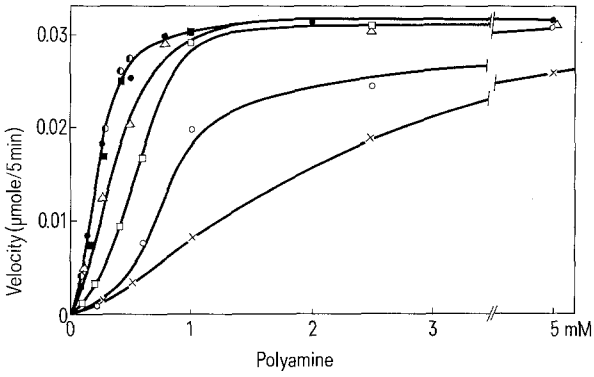
partially in several Mg^{2+}-requiring reactions[6-8] and AMP nucleosidase also requires Mg-ATP or Mg-PPi absolutely[17]. However, Mg^{2+} ions are suggested to be entirely replaced by polyamines in AMP nucleosidase reaction, since the maximal velocities attained in the presence of saturated concentrations of polyamines and Mg^{2+} ions were almost identical (data not shown).

The present study shows that polyamines express a powerful activating effect on the activity of AMP nucleosidase. Of polyamines tested, spermine, spermidine and some diamines, which are generally found in bacterial cells[1,2], showed a most striking activation of the enzyme, and furthermore, the concentrations necessary for 50% activation of the enzyme activity for these physiological amines were in the range 0.2–0.5 mM. It is thus reasonable to assume that these amines are physiological regulators of the enzyme in *A. vinelandii*. AMP nucleosidase may be a regulatory enzyme in the conversion of adenine nucleotides to inosine or guanine nucleotides[15,16] and to stabilize the adenylate energy charge[14] in *A. vinelandii*. The role of the enzyme in the control of adenine nucleotide metabolism is strengthened by the lack or very low activity of AMP deaminase and 5′-nucleotidase (EC 3.1.3.5)[12,14,15]. The present result suggests that the increase in polyamines activates the activity of AMP nucleosidase, which will result in the purine nucleotide synthesis and stabilization of energy charge in the exponential stage of bacterial growth. It should be emphasized that polyamines can regulate the purine nucleotide synthesis and the adenylate energy charge through the activation of AMP nucleosidase and AMP deaminase in prokaryotes and eukaryotes, respectively.



Effect of concentrations of polyamines and diamines on the activity of AMP nucleosidase. The enzyme was purified from *A. vinelandii*, strain 0, as described previously[15] with a slight modification: protamine treatment was replaced by aminohexyl-Sepharose (Pharmacia) chromatography. Enzyme activity was measured by estimating production of ribose 5-phosphate[18]. The reaction mixture contained 0.5 mM ATP, 1 mM AMP, 5 mM Tris-HCl buffer, pH 8.0, various concentrations of polyamines and the enzyme in a final volume of 0.2 ml. The reaction was carried out at 37 °C for 5 min and terminated by the addition of the reducing sugar reagent of Dygert et al.[18]. ◐: Spermine, ■: spermidine, ●: diaminohexane, △: cadaverine, □: putrescine, ○: propylenediamine, ×: ethylenediamine.

1 D.R. Morris and R.H. Fillingame, A. Rev. Biochem. *43*, 303 (1974).
2 H. Tabor and C.W. Tabor, Adv. Enzymol. *36*, 203 (1972).
3 W.G. Dyksa, Jr, and E.J. Herbst, Science *149*, 428 (1965).
4 C.M. Caldarera, B. Barbiroli and G. Moruzzi, Biochem. J. *97*, 84 (1965).
5 J.E. Kay and V.J. Lindsay, Exp. Cell. Res. *77*, 428 (1973).
6 S. Yoshida, S. Masaki and T. Ando, J. Biochem. *79*, 895 (1976).
7 H. Fukuyama and S. Yamashita, FEBS Lett. *71*, 33 (1976).
8 H. Tanigawa, M. Kawamura and M. Shimoyama, Biochem. biophys. Res. Commun. *76*, 406 (1977).
9 C.C. Levy, W.E. Mitch and M. Schmukler, J. biol. Chem. *248*, 5712 (1973).
10 Y. Tashima, M. Hasegawa and H. Mizunuma, Biochem. biophys. Res. Commun. *82*, 13 (1978).
11 M. Yoshino, K. Murakami and K. Tsushima, Biochim. biophys. Acta *542*, 177 (1978).
12 A.G. Chapman and D.E. Atkinson, J. biol. Chem. *248*, 8309 (1973).
13 C.L. Zielke and C.H. Suelter, in: The Enzymes, vol. 4, 3rd ed., p. 47. Ed. P.D. Boyer. Academic Press, New York and London 1971.
14 V.L. Schramm and H. Leung, J. biol. Chem. *248*, 8313 (1973).
15 M. Yoshino, J. Biochem. *68*, 321 (1970).
16 M. Yoshino and N. Ogasawara, J. Biochem. *72*, 223 (1972).
17 J. Hurwitz, L.A. Heppel and B.L. Horecker, J. biol. Chem. *226*, 525 (1957).
18 S. Dygert, L.H. Li, D. Florida and J.A. Thoma, Analyt. Biochem. *13*, 367 (1974).

Ethanol metabolism in *Drosophila melanogaster*

M.C. Deltombe-Lietaert, J. Delcour, N. Lenelle-Monfort and A. Elens

Unité de Génétique et Physiologie Cellulaire, Facultés N.D. de la Paix, 61, rue de Bruxelles, B-5000 Namur (Belgium), 4 August 1978

Summary. A quantitative study of the transformation of ethanol into acetaldehyde shows that, in *Drosophila melanogaster*, the mitochondrial ethanol oxidizing system is not very active but that the part played by catalase appears more important than expected. For a strain without alcoholdehydrogenase, ethanol is highly toxic. The presence of acetaldehyde in the culture medium is toxic for all the strains studied. But, since even a strain without any aldehydeoxidase lives normally, the metabolic production of acetaldehyde does not seem dangerous.

The physiological and ecological importance of ethanol tolerance for *D. melanogaster* has been emphasized by many authors[1-6]. Adapting itself to temperate climates, this species is considered to have modified its ecological niche in order to exploit food sources characterized by a higher ethanol concentration, as e.g. in vinification caves and breweries. A nutritional value of ethanol has even been demonstrated, since the survival time of flies without food is greater in presence of ethanol[7,8].

Ethanol tolerance appears to be related in some way to the presence of alcohol dehydrogenase (ADH)[9-13]. The survival time without food but in presence of ethanol is greater for flies from strains with a higher ADH level[14]. The correlation between survival time and ADH level is however not a

perfect one, suggesting an intervention of other factors than ADH.

Some recent publications concerning the pathways of ethanol metabolism in mammalian tissues[15-20] may help to identify some of those factors. If ethanol oxidation is generally accepted to occur primarily via ADH catalysis, a role for catalase has also been demonstrated. Furthermore, one has to consider a microsomal ethanol oxidizing system (MEOS) which also plays a part in oxidation, at least in mammalian tissues. All 3 enzymatic systems transform ethanol into acetaldehyde, the subsequent fate of which is not well-known, although aldehyde oxidase (AO) is credited with playing a major role in its conversion into acetate. Acetyl-thiokinase and acetyl-coenzyme A act in the following stages of ethanol metabolism, eventually giving products which may have an energetical action, responsible for the ADH nutritional value of ethanol (figure 1).

Pyrazole inhibits the ADH activity, and sodium-azide inhibits the catalase activity. Consequently, one may use these inhibitors in order to study, quantitatively as well as qualitatively, the pathways of ethanol metabolism, determining by elimination the part played by each of the enzymatic systems.

Similar methods have been applied by us to *Drosophila* flies. The flies were grown on axenic medium, according to David and Clavel[21] and 'deyeasted' according to Day et al.[22] before the test. Immediately before homogenization, they were immobilized by cooling them. For each experiment, 500 mg of adult flies were homogenized with a Potter homogenizer in 0.75 ml of 0.25 M sucrose Tris buffer at pH 7.5. The homogenate was centrifugated at 15,000 rpm for 30 min. 40 µl aliquots of supernatant were then placed in 5 ml vials, with 40 µl 7.5 mM ethanol, 10 µl 0.05 M Tris buffer (pH 7.5) and, when indicated, the following compounds: pyrazole 2 mM, sodium-azide 1 mM or both inhibitors together.

The vials were then sealed hermetically and incubated for 1 h in a waterbath at 30 °C. Together with each incubation batch was also incubated a 5 ml vial containing 40 µl ¼ N sucrose, 40 µl ethanol 7.5 M and 10 µl 0.05 M Tris buffer (pH 7.5). The values thus obtained are used as evaporation control and substracted from the corresponding experimental values.

After incubation, the aliquots received 10 µl 25 mM isopropanol, which is used as a reference standard in the gas chromatograph test, as its retention time is slightly superior to the retention time of ethanol. 2 µl of this mixture were then injected in the gas chromatograph.

The Varian gas chromatograph column, 7 foot in length and ⅛ inch in diameter, is filled with 150-200 mesh Q Porapak. Nitrogen was employed as a carrier gas at a flow rate of 40 ml/min. The temperature was 140 °C for the column, 180 °C for the injector and 200 °C for the hydrogen flame detector. Solutions with known amounts of ethanol served as standards.

Flies of 3 strains have been assayed. These strains had been chosen for the contrasted patterns of their enzymatic activities, determined according to Sofer and Ursprung[23] for ADH, Baudhuin et al.[24] for catalase, Courtright[25] for AO (table 1).

The concentration loss observed when both catalase and ADH are inhibited do not differ significantly from the values obtained for the evaporation control, for no one of the 3 assayed strains. One is allowed to conclude that the MEOS do not have any significant action in *Drosophila* flies, contrary to what is described for mammalian tissues.

Table 1. Enzymatic activities in the 3 strains

Strains	Enzymes Alcohol dehydrogenase	Catalase	Aldehyde oxydase
e^+ 0612a	437.37 ± 17.6	349.94 ± 27.75	7219.97 ± 546.78
184 (AO null)	465.52 ± 19.81	275.03 ± 33.61	0.00
Black n4 (ADH null)	0.00	348.31 ± 15.42	7958.86 ± 384.10

Activities in unit./g prot., with SE.

Table 2. Ethanol toxicity, in presence of various concentrations of acetaldehyde

Strains	Acetaldehyde concentration (%) 0.00		0.25		0.50		0.75		1.00	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
	LC_{50} ethanol concentrations									
612a	15.6	9.8	16.1	9.6	13.4	8.9	11.9	3.5	7.5	+
184	7.1	6.5	6.0	2.7	+	+	+	+	+	+
Black n4	2.6	2.2	2.3	1.8	0.5	+	+	+	+	+

+: 50% of the flies have been killed before the measure.

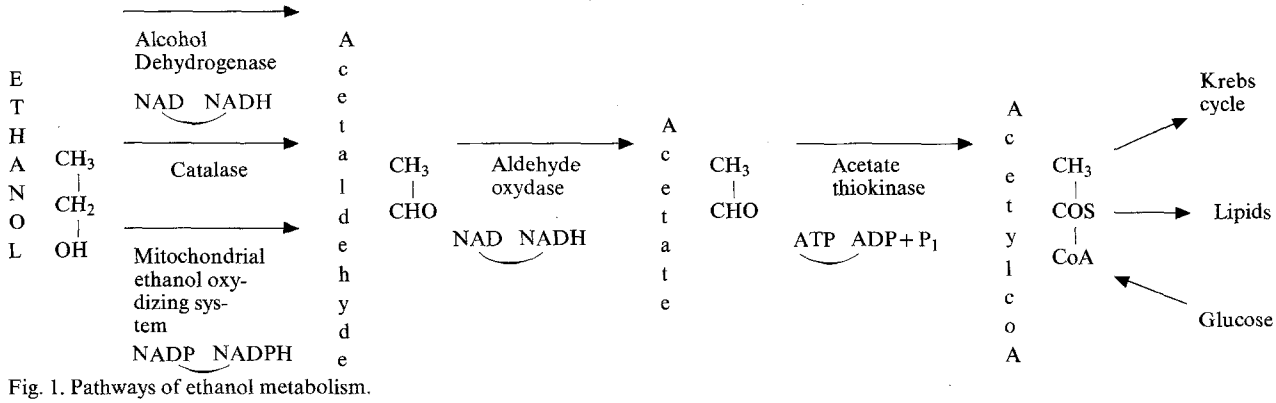


Fig. 1. Pathways of ethanol metabolism.

The total concentration loss is clearly the sum of the catalase and ADH actions, as can be seen by comparing it with the concentration losses due to ADH alone on the one hand (in presence of sodium-azide) and to catalase alone on the other hand (in presence of pyrazole). In some mammalian tissues[18] pyrazole is said to inhibit not only ADH but in a lesser degree catalase action as well. Obviously, the reactions are not the same for *Drosophila* flies, in the present case at least (figure 2).

As in mammalian tissues, the part played by catalase in oxidations is important. Such a striking role of catalase was unexpected, especially from all the above quoted publications, which had given much more attention to ADH. But so important a catalase action can probably explain how even strains with a low ADH activity are able to use ethanol as food[14].

The same 3 strains have also been submitted to tolerance experiments, according to David et al.[26], and to survival experiments without food but in presence of ethanol, according to Libion-Mannaert et al.[14]. In the tolerance experiments, the LC_{50} ethanol concentrations in hermetically closed vials were determined, for the first and second days, in presence of various concentrations of acetaldehyde. Acetaldehyde is really highly toxic, especially for the 'AO null' strain which lacks AO; ethanol is peculiarly dangerous for the 'ADH null strain' (table 2). Such results were to be expected from the spectrophotometric and gas-chromatographic records. In the survival experiments, the behaviour of the e^+612a and the 'ADH null' strains are in agreement with expectations. But the 'AO null' strain survives reasonably well (figure 3). Such observations do not fit exactly with the above proposed ethanol metabolic pathways. Another way for the transformation of acetaldehyde into acetate must exist and, as has recently been suggested[27], AO has probably not the exclusive role with which it had formerly been credited.

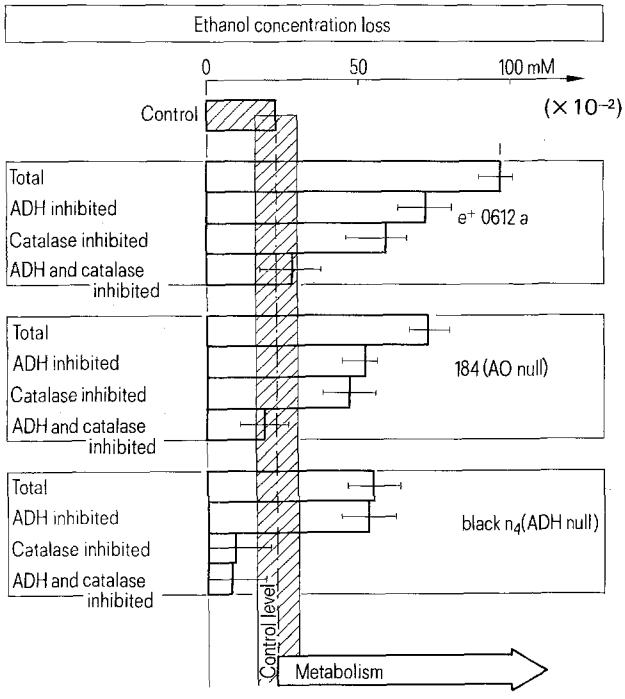


Fig. 2. Ethanol concentration loss.

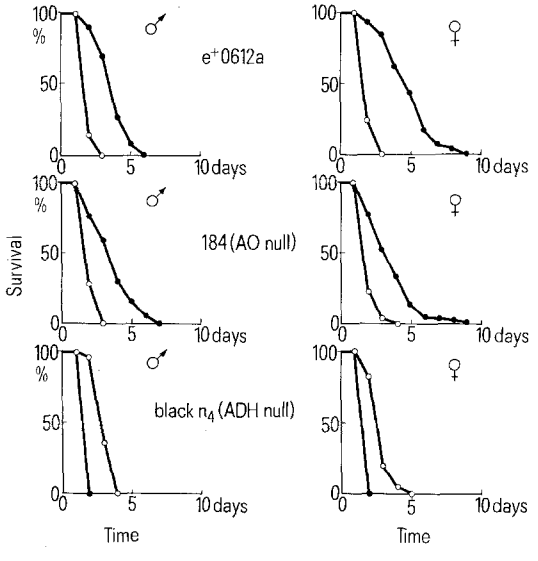


Fig. 3. Survival time of starving flies in the presence (closed symbols) or in the absence (open symbols) of ethanol.

1 J.A. McKenzie and P.A. Parsons, Oecologia, Berl. *10*, 373 (1972).
2 J.A. McKenzie and P.A. Parsons, Genetics *77*, 385 (1974).
3 J. David and C. Bocquet, C. r. Acad. Sci. Paris *279*, 1385 (1974).
4 J. David and C. Bocquet, Experientia *31*, 164 (1975).
5 J. David, C. Bocquet, M.F. Arens and P. Fouillet, Biochem. Genet. *14*, 989 (1976).
6 J. David, Ann. Biol. *16*, 451 (1977).
7 M. Libion-Mannaert, M.C. Deltombe-Lietaert and A. Elens, Abstr. 4th Eur. Drosoph. Res. Conf., Umea, Sweden (1974).
8 J. van Herrewege and J. David, C. r. Acad. Sci. Paris *279*, 335 (1974).
9 E.H. Grell, K.B. Jacobson and J.B. Murphy, Science *149*, 80 (1965).
10 E.H. Grell, K.B. Jacobson and J.B. Murphy, Ann. N.Y. Acad. Sci. *151*, 441 (1968).
11 C.L. Vigue and F.M. Johnson, Biochem. Genet. *9*, 213 (1973).
12 J. Gibson, Nature *227*, 959 (1970).
13 B. Clarke, Genetics *79*, 101 (1975).
14 M. Libion-Mannaert, J. Delcour, M.C. Deltombe-Lietaert, N. Lenelle-Monfort and A. Elens, Experientia *32*, 22 (1976).
15 E. Feytmans and F. Leighton, Biochem. Pharmac. *22*, 349 (1973).
16 C.S. Lieber, Scient. Am. *234*, 3, 25 (1976).
17 F. Lundquist, N. Grunnet, S.E. Damgaard and H.I.D. Thielen, Trends Biochem. Sci. *2*, 8, 173 (1977).
18 R. Teschke, Y. Hasumura and C.S. Lieber, Arch. Biochem. Biophys. *175*, 635 (1976).
19 R. Teschke, S. Matsukazi, K. Onishi, L.M. De Carli and C.S. Lieber, Alcoholism: Clinical and Experimental Research *1*, 7 (1977).
20 P. Havre, M.A. Abrams, R.J.M. Corrall, L.C. Yu, P.A. Szczepanik, H.B. Feldman, P. Klein, M.S. Kong, J.M. Margolis and B.R. Landau, Arch. Biochem. Biophys. *182*, 14 (1977).
21 J. David and M.F. Clavel, Bull. biol. Fr. Belg. *99*, 369 (1965).
22 T.H. Day, P.C. Hillier and B. Clarke, Biochem. Genet. *11*, 141 (1974).
23 W. Sofer and H. Ursprung, J. biol. Chem. *243*, 3110 (1968).
24 P. Baudhuin, H. Beaufay, Y. Rahman-Li, O.Z. Sellinger, R. Wattiaux, P. Jacques and C. de Duve, Biochem. J. *92*, 179 (1964).
25 J.B. Courtright, Genetics *57*, 25 (1967).
26 J. David, P. Fouillet and M.F. Arens, Arch. Zool. exp. gén. *115*, 401 (1974).
27 J. David, Recherche *89*, 482 (1978).