SAM ([$^3$H]-S-adenosyl-methionine) from CEA Saclay, 13.5 Ci/mmole in 60 µl of tris-HCl buffer 0.05 M (pH 8.6). After 45 min incubation, the reaction was stopped by the addition of 200 µl of 0.5 M borate buffer (pH 11) saturated with sodium chloride and containing *p*-synephrine, norphenylephrine and N-methylphenylethanolamine (1 µg each). N-methylated amines were extracted with 5 ml ethylacetate and centrifuged for 5 min at 10,000 × g. After reaction with dansylchloride, the 3 methylated amines were separated using the different and consecutive chromatographic systems[3]. The radioactivity was found to be associated (> 90%) with *p*-octopamine.

*Results.* As depicted in table 3, the *p*-octopamine levels are significantly higher in heads and bodies from hyperactive animals as compared to hypoactive ants. This difference was more significant in the heads (1.58 and 3.83) than in the bodies (0.67 and 1.32).

*Discussion.* Endocrine glandular activity and metabolic needs in animals are known to be closely related[12]. Moreover, it has been shown that glandular cells of the retrocerebral system of hyperactive workers contain numerous dense granules and large dense bodies[13]. Those large dense bodies may contain octopamine, as has already been postulated for insects[14]. It was therefore of great interest to try to distinguish a difference between the 2 categories of ants (hyperactive and hypoactive ants) on the basis of biochemical characteristics.

Catecholamines and serotonin are supposed to be involved in aggressive behavior in ants[15,16]. However, to our knowledge, octopamine has never been described in these animals. Moreover, the amount found in this study is high as compared to other invertebrates[5,6]. The observation of considerably larger amounts in the head may indicate its association with the CNS of ants; further investigations at the brain level are necessary. Of great interest is the relationship between the activity and the levels of octopamine. This observation is not limited to mammals[8] but applies also to invertebrates. It may indicate a general involvement of octopamine in behavior. However, the relationship between the not, as yet, well delineated biochemical functions of octopamine and higher activity in animals has still to be clarified.

Table 2. Activity coefficient of ants selected for experiments

| Hypo $k_a$ | No. of individuals | Hyper $k_a$ | No. of individuals |
|---|---|---|---|
| 1.41 | 6 | 4.50 | 6 |
| 1.33 | 9 | 4.58 | 4 |
| 1.25 | 3 | 4.66 | 6 |
| 1.08 | 2 | 4.75 | 2 |
| | | 4.81 | 1 |
| | | 4.91 | 1 |

Table 3. Octopamine content of heads and bodies of hyper- and hypo-active ants

| Heads | | Bodies | |
|---|---|---|---|
| Hypo | Hyper | Hypo | Hyper |
| 1.58 ± 0.22 | 3.83 ± 0.20* | 0.673 ± 0.05 | 1.32 ± 0.05* |

The octopamine values are expressed in µg/g of tissues ± SEM from assays on 4 pools (heads) and 3 pools (bodies) each containing 5 pieces. The various pools were formed directly from the selected ants, whose $k_a$ coefficient is given table 2. (B, C, D individuals in table 1 were used in previous trials and did not appear in table 3). (* = $p < 0.001$ as compared to hypo).


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## How can *Drosophila* flies without aldehyde oxidase detoxify acetaldehyde?[1]


M.C. Lietaert, M. Libion-Mannaert, N. Hougouto and A. Elens

*Unité de Génétique et Physiologie Cellulaire, Facultés Universitaires N.D. de la Paix, 61, rue de Bruxelles, B-5000 Namur (Belgium), 30 November 1981*



*Summary.* The flies of a *Drosophila melanogaster* strain, called 'AO null' as it lacks the enzyme aldehyde oxidase, are nevertheless able to detoxify acetaldehyde. It seems that this action could be attributed to aldehyde dehydrogenase, or some other enzyme which ressembles aldehyde dehydrogenase.


In a previous publication[2] we questioned the almost exclusive role attributed to aldehyde oxidase (AO) in the degradation of the acetaldehyde resulting from the metabolic oxidation of ethanol by alcoholdehydrogenase (ADH),

catalase, or other enzymes in *Drosophila melanogaster*. Acetaldehyde is a highly dangerous substance, which therefore must be immediately converted into nontoxic products. However, experiments in vivo have shown that a mutant strain, called 'AO null' although it has no active AO[3], is nevertheless relatively tolerant to ethanol[4-5]. Experiments in vitro have shown that, in homogenates from 'AO null' flies, the acetaldehyde degradation is significant. As this acetaldehyde degradation cannot be due to aldehyde oxidase, one has to consider the hypothesis of some other enzyme acting on acetaldehyde.

From some recent papers concerning acetaldehyde in mammalian tissues[6-8], the first enzyme one could think about as a possible candidate is aldehyde dehydrogenase (ALDH).

10-day-old flies of the strain 'AO null' (y v f ma-$1^{bz}$)[11], known to lack any active AO[2-5], were used. As in our previous experiments, the flies were grown on axenic medium[9], and were 'deyeasted' before all the tests[10].

The ALDH activity was determined according to Lundquist[11], with a slight modification of his method: pyrazole was added to the homogenate in order to inhibit ADH, which could restore some ethanol from the initially present acetaldehyde. Using this method, we have been unable to detect even a slight ALDH activity in homogenates from our 'AO null' flies (as well as in homogenates from flies of other strains).

There is, however, another way to examine a possible action of ALDH: it is to try to suppress the acetaldehyde degradation in homogenates by the use of the well known inhibitors of ALDH, as has been done for mammalian tissue homogenates. If one succeeds, a participation of aldehyde dehydrogenase in the metabolic detoxification of acetaldehyde could be considered as highly probable, although it has been impossible to demonstrate its presence using the Lundquist method.

The flies were immobilized by cooling, immediately before homogeneization in 0.05 M Tris buffer at pH 7.5. The homogenate was centrifuged at 27,000 × g at 0 °C. Aliquots of 60 µl of supernatant were placed in 5 ml vials with 10 µl 200 mM pyrazole (ADH inhibitor) and, when indicated, 10 µl of each one of the ALDH inhibitors: cyanamide 10 M[6], pyrgyline 5 M[8], and disulfiram in saturated solution[7]. Hermetically sealed, the vials were incubated for 1 h in a waterbath at 0 °C. 10 µl 40 mM acetaldehyde were then added, and the vials were again incubated for 1 h, this time at 15 °C. Together with each incubation batch was also incubated a 5 ml control vial, containing: 60 µl 0.25 M sucrose Tris buffer, 10 µl 200 mM pyrazole, and 10 µl Tris buffer. The values thus obtained are used as evaporation controls.

Artificial systems containing ALDH were used in similar conditions, as a comparison. 40 µl ALDH (600 U/ml Tris buffer), 20 µl NAD (10 mg in 2.5 ml Tris buffer), 10 µl pyrazole 200 mM and 10 µl Tris buffer were placed in 5 ml vials with, when indicated, 10 µl of the same inhibitors as previously: cyanamide 10 M, pargyline 5 M, or disulfiram (saturated solution). Hermetically sealed, the vials were incubated for 1 h at 0 °C; 10 µl 40 mM acetaldehyde were then added and reincubated for 1 h at 15 °C.

After incubation, the aliquots received 10 µl 40 mM isopropanol, which is used as reference standard in the gas-liquid chromatographic test, as its retention time is slightly superior to the retention time of acetaldehyde or ethanol. 1 µl of this mixture was injected into the gas-liquid chromatography column. This column, 213.4 cm in length and 0.32 cm in diameter, is filled with 150-200 mesh Q Porapak. The temperature was 160 °C for the column and 220 °C for the hydrogen flame detector. Nitrogen was used as carrier gas. The chromatograph is a Sigma 4 Perkin-Elmer.

The enzymatic actions were estimated by the loss of acetaldehyde concentration observed at the end of the experiment.

The results are summarized in the table. They were very similar for the fly homogenates and for the artificial systems containing ALDH. The acetaldehyde concentration loss was almost the same, as were the effects of the inhibitors. The disulfiram had very little activity, the inhibition by cyanamide was almost complete, and the action of pargyline was intermediate.

Such observations are in good agreement with the hypothesis of an active aldehyde dehydrogenase present in the 'AO null' flies. This way their tolerance to ethanol could be explained as well as their ability to use it as a 'food'[5]. They have a relatively high ADH activity level, and the acetaldehyde produced can be immediately converted into acetate, in spite of a lack of aldehyde oxidase.

The fact remains, of course, that we did not succeed in detecting any trace of aldehyde dehydrogenase in the homogenates of our flies. Perhaps we have to question the procedure used in the preparation of the homogenates or the accuracy obtained by us with the Lundquist method. It is also quite possible that the enzyme acting to degrade acetaldehyde is not ALDH but some other enzyme with similar properties.

The effect of cyanamide, pargyline, and disulfiram on the degradation of acetaldehyde by homogenates from 'AO null' flies and by control artifical systems containing aldehyde dehydrogenase

| | Acetaldehyde degradation | | | |
|---|---|---|---|---|
| | ALDH controls | | 'AO null' flies | |
| | mg/h | % of total activity | mg/h | % of total activity |
| Total activity | $6.47 \times 10^{-3}$ | 100.00 | $4.79 \times 10^{-3}$ | 100.00 |
| Cyanamide effect | $0.70 \times 10^{-3}$ | 10.82 | 0.00 | 0.00 |
| Pargyline effect | $4.22 \times 10^{-3}$ | 65.22 | $1.98 \times 10^{-3}$ | 41.37 |
| Disulfiram effect | $5.89 \times 10^{-3}$ | 91.03 | $4.75 \times 10^{-3}$ | 99.16 |

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