Non-Specific Reversal by Vitamins of Inhibition of Ethanol Oxidation by Antabuse (Tetraethylthiuramdisulfide) in the Rat. (20846)

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Acetaldehyde was found to accumulate upon administration of ethanol(1) in animals, treated with TTD (tetraethylthiuramdisulfide($[(C_2H_5)_2NCS_2]_2$). While the overall ability of the body to dissimilate acetaldehyde remains unchanged, the rate of oxidation to acetic acid is greatly retarded through the inhibitory action of TTD. The nature of this inhibition is not yet clear. The interference with the oxidation of ethanol in TTD-treated animals is coupled with toxic symptoms. According to Lecoq(2), the toxicity is due to a disturbance of pyruvate metabolism rather than to the accumulation of acetaldehyde. This conclusion is based on the observations that injection of pyruvate produced the same symptoms as ethanol in TTD-treated animals, and that the toxic effects of both pyruvate and ethanol could be reversed by administration of nicotinamide and adenine. This author found that thiamine, but not riboflavin, could also reverse the toxicity of pyruvate and ethanol in TTD-treated animals. Ascorbic acid was found to counteract the toxicity of ethanol, but not the pyruvate(3). Lecoq *et al.* also found meso-inositol, cysteine-HCl or sodium succinate to be ineffective(4). Cortisone had slight and ACTH no effect at all. The reversibility of the inhibition of acetaldehyde oxidation by TTD *in vitro* by Racker's aldehyde dehydrogenase system on additions of diphosphopyridine nucleotide, glutathione, and to a lesser extent ascorbic acid was reported by Graham(5).

Because of the lack of data concerning acetaldehyde blood level changes following vitamin treatment in TTD-ethanol-injected animals, we have explored this problem in intact rats. The damage caused to the metabolic capacity of the liver following continuous administration of TTD was also investigated. In a preliminary communication the reversal of the effect of TTD by vitamins was shown to be competitive and non-specific(6). The details of the work are presented here.

Material and methods. Male rats of the Long-Evans strain, weighing 250-300 g (unless otherwise stated) were used. They were kept on a Purina Lab Chow diet with water given *ad libitum*. The commercial tetraethylthiuramdisulfide (obtained through the courtesy of Sharples Chemical Co., Philadelphia, Pa.) was crystallized from asym. propylene glycol. On cooling, it crystallized in long needles melting at 70°. The ethanol used was distilled over sodium and kept under anhydrous condition prior to its dilution with water. The determination of the blood acetaldehyde concentration was carried out according to the spectrophotometric method of Burbridge *et al.*(7). The absorption of acetaldehyde semicarbazone was measured at 224 mμ. For analysis 2 ml blood was withdrawn with a heparinized needle from the heart of unanesthetized rats and incubated in an all glass modified Conway cell. Liver homogenates were checked for the degree of enzyme inhibition following the administration of TTD by the Thunberg methylene blue method. Changes in the methylene blue concentration were measured by the light transmission at 660 mμ in a Klett-Summerson photometer(8). The inhibition was expressed in % by the equation: % inhibition = (C-X)100/C, where X is the change in transmission of the TTD-treated and C the change in the transmission of the controls.

Results. Each of the 5 rats per group received 20 mg of TTD in one ml of asym. propylene glycol. (kept at 38°C) intraperitoneally for 2 successive days. A single dose of 40 mg killed the animals. On the second day ½-hour following the TTD injection, the vitamins were administered intraperitoneally in 1.0 ml of aqueous solution. The controls were given 1.0 ml of distilled water. One

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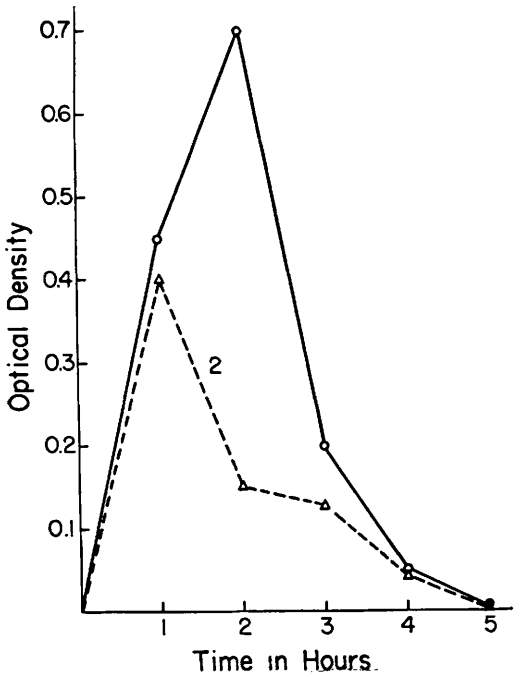

FIG. 1. Effect of nicotinamide on blood levels of acetaldehyde in antabuse-treated rats. Curve 1. Animals of group given 20 mg TTD for 2 days followed by 0.197 g ethanol. Curve 2. Same but with 20 mg nicotinamide.

half-hour later all the animals received 0.197 g of ethanol in 1.0 ml of water intraperitoneally. This concentration of ethanol was tolerated by the rats without mortality and produced measurable amounts of blood acetaldehyde within the first 15 minutes. Fig. 1, curve 1, shows the change in acetaldehyde concentration with time. The peak of acetaldehyde accumulation occurred at about 2 hours and disappeared at about 5 hours. The injection of 20 mg of nicotinamide (1.64×10^{-4} mole) decreased the blood acetaldehyde (Fig. 1, curve 2). At 1.64×10^{-5} molar level the above effect of nicotinamide is not reproducible.

The *in vivo* action of nicotinamide does not appear to be specific and is produced also by other vitamins. Table I shows the results obtained with other vitamins at 2 and 20 mg levels respectively. The lower values obtained with ascorbic acid are in agreement with the *in vitro* observations of Graham(5) that, in restoring the aldehyde dehydrogenase activity following TTD inhibition, a greater molar ratio of ascorbic acid to TTD is required than with either diphosphopyridine nucleotide or glutathione. TTD given alone(9) or with asym. propylene glycol. does not lead to a rise of the blood acetaldehyde.

The antidotal effect of nicotinamide following alcohol administration was tested in 24 male rats weighing 400-500 g in 2 experiments. The first experiment employed 8 rats and the second 16. The animals were treated with two 20 mg doses of TTD on successive days. On the second day they received 0.25-1.0 ml pure ethanol intraperitoneally followed half an hour later by 40 mg of nicotinamide in 1.0 ml of water. No reversal of either the toxic symptoms or of the aldehyde levels was observed.

In vitro experiments showed that TTD exerted a powerful inhibition on SH enzymes, *e.g.*, rhodanese(6) and succinic dehydrogenase (5). An experiment was designed to test whether TTD would damage enzymes *in vivo* upon continued administration. Fifteen male rats were injected daily with 20 mg of TTD in 1.0 ml of asym. propylene glycol. for 21 consecutive days. No mortality was observed. At the end of the period the animals were killed and homogenates of their livers were tested for impairment of their ability to reduce methylene blue in the presence of succinate, citrate and ethanol. The inhibition of the dehydrogenase activity was 38% for citrate, 60% for ethanol and 45% for succinate.

Discussion. In the above experiments it was found that about 1/10 of the amount of TTD and 1/25 of the amount of ethanol employed by Lecoq(3) caused little toxicity, but produced a significant accumulation of acetaldehyde. All the vitamins assayed could reverse the accumulation of acetaldehyde produced by TTD. Injection of chemicals like asym. propylene glycol. or cystine had no influence.

The generalized effect of TTD, on the basis of these experiments, is thought to be produced by its action on SH-groups. We also found in two experiments that 20 mg of calcium pantothenate reversed the action of TTD. The non-specific effect of the vitamins would by no means be surprising since, al-

TABLE I. Reversing Effect of Vitamins *In Vivo* on Inhibition of Acetaldehyde Oxidation by TTD.*

Group	Avg aldehyde blood levels (mg %) (a)	(b)	mml vit. required for 100% reversal of 1 mml TTD†
TTD + ethanol	1.38	1.22	—
+ nicotinamide + ethanol	1.20	.58	2.3
+ pyridoxine HCl + "	1.14	.57	1.4
+ riboflavine + "	1.24	.56	.8
+ thiamine HCl + "	1.25	.38	.6
+ folic acid + "	1.30	.60	.6
+ ascorbic acid + "	1.35	.87	3.0
Ethanol alone‡			
TTD " ‡			

* 2 hr after intraper. inj. of 0.197 g ethanol. Each figure represents 5 animals. (a) 2 mg; (b) 20 mg vit. inj.

† Calculations based on column (b).

‡ Below limit of sensitivity.

though the enzymatic processes involved in the oxidation of alcohol to acetic acid are not as yet well understood, there is every indication of the existence of several pathways of alcohol oxidation.

The amount of the vitamins required to yield reversal of TTD is shown in Table I. At present no explanation is offered for the inability of the vitamins to reverse the TTD-effect at their lower concentration.

Summary. In TTD-treated rats certain vitamins are capable of reversing the inhibition of TTD on alcohol oxidation at the acetaldehyde level when injected prior to ethanol administration. The effect of two 20 mg doses of TTD can be reversed by 20 but not by 2 mg of the vitamins. Chemicals like propylene glycol. and cystine did not produce reversal. Injection of nicotinamide into TTD-treated rats after ethanol does not relieve or reverse the toxic symptoms. There is damage to the liver of the TTD-treated rats after 21 days, as shown by its impaired ability to transfer hydrogen anaerobically to methylene blue using succinate, citrate and ethanol as substrates. The damage is thought to be produced through the affinity of TTD for the sulfhydryl sites of the enzymes.

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