at least in part, for the maintenance of the polymorphism at this locus.

The application of biochemical techniques to population genetics has provided a means of addressing the neutralist-selectionist controversy by directly examining the functional properties of the gene products in question. This approach has been applied to many gene-enzyme systems in a variety of organisms with varying degrees of success[3]. One such example is the alcohol dehydrogenase system of *Drosophila melanogaster.* A number of biochemical properties of the 2 major electrophoretic variants, ADH^{Fast} and ADH^{Slow}, have been examined by a large number of researchers in an attempt to understand, in a functional sense, the polymorphic nature of this locus. Description of the variation in temperature stability[4,5], enzyme activity[6], enzyme concentration[7], and kinetic parameters[8] have been used to establish a correlation between these functional properties of the gene products and organismal phenomena, life stage mortality[9,10], the existence of gene frequency clines[11] etc. We report here, a study in which there exists a direct correlation between properties of the alcohol dehydrogenase gene products in *D. melanogaster* and adult flies ability to utilize and survive in an alcohol environment.

Materials and methods. Adult survivorship-mortality studies

were performed on 2 isogenic strains, S-1 and F-1 (constructed such that they were homozygous for their 2nd, 3rd and X chromosomes), as described by McDonald et al.[12]. Adult flies, 6-10 days post eclosion, were lightly etherized and sorted according to sex into 10 cm vials. Survivorship-mortality studies were initiated, after the flies were awake for 1-2 h, by introducing into each vial a 2.5×2.5 cm Whatman No. 1 filter paper, to which either 1 ml of H_2O (control) or 1 ml of ethanol-H_2O of varying concentrations had been absorbed. The vials were sealed with parafilm and percent mortality recorded on a regular basis, every 5 h the first day and every 8 h each day thereafter. Hours to 50% mortality was chosen for a standard of comparison and determined graphically by plotting percent mortality vs h of exposure to H_2O or ethanol-H_2O. Each determination was represented by 6-10 vials (3-5 vials ♂♂ and 3-5 vials ♀♀). Purification of alcohol dehydrogenase (E.C. 1.1.1.1.) and enzyme activities were determined according to McDonald et al.[13]. Kinetic parameters and immunological determinations of enzyme concentrations were according to McDonald et al.[8].

Results and discussion. Hours to 50% mortality are presented in table 1. From this data 3 points can be made. First, as ethanol concentration increases from 0 to 4% for S-1 and 0 to 6% for F-1 so does the h to 50% mortality value. These ethanol concentrations, in all likelihood, represent concentrations which have limited toxic effects and the increase in longevity is due to the flies ability to utilize the ethanol present as a carbon source. Secondly, at ethanol concentrations greater than 4% for S-1 and greater than 6% for F-1 the ability to use the alcohol present as a carbon source begins to be overridden by the toxic nature of the ethanol. In such cases, the h to 50% mortality approaches and/or becomes significantly less than control value again demonstrating the toxic nature of higher concentrations of ethanol. Lastly, when the values for S-1 and F-1 are compared by the Student's t-test another observation is evident. At lower concentrations of ethanol strain S-1 does significantly better (0.5% ethanol) or as well as strain F-1 (1-4% ethanol), while at higher concentrations of ethanol (6-10%) the trend is reversed and strain F-1 survives significantly longer than strain S-1. This last point is of considereable importance when examined with regard to the biochemical properties of the 2 proteins in question, table 2. The relative importance of both Michaelis constant (K_m) and V_{max} or enzyme concentration in the process of biochemical adaptation have been recognized[3,14]. The Michaelis constant becomes a relatively more important parameter under conditions where the substrate concentration is equivalent to or less than K_m. However, at substrate concentrations appreciably greater than K_m the important parameter then becomes V_{max} or the enzyme concentration. The relative contribution of these two parameters is given by the Michaelis-Menten equation, $v = V_{max}[S]/K_m + [S]$. Even though we are limited in that we don't know the exact intracellular substrate concentrations of ethanol and NAD under our experimental conditions the data are nevertheless generally consistent with the above predictions. Under low ethanol conditions, where K_m is the important parameter, strain S-1, which has a lower K_m for ethanol, survives significantly longer or as long as strain F-1. At higher concentrations, where the important parameter is now V_{max}, strain F-1, which possesses a higher V_{max} (greater enzyme concentration) survives significantly longer than strain S-1. Our survivorship-mortality data suggest that, NAD concentration is probably less than saturating, for according to the Michaelis-Menten equation. $V_{max}f/V_{max}s$ must be ≤ 1.15, in order that S-1 flies be catalytically more efficient when $[\text{ethanol}] \approx K_m$. It has been reported that in mammals cellular NAD levels drop precipitously upon the ingestion of ethanol[15] and the same is likely to be true in flies. We are presently in the process of estimating NAD levels in these flies in alcohol stress and nonstress conditions. Although further testing will be required before definitive answers can be obtained, our results are consistent with the view that a form of environmentally dependent balancing selection may in part, be responsible for the maintenance of the polymorphism at the Adh-locus in *Drosophila melanogaster.*

Table 1. Survivorship - mortality studies on 2 isogenic strains (S-1 and F-1) of *Drosophila melanogaster*

Ethanol concentration	Hours to 50% mortality Strain S-1	Strain F-1
Control	53.08 ± 1.44	53.08 ± 3.20
0.5%	73.50 ± 1.72	63.23 ± 2.56**
1.0%	86.17 ± 1.49	86.23 ± 3.98
2.0%	96.20 ± 6.17	101.90 ± 4.87
4.0%	121.67 ± 6.66	107.98 ± 12.63
6.0%	80.67 ± 8.45	124.73 ± 15.87*
8.0%	56.67 ± 4.11	96.56 ± 8.75*
10.0%	26.83 ± 1.87	71.48 ± 13.19**

Student's t-test. * $p < 0.01$; ** $p < 0.001$.

Table 2. Biochemical properties of alcohol dehydrogenase purified from 2 isogenic strain of *Drosophila melanogaster*

Strain	$K_{m_{NAD}}$ (mM)	$K_{m_{NAD}}$ (mM)	Relative V_{max}	Relative amount
S-1	4.02 ± 0.03	0.040 ± 0.001	1.00	1.00
F-1	5.20 ± 0.06	0.041 ± 0.002	2.60	2.71

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2 Present address: Department of Genetics, University of Santiago, Santiago (Spain).
3 P.W. Hochachka and G.N. Somero, Strategies of Biochemical Adaptation. W.B. Saunders Co., Philadelphia-London-Toronto 1973.
4 B. Sampsell, Biochem. Genet. *15,* 971 (1977).
5 S. Anderson, M. Santos and J. McDonald, Drosoph. Inf. Serv. *55,* 13 (1980).
6 A.J. Birley and B.W. Barnes, Heredity *13,* 413 (1973).
7 N. Lewis and J. Gibson, Biochem. Genet. 16, 159 (1978).
8 J. McDonald, S. Anderson and M. Santos, Genetics *95,* 1013 (1980).
9 A. Kamping and W. van Delden, Biochem. Genet. *16,* 541 (1978).
10 E. Bijlsma-Meeles, Heredity *42,* 79 (1979).
11 C.L. Vigue and F.M. Johnson, Biochem. Genet. *9,* 213 (1973).
12 J.F. McDonald, G.K. Chambers, J. David and F.J. Ayala, Proc. natl Acad. Sci, USA *74,* 4562 (1977).
14 P.H. Crowley, J. theor. Biol. *50,* 461 (1975).
15 R. Thurman, T. Yonetani, J. Williamson and B. Chance, eds, Alcohol and Aldehyde Metabolizing Systems. Academic Press, New York 1974.