

**Results and discussion.** Figure 1 shows the time course of 3-0-methylglucose uptake by 2 populations of mature amphibian oocytes. In both group A (wet weight:  $2.92 \pm 0.02$  mg; %  $H_2O$ :  $44.8 \pm 0.2$ ) and group B (wet weight:  $2.10 \pm 0.01$  mg; %  $H_2O$ :  $45.7 \pm 0.0$ ) solute uptake was gradual, approaching the extracellular concentration of 3-0-MG after approximately 27 h of incubation. In contrast, the uptake of the non-metabolizable amino acid,  $\alpha$ -aminoisobutyric acid, exhibits the properties of concentrative uptake and competitive flux inhibition<sup>5</sup>. As illustrated in figure 1, the transport of 3-0-methylglucose across the oocyte plasma membrane appears to be a diffusional process and does not resemble the energy-dependent concentrative uptake of sugars exhibited by the cells of the small intestine and the kidney<sup>7</sup>.

The time courses of uptake of this non-metabolizable monosaccharide in the nucleus and cytoplasm as determined by microdissection were similar to that in the whole oocytes (figure 2B). Both compartments exhibited a gradual uptake of 3-0-MG, approaching and exceeding the extracellular concentration after approximately 27 h of incubation. During the 27 h of influx in 24 mM 3-0-MG, the concentration ratios for the nucleus ( $C_n/C_o$ ) and cytoplasm ( $C_c/C_o$ ) with respect to the 3-0-MG Ringer's were  $1.48 \pm 0.08$  and  $0.92 \pm 0.08$ , respectively.

During the influx the nucleocytoplasmic ratio,  $K_{n/c}$ , remained constant at  $1.54 \pm 0.08$  (figure 2A). This suggests that any diffusional delay at the nuclear envelope is small relative to that at the plasma membrane and that saturable carriers are not involved in the nucleocytoplasmic transport process. The observed kinetics can be explained by the permeation of 3-0-MG across the nuclear en-

velope by diffusion processes. The nucleocytoplasmic asymmetry for 3-0-MG is consistent with previous demonstrations that the nuclear envelope is not a permeability barrier for the disaccharide sucrose<sup>8-10</sup>. An exception is the observation that the nuclear envelope of intestinal cells appears to be a barrier to galactose permeation<sup>11</sup>.

In the absence of selective mechanisms for sugar permeation and uptake across the nuclear membrane, other processes must exist to explain the observed asymmetries. Horowitz and Moore<sup>12</sup> have proposed that the nucleocytoplasmic asymmetries observed for other solutes, including glycerol<sup>6</sup>, sucrose<sup>8,10</sup>,  $\alpha$ -aminoisobutyric acid<sup>5</sup>, inulin<sup>12</sup> and dextran<sup>13</sup> are the result of nonmembrane processes. In particular, the determinants of these nucleocytoplasmic solute asymmetries appear to be equilibrium processes such as macromolecular binding and the related phenomenon of differential solubility in the water of nucleoplasm and the cytoplasm. While this study does not specify the mechanism(s) responsible for the partition of 3-0-methylglucose between the nucleus and cytoplasm, the results can be explained by the partial exclusion of the solute from the water in the cytoplasm.

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## Ethanol: Larval discrimination between two *Drosophila* sibling species<sup>1</sup>

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**Summary.** Newly hatched larvae of *D. melanogaster* preferentially migrate to agar containing ethanol, whereas its sibling species *D. simulans* shows no initial preference. This can be related to the ecological biology of resource utilization in the wild.

Little is known of the ecological biology of the majority of *Drosophila* species. Even the cosmopolitan siblings *melanogaster* and *simulans*, although finding very widespread use in genetical research, have not been studied in great depth ecologically. It has been shown that *melanogaster* adults and larvae are more tolerant to ethanol in the laboratory and in nature than *simulans*<sup>2</sup>, apparently because the former species is better able to utilize ethanol as a food resource than the latter; *melanogaster* adults have also been shown to migrate towards wine fermentation tanks during vintage while *simulans* adults move in the opposite direction<sup>3</sup>. Although resources exploited at the larval stage are of obvious importance for the development of *Drosophila* species, few reports consider this stage<sup>4</sup>. We report here on a behavioural difference between these 2 species whereby newly hatched first instar *melanogaster* larvae preferentially migrate to agar containing ethanol, while the movement of *simulans* larvae is initially independent of ethanol.

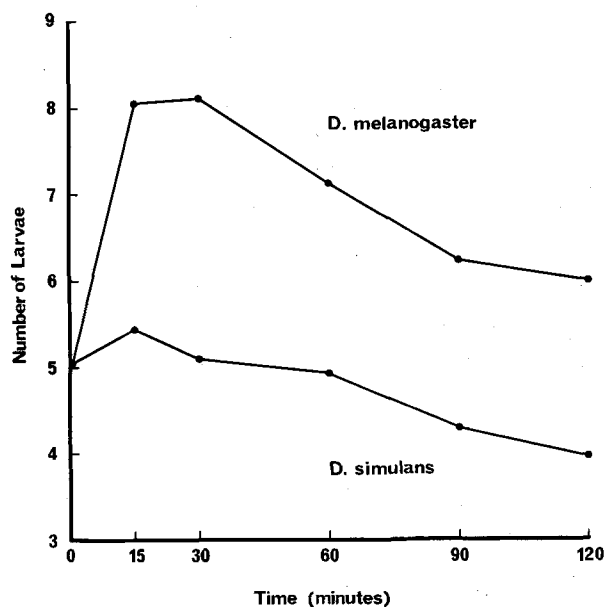
10 newly hatched larvae were placed centrally on a Petri dish containing agar; 1 semicircle of the agar contained 6% ethanol. The relative numbers on the 2 sectors were

noted for periods up to 2 h. We tested 6 isofemale strains of *simulans* and 5 of *melanogaster* derived from a Melbourne population (10 replicates per strain). There was no overlap across species and means across strains were therefore pooled; they are plotted as mean numbers choosing agar with ethanol up to 120 min from the start of the experiment in the figure.

*D. melanogaster* larvae showed a clear initial preference for ethanol which slowly diminished with time. The larvae crawled about on the agar presumably in search of food which, apart from the ethanol<sup>5</sup>, was not provided; the slow fall in the number on the ethanol-containing agar with time thus appears reasonable. *D. simulans* larvae on the other hand showed no initial preference, although there was a slow fall in the number of larvae on ethanol-containing agar with time, suggesting that *simulans* larvae slowly begin to exhibit a tendency towards alcohol avoidance.

These results parallel those acquired for oviposition; *melanogaster* frequently shows a preference for ethanol-containing media while *simulans* tends to oviposit on media without ethanol<sup>2</sup>. However, the results are far more un-

equivocal than oviposition data<sup>6</sup>, suggesting a much higher level of specificity for larval behaviour. This is consistent with the exploitation of a variety of foods by a cosmopolitan species since, following hatching, larvae can



Mean number of larvae out of 10 on ethanol containing agar up to 120 min for the 2 sibling species.

move to the most beneficial resources available to them. However, in many rare endemic species, quite specific oviposition stimuli presumably occur because of highly specific larval resource exploitation, often involving parts of particular plant species<sup>7</sup>.

The known ecological differences between these 2 species are principally quantitative rather than qualitative, both species apparently using rather similar resources<sup>8</sup> with the single exception of ethanol. However, since studies concentrating on larvae are rare, future research could alter this situation. The comparative study of resource utilization by larvae of different *Drosophila* species remains an open field, and detailed investigations in particular of sibling species may yield interesting information concerning evolutionary divergence.

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## Adenylate cyclase activation by trypsin in KB cell cultures

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**Summary.** Trypsin commonly used for cell dispersion increases adenylate cyclase activity of KB cells. It acts on catalytic receptors, since the apparent  $K_m$  for ATP is lowered, and it alters the dependence of adenylate cyclase on  $Mg^{++}$  ions.

Evidence has accumulated suggesting that cyclic AMP can influence cell divisions in a number of cell types in culture<sup>1,2</sup>. Certain agents, such as pronase, which are known to act on the cell surface, can initiate cell divisions. Bombick and Burger<sup>3</sup> have shown that the action of such agents can be blocked by the addition of dibutyryl cyclic AMP. It seems that extracellular conditions may control cell divisions by affecting the membranal components of the cyclic AMP system, i.e. the adenylate cyclase. This paper describes the effects on adenylate cyclase of procedures commonly used to detach cells from flasks during cell cultures.

**Materials and methods.** KB cells were grown in 75 cm culture surface Falcon flasks containing 20 ml of Eagle's minimal essential supplemented with 10% calf serum, 0.25% sodium bicarbonate and 0.005% aureomycin (pH = 7.1). Approximately  $8 \times 10^6$  cells were seeded from confluent cells dispersed by one of the 3 commonly used methods: scraping with glass taws, treatment with 0.25% trypsin for 2 min, or treatment with 2.5% ethylene diamine tetraacetic sodium salt (EDTA) for 10 min.

Changes of media for the feeding of culture were made 42 h later. In these conditions, confluency is obtained 48 h later. For cell numerations, cell viability was routinely tested by eosine dye exclusion. For adenylate cyclase assay cells were harvested by scraping in 25 mM Tris-HCl pH = 7.6 supplemented with 1 mM  $MgCl_2$  and 250 mM saccharose, broken at 4°C and centrifugated at 600 × g for 10 min, the pellet was routinely used for assays. 3 culture flasks were pooled for each enzyme activity determination. Assay reaction constituents<sup>4</sup> included 2 mM ( $\alpha^{32}P$ )-ATP 1  $\mu Ci$ , 1 mg/ml creatine phosphokinase, 20 mM creatine phosphate, 1 mg/ml bovine serum albumine, 10 mM  $MgCl_2$  and enzyme (approximately

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