The utilization of ethyl acetate vapor by *Drosophila buzzatii* adults

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Summary. Ethyl acetate is utilized by *Drosophila buzzatii* adults under some physiological conditions, reducing mortality rate and body weight loss. Ethanol has similar effect.

Adult *Drosophila* can utilize atmospheric ethanol[2-5], acetaldehyde[6] and acetic acid[7] to increase longevity. Atmospheric ethanol was used as a substrate to study differences between alcohol dehydrogenase allozymes[3,8,9]. Ethyl acetate and other short chain esters may be postulated to have similar usefulness in studying esterase allozymes. Ethyl acetate was detected in necrotic tissue of *Opuntia stricta,* a natural habitat of *Drosophila buzzatii*[10].

Polymorphic esterase-1 in *D. buzzatii* can hydrolyse ethyl acetate[10], as can Est-6 in *D. melanogaster*[11]. In this paper, an attempt to study the response of *D. buzzatii* adults to ethyl acetate vapor will be reported. Since it was desirable to compare the effect of ethyl acetate with that of ethanol, ethanol was included in some experiments.

The experimental unit was a 250-ml glass beaker, whose air space was estimated to be 309.2±1.4 ml. Each beaker contained 4 30-ml glass vials, with each vial stoppered by terylene cloth. 20 flies of one sex were added to each empty vial, and allowed to recover from etherization overnight (15 h) at 20 °C. Next morning, the vials were added to the beakers, and 10 µl ethyl acetate or ethanol was injected into each beaker, which was covered immediately with a plastic film ('parafilm') to create a closed system. The beakers then were maintained at 25 °C. Control was the absence of any chemical. As such, the relative humidity inside the closed beaker at the beginning of the experiment was that of the ambient environment of 30-40%, but during the experiment, it was not controlled.

In the 1st experiment, flies 1-day-old on the introduction to the system were used to set up 11 replicate beakers for each of ethyl acetate, ethanol and control. Mortality was recorded every 12 h (fig. 1). Ethanol resulted in the lowest mortality rate, with ethyl acetate intermediate. Mean longevity of control flies was 3.44±0.14 days as compared with 4.92±0.14 (ethyl acetate) and 5.61±0.14 (ethanol). In the 2nd experiment, 15 replicate beakers were set up for each of the above 3 treatments, again using 1-day-old flies. Everyday, 3 replicates of each treatment were terminated and a random sample of 10 surviving flies in each vial was weighed (fig. 2). Even though the weights from different treatments were overlapping on any day, there was a clear trend for slower rate of weight loss in ethanol than control with ethyl acetate intermediate.

In the 3rd experiment, well fed 5-day-old flies were used to set up 12 replicate beakers for each of ethyl acetate, ethanol and control. Mortality was recorded daily for 7 days. Ethanol was very toxic as was ethyl acetate to a lesser extent up to day 5. Ethanol and ethyl acetate caused substantial mortality in the first 24 h, 48.54% (confidence interval (CI) of 45.72-51.37%) and 14.46% (CI= 12.53-16.58%), respectively, as compared with 5.15% (CI=3.97-6.47%) for the control. Even though differences between treatments were continuously reduced, the original ranking was maintained for the next 4 days. In the last experiment, starving 3-day-old flies were used to set up 32 replicate beakers for each of ethyl acetate and control. Mortality was recorded every 12 h (fig. 1). As in younger flies, ethyl acetate reduced the mortality rate, resulting in higher mean longevity (3.2±0.1 days) as compared with 1.79±0.1 days for the control.

In all 4 experiments, measurements were done on the 2 sexes separately. In experiments 1 and 2, the 2 sexes were included in the same beaker (2 vials per sex). In the last 2 experiments, all 4 vials of a beaker contained flies of only 1 sex, with males in half the beakers, and females in half. Therefore, in analyses of variance, the main effect due to sex and the sex X chemical treatment interaction were partitioned. Except in the 1st experiment, females always lived longer than males. In the 3rd experiment, mean longevity of females was 2.57±0.1 days as compared with 2.41±0.1 days for males. In the last experiment, after 5 days, mortality of males was 39.01% (CI=37.66-40.36%) as compared with 29.67% (CI=28.41-30.94%) for females. Also, in the 2nd experiment, females were heavier than

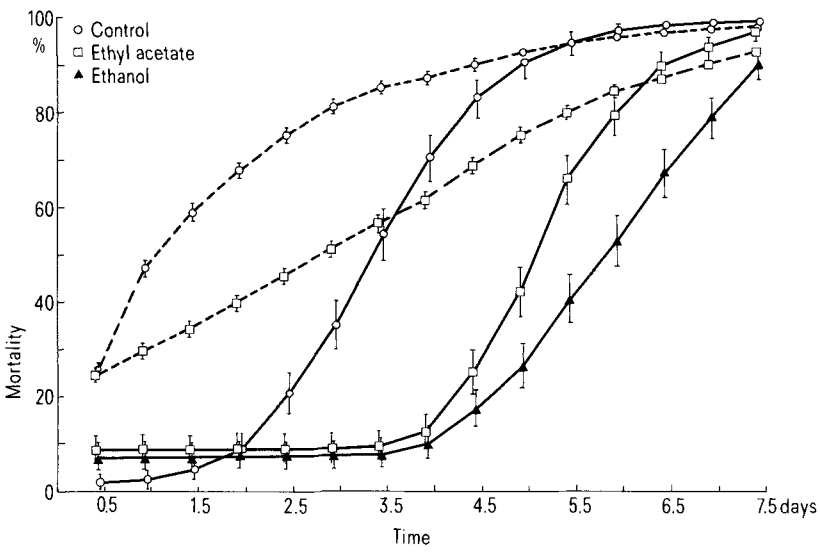

Figure 1. Mortality curves. Ethanol (△), ethyl acetate (□), control (○). Vertical lines are 95% confidence intervals, calculated from the residual errors of ANOVA of angular transformed data. Solid lines are from experiment 1 in which flies were 1-day-old on the introduction to the system. Each point is the mean of 44 measurements. Broken lines are from the last experiment using starving 3-day-old flies. Each point is the mean of 128 measurements.

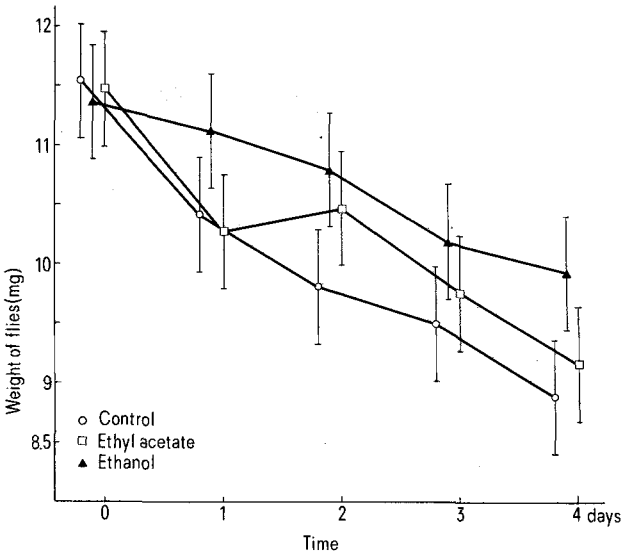

Figure 2. Body weights of sample of 10 flies (mg) of each of days 0-4. Ethanol (△), ethyl acetate (□), control (○). Vertical lines are 95% confidence intervals calculated from residual errors of ANOVA. Each point is the mean of 12 measurements.

males. The average weight of a sample of 10 females was 11.25 ± 0.14 g as compared with 9.38 ± 0.14 g for males. But the sexes did not respond differently to different chemical treatments, as indicated by the statistically non-significant sex x chemical treatment interactions in all experiments.

It seems as if the response of adult flies to ethanol and ethyl acetate vapor depended on the net balance between the toxicity of the chemical and its benefit as an energy source. That benefit must be evaluated according to the physiological need of the fly. The young or starving fly does not have a large energy reserve, and therefore can utilize the chemical vapor as an emergency energy source.

The well fed fly does not need such benefit, hence the toxicity effect dominated the response. Even though the dual toxic and beneficial effects of ethyl acetate and ethanol vapor were demonstrated, the experimental unit was very stressful. However, the intrinsic stresses, e.g. starvation and desiccation, were similar for all treatments.

Using the same system, 8 other esters (all acetates from propyl to amyl, both n- and i-, ethyl propionate and ethyl n-butyrate) also were studied. Except for amyl acetate, young flies were able to utilize these esters to increase longevity[12]. Genetic studies of polymorphic esterases are seriously circumvented by the lack of information about the natural substrate[10,13]. Atmospheric short chain esters offer an opportunity to study possible differences between esterase allozymes. Using young *D. buzzatii* flies, different esterase phenotypes responded differently to some esters (n- and i-propyl acetate, n- and i-butyl acetate and ethyl propionate)[12].

1 This work was done under grants from the Australian Research Grants Committee and U.N.E. Internal Research Grants to Prof. J.S.F. Barker.
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0014-4754/83/040419-02$1.50 + 0.20/0

Large scale isolation of zonae pellucidae from ovarian oocytes of mice

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Summary. A large scale method for the isolation of zonae pellucidae (ZP) from mouse ovaries is described. It involves the squashing of the ovaries on a screen (70 × 70 M mesh) and sedimentation of the material in a discontinuous gradient. It is possible to obtain free ZP in useful quantities in about 1 h's work.

Several methods for large scale isolation of zonae pellucidae (ZP) from large mammals have been reported, for example pig ovaries[1-3] and cow ovaries[3,4]. However, no large-scale method for the isolation of ZP from mice have been reported (Dunbar, personal communication). The procedure reported by Bleil et al.[5] cannot be considered a large-scale one (it yields about 50 ZP per h of work).

In this communication a simple method for the isolation of large amounts of entirely free ZP from mice ovarian oocytes is described. It consists of the squashing of the ovaries on a wire screen, followed by washing off the squashed material in a phosphate buffered saline solution (PBSS). After 2 centrifugation steps, the material is deposited on the top of a discontinuous gradient, composed of 2 layers. After 20-30 min sedimentation, free ZP are recovered from the upper half of the intermediate 8% sucrose layer.

Materials and methods. 18-25-day-old white mice were sacrificed by cervical dislocation. The ovaries were removed and freed from the Fallopian tubes and retroperitoneal fat cushion, washed in a phosphate buffered saline solution (PBSS), (NaCl 7‰; pH: 7.2), maintained at 4 °C, and then placed on a piece (2.5 × 2.5 cm) of wire screen, (stainless steel, 70 × 70 M mesh).

Ovaries were squashed with a spatula and the lower face of the mesh was washed in a culture dish containing PBSS in