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Effect of 2,4-Dinitrophenol on the Rate of Ethanol Elimination in the Rat *in vivo*

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Videla & Israel (1970) have reported that the rate of ethanol metabolism in rat liver slices is markedly increased by mitochondrial uncouplers. This would indicate that the rate of NADH reoxidation rather than the amount of alcohol dehydrogenase is the rate-limiting step in the oxidation of ethanol. It is not known, however, if the same holds for the intact animal. Dinitrophenol has been shown to increase markedly the rate of ethanol elimination in the dog *in vivo* (Harger & Hulpieu, 1935; Newman & Tainter, 1936; Ewing, 1940). However, there is a difference of opinion as to whether this is caused by increased rate of metabolism or increased excretion of ethanol through the lungs, as dinitrophenol is known to augment the rate of respiration (Newman, 1941). An increased cutaneous evaporation might also be responsible for part of this effect in those species that sweat.

We have studied the effect of dinitrophenol on the rate of ethanol elimination in the rat *in vivo*. The amount of ethanol expired or lost by cutaneous evaporation was also determined at the same time. Male Wistar rats (90–100g) kept without food for 20–24h were employed. The rats were given 2,4-dinitrophenol by intraperitoneal injection (3.4mg/100g body wt.). Ethanol was also given intraperitoneally 30min after the dinitrophenol injection. Two doses of ethanol were employed: 112mg [2ml of 5.6% (w/v) solution]/100g body wt. and 304mg [2ml of 15.2% (w/v) solution]/100g body wt. Immediately after the ethanol injection each animal was placed in a closed glass metabolic chamber through which a stream of air was passed. Ethanol lost by expiration or evaporation was recovered from the stream of air in two bubbler tubes in series containing water and crushed ice. At the times indicated the animals were killed by a blow on the neck and they were homogenized completely, including faeces and urine, in ice-cold water to a final volume of 1000ml. An industrial Waring Blendor was used for this purpose. Ethanol in the deproteinized homogenate, and in the mixed liquid in the bubbler tubes (made up to a final volume of 100ml), was determined enzymically as described by Hawkins, Kalant & Khanna (1966). The recovery of ethanol in the homogenate at zero time (5–10s after injection) was $92.5 \pm 1.1\%$ (8) for the dose of 304mg/100g body wt. and $91.0 \pm 1.1\%$ (4) for the dose of 112mg/100g body wt. These values were employed in calculation of the rate of ethanol elimination in the animals. Dinitrophenol did not affect the recoveries at zero time. To avoid any systematic error in the experiments, one control and one dinitrophenol-treated rat, weight-paired within 2–3g, were carried through the procedure at the same time. New ethanol solutions were made up and a new standard curve for the determination of ethanol was done for each pair.

In each of the 20 pairs of rats studied the dinitrophenol-treated rat showed a greater disappearance of ethanol than did the control. The increase ranged between 20 and 30%. If the rate of ethanol disappearance is obtained by difference between the 60min and 120min values for residual ethanol after the larger dose, the dinitrophenol-treated rats have an approximately 50% greater rate. The pooled results are shown in Table 1. The amount of ethanol lost by expiration and evaporation ranged between 2.7 and 8.2% of the total amount of ethanol eliminated, depending on the initial dose given. This is in the range of the values reported by Van Harken & Mannering (1969), who used a similar technique. As expected, dinitrophenol increased the amount of ethanol lost in the expired air. However, this increase cannot account for more than 7–25% of the dinitrophenol effect, thus indicating that a true metabolic effect occurs.

The effect of dinitrophenol found *in vivo* is less marked than that previously reported in rat liver slices incubated with 0.1mM-dinitrophenol (Videla & Israel, 1970). The dose of dinitrophenol given in the present study would correspond to a concentration of about 0.2mM if dinitrophenol were distributed evenly in the body water. This is not likely to be true since dinitrophenol is extremely soluble in lipids, so that the amount of dinitrophenol available to interact with the liver mitochondria is likely to be much less. Owing to the toxicity of dinitrophenol larger doses cannot be administered. Although in the present study none of the rats injected with dinitrophenol died, a preliminary experiment showed that doubling the dose killed three out of four animals injected. Thus, even though this cannot be definitely ascertained, it is likely that in the experiments reported here the uncoupling effect of dinitrophenol was not maximal.

Table 1. *Effect of dinitrophenol on the rate of ethanol elimination in the rat* in vivo

The values are given as means ± S.E.M. of the numbers of determinations given in parentheses; P values were obtained by the t test for paired data.

Time after injection ...	45 min	60 min	120 min
Dose of ethanol	121 mg/100 g	304 mg/100 g	304 mg/100 g
Ethanol disappeared (mg/100 g body wt.)			
Control	27.8 ± 2.3 (6)	49.3 ± 5.7 (7)	80.5 ± 5.1 (7)
Dinitrophenol-treated	35.1 ± 1.0 (6)	59.6 ± 5.3 (7)	104.5 ± 7.7 (7)
	(Δ = +7.3; P < 0.01)	(Δ = +10.3; P < 0.025)	(Δ = +24.0; P < 0.05)
Ethanol expired (mg/100 g body wt.)			
Control	0.75 ± 0.04 (5)	2.20 ± 0.32 (5)	6.59 ± 0.82 (5)
Dinitrophenol-treated	1.17 ± 0.23 (5)	4.64 ± 1.67 (5)	10.73 ± 0.81 (5)
	(Δ = +0.42; P < 0.1)	(Δ = +2.44; P < 0.05)	(Δ = +4.14; P < 0.02)

An increase in the rate of ethanol metabolism by dinitrophenol in the rat *in vivo* suggests that the rate-limiting step in the oxidation of ethanol is the reoxidation of NADH to NAD^+ rather than the activity (maximum activity when assayed with saturating concentrations of NAD^+) of alcohol dehydrogenase. This would explain why pyruvate and fructose, two compounds that can increase the rate of extramitochondrial oxidation of NADH, increase the rate of ethanol metabolism *in vivo* (Lundquist & Walthers, 1958; Pawan, 1968; Westerfeld, Stotz & Berg, 1942) and *in vitro* (Thieden & Lundquist, 1967; Smith & Newman, 1959; Videla & Israel, 1970) (see also Lundquist, 1970).

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