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DROSOPHILA ALCOHOL DEHYDROGENASE: DETOXIFICATION OF ISOPROPANOL AND ACETONE, SUBSTANCES NOT USED IN ENERGY METABOLISM

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1. Introduction

Population geneticists, using the *D. melanogaster* polymorphism as a model, have demonstrated that environmental conditions and specially alcohol treatments can modify the frequencies of alcohol dehydrogenase (ADH) alleles (Gibson, 1970; Bijlsma-Meeles and Van Delden, 1974; Clarke, 1975; Oakeshott, 1976; Van Delden *et al.*, 1975, 1978) and favour the most active enzyme.

Previous results have shown that ADH is a key enzyme both for ethanol detoxification (David *et al.*, 1976, 1978) and for metabolic utilisation (Van Herrewege and David, 1974, 1978; Libion-Mannaert *et al.*, 1976). It was argued (David *et al.*, 1979) that, in nature, species could be preferentially adapted either to detoxify ethanol or to use small concentrations as a resource. Recently, both traits were shown, in *D. melanogaster*, to have a partial genetic independence (Van Herrewege and David, 1980).

Drosophila ADH uses several primary or secondary alcohols as substrates (Vigue and Johnson, 1973; Day *et al.*, 1974; Chambers *et al.*, 1978). It is generally found that primary alcohols are transformed into aldehydes, while secondary alcohols are transformed into ketones. For example, ethanol is transformed into acetaldehyde which is further metabolized into acetate and used in the Krebs cycle (Clarke, 1975; David *et al.*, 1976; David, 1977; Deltombe-Lietaert *et al.*, 1979). On the other hand, ketones do not seem to be further metabolised (Papel *et al.*, 1979; Van Herrewege *et al.*, 1980), although some contrary results have been published (Oakeshott, 1977). Moreover, ketones are usually more toxic than the corresponding alcohols, so that treating live flies with long chain unsaturated secondary alcohols is a classical way for screening ADH null mutants (Sofer and Hatkoff, 1972; O'Donnel *et al.*, 1975): flies without ADH activity do not produce the toxic ketone and survive, while normal flies are killed.

From the above data, we would expect that Drosophila would not be able to detoxify secondary alcohols, which cannot be used in energy metabolism. The well known fact that, *in vitro*, ADH is much more active on secondary alcohols, like isopropanol (Vigue and Johnson, 1973; Day *et al.*, 1974) may be considered as an evolutionary paradox. It has recently been shown that various primary alcohols are effectively used as energy sources, while secondary ones are not (Van Herrewege *et al.*, 1980).

Moreover, acetone, which is produced from isopropanol, has been shown to be an enzymatic poison for ADH, rapidly decreasing the ADH activity (Papel *et al.*, 1979) in live flies.

However, various investigators have obtained different results. McDonald and Avise (1976) observed a positive correlation between ADH activity of various species and their tolerance to isopropanol. Van Delden *et al.* (1975) observed an increase in the frequency of the most active allele of ADH in cultures treated with isopropanol. David *et al.* (1976) also observed that ADH positive flies were slightly more tolerant to isopropanol and isobutanol than ADH negative flies. We have investigated the possible role of ADH for the detoxification of isopropanol and acetone.

2. Material and methods

We used as a wild type reference a French, Colmar strain, which had been selected for an increased ethanol tolerance during 54 generations (David *et al.*, 1977). At the end of the selection, alcohol tolerance measured by the concentration killing 50 per cent of adults after two days of treatment (L.C. 50), was about 28 per cent of ethanol. Selection was then interrupted for two years and the tolerance decreased to about 22 per cent. As ADH negative flies, we took a strain homozygous for the Adh^{n4} allele (Sofer and Hatkoff, 1972) kindly provided by Prof. W. Sofer and used in previous experiments (David *et al.*, 1976, 1978).

Prior to the experiments, genetic backgrounds were made homogeneous by crossing the two strains, and the F1 adults intercrossed to produce F2 flies. Virgin F2 females were isolated and individually backcrossed to Colmar males. The genotypes of the female progeny were assessed by starch gel electrophoresis and only cultures from negative females kept. This procedure was repeated for ten successive backcrosses and, at the end, an homozygous ADH negative strain was isolated. With such a procedure, the ADH negative allele and some adjacent parts of the second chromosome were introduced into the genetic background of the Colmar strain. Ethanol tolerance of these flies was found to be lower than 2 per cent, thus confirming the major role of ADH for this process.

For studying the effects of isopropanol and acetone, experimental flies were reared at 25°C on a killed yeast medium (David and Clavel, 1965). After emergence, adults were distributed in groups of 10 for utilisation tests or in groups of 20 for toxicity tests, and aged for 3 or 4 days. They were then transferred to air tight vials and dead flies recorded once or twice daily. For a metabolic test, a water solution with a low concentration of the possible nutrient was put into the vial. For a toxicity test, higher concentrations were used and added to a 3 per cent sucrose solution. More detailed technical information may be found in previous papers (David *et al.*, 1974, 1976, 1978).

3. Result

(i) *Metabolic utilisation*

Survival curves of the two genotypes in the presence of a very low, non-toxic concentration (0·4 per cent) of isopropanol, acetone and ethanol are given in fig. 1. With isopropanol and acetone, the curves for the two

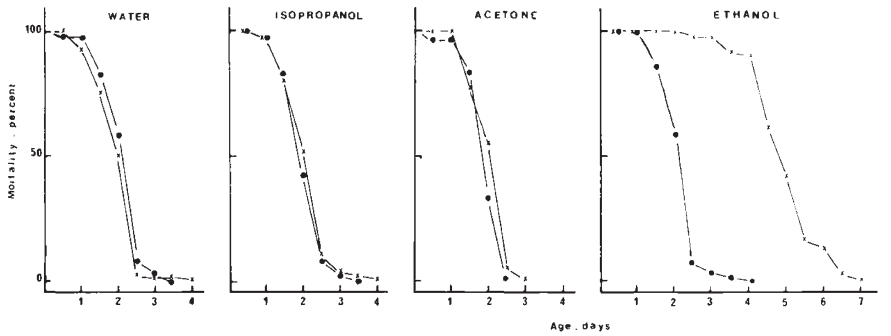

FIG. 1.—Survival curves of ADH negative (●) and ADH positive (×) flies in the presence of water alone or a solution of a low (0·4 per cent) non-toxic concentration of isopropanol, acetone or ethanol. Each curve is based on 80 flies (40 ♂ and 40 ♀; both sexes pooled).

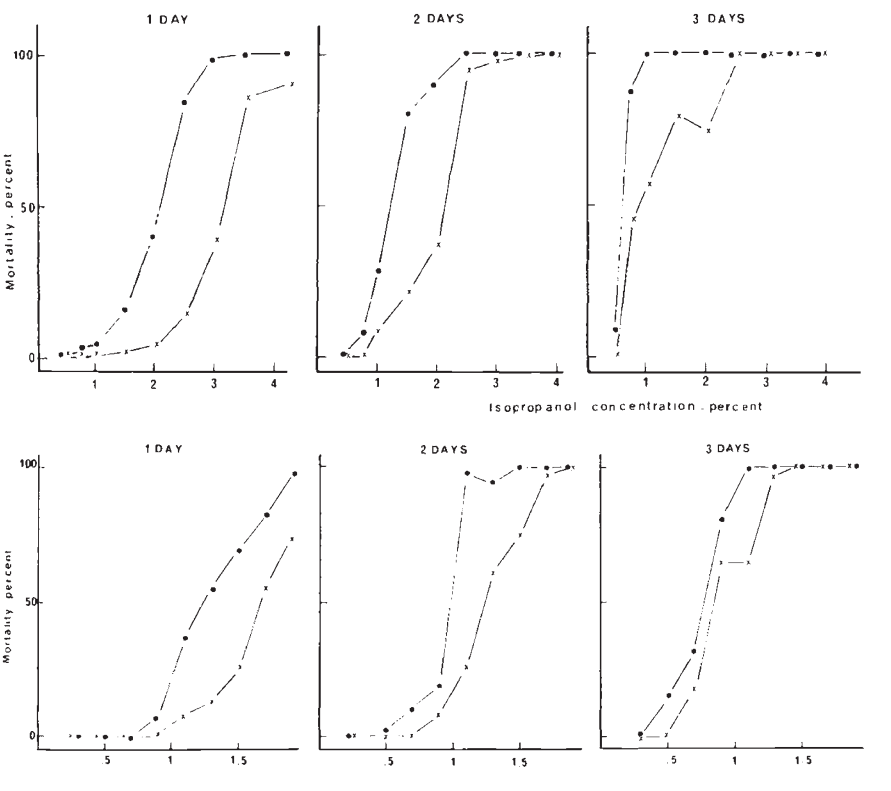

FIG. 2.—Comparison of the toxicity of isopropanol or acetone for ADH negative (●) and ADH positive (×) flies. Mortality curves are shown after 1, 2 or 3 days of treatment. Chemicals, at the indicated concentration, were added to a solution of 3 per cent of sucrose. For each concentration, at least 80 adult flies (both sexes pooled) were used.

kinds of flies are almost identical and also very similar to those obtained with water only. Mean life duration is about 42 hours and the differences are not significant. With 0·4 per cent ethanol, on the other hand, a clear difference exists between the two strains: ADH negative flies survived 45 hours, a value almost identical to that observed on water while ADH positive flies survived for 110 hours. These results confirm that only ethanol may be used as a food and also that ADH is necessary for this process.

(ii) *Toxicity*

Experimental results for isopropanol are shown in fig. 2. Mortality percentages after 1, 2 and 3 days of treatment are always much lower for ADH positive flies. For example, after two days of treatment, the L.C. 50 is 1·2 per cent of isopropanol for ADH negative flies and 2·2 for wild type flies. Similar experiments were done with acetone and the results are presented in fig. 2. Again, ADH negative flies were found to be much more sensitive than normal flies. A comparison of the L.C. 50 for the two toxic substances after 1, 2 and 3 days of treatment is given in fig. 3. Several

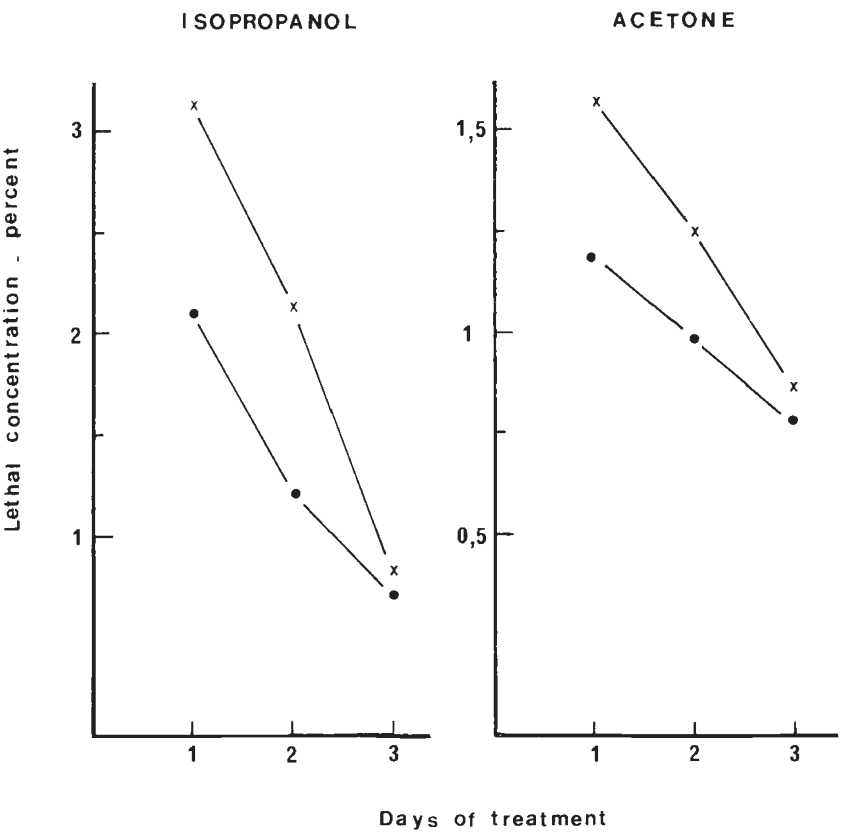

FIG. 3.—Variations of the toxicity of isopropanol and acetone, expressed as the L.C. 50 (lethal concentration 50) after different durations of treatment. ●: ADH negative flies; ×: ADH positive.

conclusions can be derived. The toxicity increases with duration of the treatment; for each genotype, the decrease is almost linear, and the slopes of the regression lines are higher for ADH positive than for ADH negative flies, so that the difference between the two genotypes tends to zero on the third day. Finally, acetone toxicity is almost twice the isopropanol toxicity in one day old flies, while the L.C. 50 of the two products becomes similar after 3 days.

4. Discussion and conclusion

With the experimental procedure followed, it is impossible to discriminate between effects due to the locus of interest and effects of linked unknown genes. It is however highly probable that the differences observed here can be ascribed to ADH. Our data confirm and extend previous work (David *et al.*, 1976). ADH plays a significant role in the detoxification of isopropanol and acetone, two products which are, however, not used in energy metabolism. These results also provide a physiological basis for the results of Van Delden *et al.* (1975) and McDonald and Avise (1976) who observed a higher tolerance of isopropanol in flies with higher ADH activity.

If acetone is used by ADH as a substrate, it should be transformed in the live fly by a reverse reaction into isopropanol, which is less toxic. Moreover, we know that acetone will rapidly inhibit ADH activity (Papel *et al.*, 1979). The observation (fig. 3) that, after 3 days of treatment, the wild type and the ADH negative flies show almost the same L.C. 50 can be attributed to such a phenomenon: in both types of flies, ADH activity must be close to zero. Detoxification of isopropanol by ADH raises, however, a physiological paradox. Although transforming the alcohol into a more toxic ketone, ADH increases the physiological tolerance to isopropanol. Our results confirm and explain a previous conclusion (Van Herrewege and David, 1980): detoxification and utilisation of alcohols as an energy source may involve different processes.

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