Rate of Ethanol Metabolism in Fed and Starved Rats after Thyroxine Treatment

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When ethanol is consumed, the major part is oxidized in the liver. Some ethanol may also be oxidized by the alcohol dehydrogenase (EC 1.1.1.1.) present for example in the kidney. A small amount is not oxidized at all but is excreted as such.[1] Availability of the active enzyme in sufficient amount and reoxidation of $NADH_2$ to NAD are both necessary for optimal oxidation of ethanol in the liver.[1,2] In the rat, liver alcohol dehydrogenase activity begins to increase after birth and cannot be enhanced by short-term administration of ethanol or by injections of triamcinolone or hydrocortisone.[3] Hawkins, Kalant and Khanna,[4] however, have reported a significant increase in alcohol dehydrogenase activity in rats after chronic ethanol administration.

Thyroid hormone treatment stimulates RNA and protein synthesis in the liver and the activities of certain oxidative enzymes are consequently increased,[5-7] but the effect on liver alcohol dehydrogenase has been little studied. Administration of thyroid hormones *in vitro*, however, is known to bring about a decrease in liver alcohol dehydrogenase activity.[8,9] The effects of thyroid hormone treatment on blood ethanol elimination are variable, and conflicting results have been reported.[10,11]

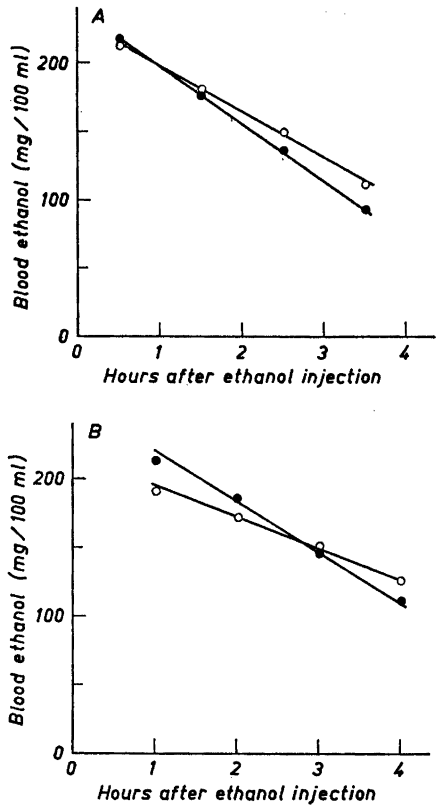

Fig. 1. Blood ethanol concentrations after an intraperitoneal injection of ethanol in fed and starved rats. Each point represents the mean of 10 animals. A. Fed rats. B. Starved rats. Open circles: control rats. Closed circles: thyroxine-treated rats.

An analysis was made of the blood ethanol concentration after an intraperitoneal injection of ethanol to female albino rats of the Wistar strain, weighing 120–200 g. The dose of ethanol administered was 2 g/kg as a 20 % w/v solution. 40 μg of L-thyroxine was given to half of the experimental animals daily by subcutaneous injection for seven days. The control rats received the same volume of saline. The animals were fed a conventional laboratory chow *ad libitum*, except for one group, which was starved for 48 h before the experiments. Blood samples for ethanol determinations were taken from the tip of the tail. Ethanol was determined by the enzymic method of Bonnichsen.[12] Alcohol dehydrogenase activity was determined from liver homogenates as described by Räihä, Koskinen and Pikkarainen.[3] The ethanol-oxidizing capacity of liver slices was studied by a technique described earlier.[13] Protein determinations were made by a modified biuret method.[6]

Blood ethanol concentrations after injection of ethanol to fed and starved rats are presented in Fig. 1. The disappearance rates calculated from the linear part of the curves for each individual rat (Table 1) indicate a more rapid rate in fed, thyroxine-treated rats than in corresponding controls. Starvation retarded the elimination

Table 1. Rate of blood ethanol disappearance, liver alcohol dehydrogenase activity, and the ethanol-oxidizing capacity of liver slices in the control and thyroxine-treated rats. The results are expressed as means±S.E.M. of 10 experiments.

	Ethanol disappearance mg/100 ml blood/h		Liver alcohol dehydrogenase activity mIU/g wet wt.		Ethanol-oxidizing capacity of liver slices mg/g wet wt./h	
Fed rats						
controls	36.0±2.5	$p<0.05$	1010±52	$p<0.001$	1.31±0.07	$p<0.05$
thyroxine-treated	44.0±2.2		630±29		1.09±0.06	
Starved rats						
controls	21.0±1.4	$p<0.001$	1030±85	$p<0.001$	—	
thyroxine-treated	37.0±1.4		660±35		—	

rate in the control rats by 42 % ($p<0.01$), whereas its effect was much smaller (16 %, $p<0.02$) in the thyroxine-treated rats.

The liver alcohol dehydrogenase activity was found to decrease markedly in the thyroxine-treated animals. After a treatment period of one week the activity was 57 % of the initial level. Starvation for 48 h did not significantly influence the activity in either of the experimental groups. When the alcohol dehydrogenase activities were expressed in mIU/g of soluble protein, the results were similar. The ethanol-oxidizing capacity of liver slices was found to decrease by 17 % after treatment with thyroxine (Table 1).

The diminished alcohol dehydrogenase activity and the decreased ethanol-oxidizing capacity of liver slices suggest that liver ethanol metabolism was slower than normal in the thyroxine-treated rats. A parallelism in the changes in these two parameters has also been shown in the rat after birth.[9] The increased rate of blood ethanol disappearance, however, does not correlate with the findings in the liver preparations. A similar lack of correlation between liver alcohol dehydrogenase activity and blood ethanol elimination has been reported in other instances: For example, in the case of two strains of mice with different levels of liver alcohol dehydrogenase activity,[14] in the horse, in which liver alcohol dehydrogenase activity is 10-fold that of man, but eliminates ethanol much less rapidly than in man,[15] and also in human subjects with hepatic diseases.[16] Thus it is obvious that other factors besides hepatic alcohol dehydrogenase determine the disappearance rate of blood ethanol.

Starvation has been shown to decrease ethanol metabolism in liver slice experiment.[2] We found a great retardation in blood ethanol elimination after starvation. The mechanism proposed to be responsible for the retardation is the greatly increased liver $NADH_2/NAD$ ratio during ethanol oxidation in starved animals.[2] If this explanation is valid, the present results of the smaller effect of starvation on the ethanol disappearance rate are understandable, since we have earlier found, in experiments with liver slices, that the lactate/pyruvate ratio, which reflects the cytoplasmic $NADH_2/NAD$ ratio, increases significantly less, after similar treatment with thyroxine, than in control rats.[13]

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Lead Tetraacetate Oxidation of Cedrol. Syntheses of 8,14-Cedranoxide and 14,8-Cedranolide

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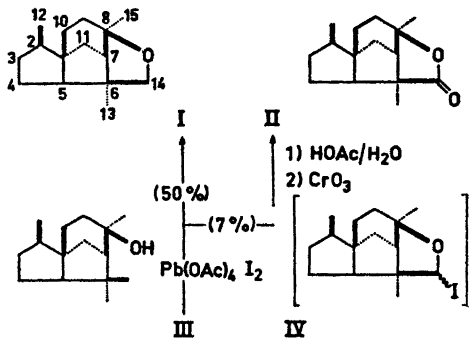
Recently a sesquiterpene oxide[1] and a lactone,[2] from *Juniperus foetidissima* Willd.[1] were characterised and shown to be the cedrane derivatives, 8,14-cedranoxide *I* and 14,8-cedranolide *II*.[2] We now report the syntheses of these constituents *via* transannular lead tetraacetate oxidations of cedrol *III*,* the structure of which has been previously ascertained by a total synthesis.[3]

Transannular oxygenations of saturated carbon atoms with oxygen species generated by lead tetraacetate are well known, particularly for the syntheses of various steroid oxides.[4] The C(14)-methyl group in cedrol *III* is suitably orientated for an attack by a similar 8 S oxygen species. Preliminary oxidation experiments on cedrol *III* in benzene confirmed that upon prolonged treatment traces of the oxide *I* were formed. The reaction rate was very low and side reactions were observed. However, using the method of Heusler *et al.*,[5] in which the oxidation is carried out in the presence of iodine, the oxide *I* was formed in high yield (50 %). Treatment of the crude lead tetraacetate oxidation product with aqueous acetic acid followed



by a chromic acid oxidation yielded, apart from the oxide *I* (50 % yield), small amounts of 14,8-cedranolide *II* (7 % yield), probably formed *via* an intermediate iodoether *IV*, since the oxide *I* was found to be stable under these reaction conditions.

Experimental. Lead tetraacetate (4 g) and calcium carbonate (2 g) were heated under reflux in cyclohexane (125 ml) for 10 min. Iodine (1 g) and cedrol (300 mg; m.p. 86–87°; $[\alpha]_D$ +10.1°) were added and the mixture was refluxed for 2.5 h. After cooling, the precipitate was filtered off and washed with ether. The combined organic solutions were washed consecutively with 5 % aqueous potassium iodide, 5 % aqueous sodium thiosulphate, and water. The organic phase was dried (Na_2SO_4) and the solvents evaporated. The residue was adsorbed from light petroleum (b.p. 40–60°) on to a silica gel column (30 g). Ether (4 %) in light petroleum eluted 8,14-cedranoxide (150 mg, yield 50 %). Rechromatography on silica gel (15 g) with ether (4 %) in light petroleum followed by distillation (bath temp. 100°; 0.1 mm Hg) yielded a pure product identical in all respects ($[\alpha]_D$, IR, NMR, mass spectrum, TLC, and GLC) to those of natural 8,14-cedranoxide.[1,2] The rotations of our synthetic and natural 8,14-cedranoxide samples were considerably higher ($[\alpha]_D$ −96°, *c* 1.91 in $CHCl_3$) than the value previously reported[1] ($[\alpha]_D$ −69°, misprint?) for the natural product.

* A compound which must be identical to 8,14-cedranoxide has recently been prepared from cedrol *via* similar transannular oxygenations (Blumenthal, J. H., Stork, G. and Theimer, E. T. *U.S. patent* 3,281,432/1966).