

activation of human fat cell adenylate cyclase<sup>2</sup>. Although adenylate cyclase was not directly investigated, the present results argue strongly in favour of such a mechanism, which, as shown previously, does not involve the destruction of  $\alpha$ -adrenergic receptors<sup>2</sup>.

The present study also shows that such a 'permissive' effect of trypsin does not apply only to catecholamine-induced lipolysis. In fact, we found that the trypsin-treatment of human fat cells also increased the lipolytic response and the cAMP accumulation induced by theophylline concentration producing half-maximal stimulation of lipolysis. Nevertheless, the maximal lipolytic response of human fat cells to theophylline was found unaltered after trypsin-treatment.

The above-described modifications induced by trypsin in human fat cells are comparable with those exerted by growth hormone (GH) + glucocorticoid in the rat ones<sup>7</sup>. In fact, it was reported that these hormones increase norepinephrine- and theophylline-induced lipolysis, but fail to affect both the dibutyryl-cyclic AMP-induced one and the maximal lipolytic response of rat adipocytes to theophylline and catecholamines<sup>7,8</sup>. Furthermore, despite its non-effectiveness on the basal adenylate cyclase activity of rat fat cell ghosts, GH was shown to increase the sensitivity of this enzyme to catecholamines<sup>9</sup>, an effect which was prevented by inhibitors of RNA-synthesis<sup>9</sup>. It was thus concluded that GH affects lipolysis through increased synthesis of a plasma membrane protein(s) which may enhance the ability of catecholamines to activate adenylate cyclase<sup>9</sup>.

In the case of human fat cells, synthesis of such a protein could also explain the modifications of lipolysis found after trypsinisation. In a previous report<sup>2</sup>, we suggested that trypsin inactivates a membranous protein(s) which may usually prevent the binding of catecholamines to human fat cells or inhibit the transmission of the appropriate signals from the  $\beta$ -receptors to adenylate cyclase. Recent experiments<sup>10</sup> have shown, however, that catecholamine-binding to human fat cells was unimpaired after trypsin-treatment.

Anyhow, such modifications in plasma membrane proteins would not explain the present results concerning the 'permissive' effect of trypsin on theophylline-induced lipolysis. In fact, because of the well-known inhibitory action of theophylline upon PDE<sup>3</sup> and of the cytosolic localization of these enzymes in human fat cells<sup>11</sup>, it appears unlikely that the 'permissive' effect of trypsin on theophylline-induced lipolysis could result from a direct action of trypsin on PDE. Explanation of this 'permissive' effect requires the establishment that the inhibition of PDE may be the only way of action of theophylline on lipolysis in human fat cells.

- 7 J. N. Fain and R. Saperstein, in: *Adipose tissue, Regulation and Metabolic Functions*, p. 20. Ed. B. Jeanrenaud and D. Hepp. Academic Press, New York 1970.
- 8 J. N. Fain, *Endocrinology* 82, 825 (1968).
- 9 J. N. Fain, *Pharmac. Rev.* 25, 67 (1973).
- 10 Y. Giudicelli, unpublished data.
- 11 S. S. Solomon, *J. Lab. clin. Med.* 79, 598 (1972).

## Feeding an insect through its respiration:

### Assimilation of alcohol vapors by *Drosophila melanogaster* adults

J. Van Herrewege and J. R. David<sup>1</sup>

*Laboratoire d'Entomologie expérimentale et de Génétique (associé au C. N. R. S.), Université Claude Bernard, 43, bd du 11 Novembre 1918, F-69621 Villeurbanne (France), 6 July 1977*

**Summary.** Ethanol given to otherwise starved *Drosophila* adults can increase their survival from 2.5 to 8 days. Similar results were obtained when only alcohol vapor was accessible to the flies, demonstrating the possibility of significant feeding through their respiration. This physiological capacity could be useful in wild conditions.

Among all presently known *Drosophila* species, *D. melanogaster* is characterized by a very high tolerance to ethanol<sup>2</sup> (and unpublished data) which allows its development on the surface of fermenting wine jars, on substrates often containing 10% of alcohol. This tolerance is due to a very active alcohol dehydrogenase (ADH)<sup>3</sup>: after its detoxification, alcohol is used as an energy source as shown by the increase in lifespan of starved adults<sup>4,5</sup>.

Since alcohol is volatile, long experimental tests are difficult to run and it is not easy to know the exact concentration which reaches the flies. In our previous studies, toxicity measurements were made by placing the adults in air tight plastic tubes containing an alcoholic solution adsorbed on cellulose wool<sup>2,3</sup>. For the study of alcohol utilization, flies were kept in ventilated cages<sup>4</sup> and the alcoholic solution was incorporated to an agar gel. In these experiments, the flies were able to ingest alcohol but it was also supposed that some alcohol could also penetrate directly into the body through respiration.

The present work was undertaken to test this hypothesis: we show that the feeding value of ethanol is almost the same when only vapors are provided as when adults have a direct access to the solution. Two experimental tech-

niques were compared. In the first, flies were kept in a double, ventilated cage. In the second, they were put in the upper compartment of an air-tight tube. Schemes and further explanations are given in figure 1. With both techniques, control experiments were made in which flies had a direct access to the alcoholic solution.

The results, obtained with  $F_1$  heterozygotes issued from a cross between 2 laboratory strains, are shown in figure 2. With the ventilated cage technique (figure 2A), life duration of controls increased with alcohol concentration up to an optimum of about 10% and then decreased almost linearly. When flies had no access to the alcoholic solution, a fairly similar curve was observed but the toxic effects

- 1 We thank R. Grantham for help with the manuscript.
- 2 J. David, P. Fouillet and M. F. Arens, *Archs Zool. exp. gén.* 115, 401 (1974).
- 3 J. David, C. Bocquet, M. F. Arens and P. Fouillet, *Biochem. Genet.* 14, 989 (1976).
- 4 J. Van Herrewege and J. David, *C. r. Acad. Sci. Paris* 279, 335 (1974).
- 5 M. Libion-Mannaert, J. Delcour, M. C. Deltombe-Lietaert, N. Lenelle-Montfort and A. Elens, *Experientia* 32, 22 (1976).

were less pronounced: even with an ethanol concentration of 64% in the inaccessible compartment of the cage, a significant increase of survival duration was observed.

With air-tight tubes (figure 2B) an increase in lifespan was also observed with low concentrations of alcohol. As previously, an increase in life expectancy due to alcohol vapor alone was observed and the slope of the decreasing part of the curve was also lower than for the controls. Differences with the results of figure 2A are to be noted: a) the optimum concentration was much lower, about 4% instead of 10%; b) the maximum survival was lower, about 140 h instead of 200; c) the maximum lifespan was significantly shorter when the flies received only alcohol vapor.

Our results confirm that alcohol is easily used by *Drosophila* adults, although the maximum increase in lifespan is much less than with a sugar<sup>6</sup>. This difference is probably due to toxic effects of high alcohol concentrations. Differences associated with technique seem easy to interpret: with ventilated cages, some of the alcohol evaporates and is lost, so that higher concentrations are needed to reach optimum and toxic levels. With the closed tube technique,

the lower maximum lifespan could be due to the fact that, in this case, flies were submitted not only to alcohol but also to a noxious effect of a partial anoxia.

The most interesting result is the good survival observed when only alcohol vapor is available. Only in one case (air-tight tubes) was the maximum lifespan shorter than with control flies. This difference could be explained by the fact that, in this case, adults which were submitted to alcohol vapor, had no water to drink.

When alcohol is ingested it is probable that, in *Drosophila* as in mammals, it crosses the intestinal barrier and enters the blood. Furnishing the vapor avoids this barrier and allows direct dissolution into the hemolymph. Therefore, alcohol vapor can be metabolized and used for energy production; such a process could probably occur with several other volatile compounds and in many different species. *D. melanogaster* adults are probably the best experimental material for demonstrating this phenomenon because they tolerate high concentrations of alcohol and because their life expectancy is greatly increased by an energy source only.

It has recently been shown that ethanol is a very significant parameter in *Drosophila* ecology<sup>7</sup>. Adults are attracted by ethanol and other products of the alcoholic fermentation which act as odoriferous signals for feeding and oviposition sites. When a site is discovered, *Drosophila* adults fill their crop, ingesting many yeast cells. They also often stay for a long time on the food, entering the small cavities of fermenting fruits. It is likely, although no precise measurements are available, that, in such cavities, the concentration of alcohol vapor can reach a fairly high level. The capacity of *Drosophila* adults to use nutritive vapors could be significantly useful to wild flies, as has recently been suggested<sup>8</sup>.

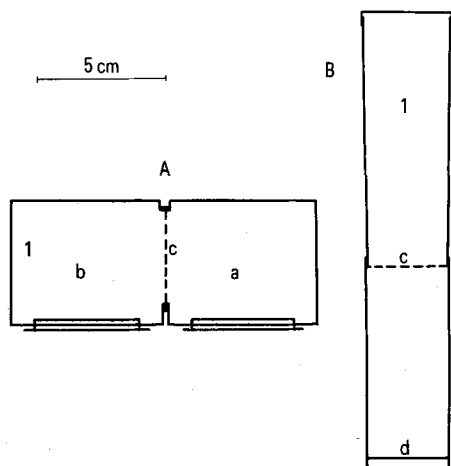


Fig. 1. Techniques used for testing the nutritive value of alcohol vapor. A Ventilated cage; B air tight tube. (a Wire screen; b agar and water; c agar gel with alcohol solution; d alcohol solution; in all cases, flies were kept in the compartment 1.)

6 J. Van Herreweghe, C. r. Acad. Sci. Paris 276, 2565 (1973).

7 J. A. McKenzie and P. A. Parsons, Oecologia, Berl. 10, 373 (1972).

8 W. T. Starmer, W. B. Weed and E. S. Rockwood-Sluss, Proc. nat. Acad. Sci. USA 74, 387 (1977).

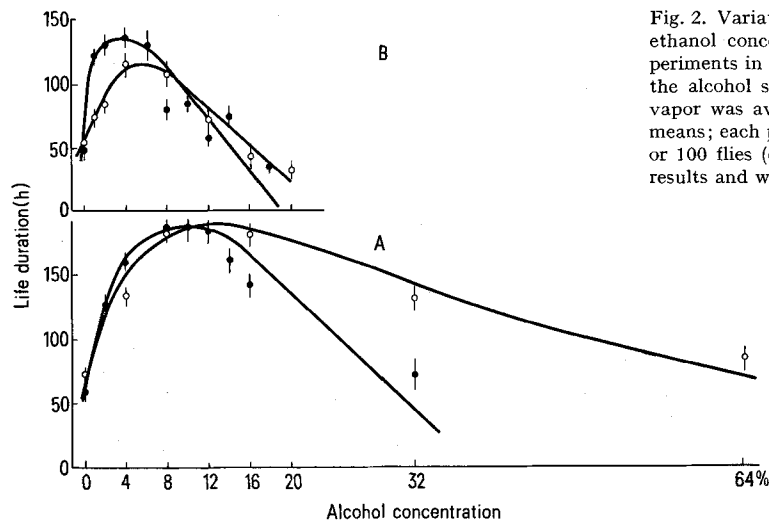


Fig. 2. Variation of lifespan of *Drosophila* adults as a function of ethanol concentration. A Experiment in ventilated cages; B experiments in air tight tubes. ●, Control flies having direct access to the alcohol solution; ×, experimental flies to which only alcohol vapor was available. Vertical bars indicate confidence intervals of means; each point corresponds to results on 200 flies (control curve) or 100 flies (experimental curves); males and females gave similar results and were averaged.