The Increased Rate of Ethanol Removal from Blood of Clofibrate-Treated Rats

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After ethanol injection similar metabolite changes were found in livers of clofibrate-treated and non-treated rats. The rate of ethanol removal from blood of clofibrate-treated rats was increased by 50%, but this was directly proportional to the increased liver size caused by clofibrate. No other difference needs to be postulated to explain the increased rate of ethanol removal from blood.

A decrease in the cytoplasmic free $[NAD^+]/[NADH]$ ratio of liver is characteristic during ethanol metabolism (Krebs, 1968). Treatment of rats with thyroxine decreases, or abolishes, this response although it does not increase the rate of ethanol removal in man, dog or rat (Rawat & Lundquist, 1968; Kalant *et al.*, 1962; Lindros, 1972). Clofibrate [ethyl α -(*p*-chlorophenoxy)isobutyrate] causes some thyroxine-like effects; it increases the activity of mitochondrial α -glycerophosphate dehydrogenase and liver O_2 consumption (Westerfeld *et al.*, 1968; Ruegamer *et al.*, 1969). Kähönen *et al.* (1971) reported that clofibrate also increased the rate of ethanol metabolism *in vivo*. Further, they found no change in the $[lactate]/[pyruvate]$ ratio in perfused livers of clofibrate-treated rats. However, the mechanism by which the rate of ethanol removal was increased is not clear from their results. Clofibrate is known to cause a substantial increase in liver size which might be expected to contribute to the observed changes in the rate of ethanol removal (Hess *et al.*, 1965; Kurup *et al.*, 1970; Fallon *et al.*, 1971). We have therefore examined more closely the effect of clofibrate on the metabolism of ethanol by rat liver *in vivo*.

Experimental

Male albino rats of the Wistar Furth strain, weighing 200–250 g, were used. Free access to food and water was available. Clofibrate was a gift of Ayerst Laboratories, New York, N.Y., U.S.A. Treatment was by daily intramuscular injections (600 mg/kg body wt.) for 9 days. Livers were removed from unanaesthetized rats, freeze-clamped, powdered under liquid N_2 and extracted with $HClO_4$ as previously described (Veech *et al.*, 1972). The following metabolites were measured by standard enzymic techniques: ADP and AMP (Adam, 1963); NH_4^+ (Kirsten *et al.*, 1963); ATP (Lamprecht & Trautschold, 1963); citrate (Moellering

& Gruber, 1966); dihydroxyacetone phosphate (Bücher & Hohorst, 1963); ethanol (Dickinson & Dalziel, 1967); glucose (Slein, 1963); glucose 6-phosphate (Hohorst, 1963a); glutamate (Bernt & Bergmeyer, 1963); α -glycerophosphate (Hohorst, 1963b); lactate (Hohorst, 1963c); malate (Hohorst, 1963d); α -oxoglutarate (Bergmeyer & Bernt, 1963) orthophosphate (Guynn *et al.*, 1972); pyruvate (Bücher *et al.*, 1963).

Free nicotinamide nucleotide ratios were calculated from the formulae: cytoplasmic $[NADP^+]/[NADPH] = [\alpha\text{-oxoglutarate}][CO_2]/[isocitrate]K$, where $K = 1.17 M$ (Londesborough & Dalziel, 1968); cytoplasmic $[NAD^+]/[NADH] = [pyruvate]/[lactate]K$, where $K = 1.11 \times 10^{-4}$ (Williamson *et al.*, 1967); mitochondrial $[NAD^+]/[NADH] = [\alpha\text{-oxoglutarate}][NH_4^+]/[glutamate]K$, where $K = 3.87 \times 10^{-6} M$ (Williamson *et al.*, 1967). The concentration of isocitrate was assumed to be 5.65% that of citrate (Veloso *et al.*, 1973) and tissue CO_2 was taken to be 1.17 mM (Krebs & Veech, 1969).

To measure the rate of ethanol removal *in vivo*, rats were anaesthetized with diethyl ether and catheters were placed in the femoral artery and inferior vena cava (Hawkins *et al.*, 1971). At 1.5 h after awakening, ethanol (10 mmol/kg body wt.) was injected into the inferior vena cava. Arterial blood samples (0.2 ml) were taken at 5 min intervals for 30 min. During this period ethanol disappearance obeyed zero-order kinetics. A straight line was drawn through the points by using the method of least squares and the rate of ethanol removal was calculated from the formula:

$$\frac{([ethanol]_{0 \text{ min}} - [ethanol]_{30 \text{ min}})}{[ethanol]_{0 \text{ min}}} \times \frac{(\mu\text{mol injected})}{30 \text{ min}} \times \frac{1}{\text{wt.}}$$

'wt.' refers to either whole body weight or liver weight.

Alcohol dehydrogenase activity was measured at 38°C in the supernatant fraction of liver homogenized in 9 vol. of 0.1 M-potassium phosphate buffer,

pH 7.2, and centrifuged at 40000g for 20 min. Activity in the forward direction was determined with 5 mM-ethanol and 1 mM-NAD⁺ as substrates, and the reverse reaction as described by Krebs & Perkins (1970).

Results and discussion

The patterns of metabolite changes that occurred after ethanol injection were similar in both normal and clofibrate-treated rats. There were marked increases in the concentrations of α -glycerophosphate and lactate, whereas the concentrations of 3-phosphoglycerate, phosphoenolpyruvate and pyruvate were decreased (Table 1).

Almost all of the observed metabolite changes could be ascribed to the markedly decreased cytoplasmic [NAD⁺]/[NADH] ratio and the near-equilibrium relationship maintained between 3-phosphoglycerate and α -glycerophosphate as shown by the following equation:

$$\frac{[\alpha\text{-Glycerophosphate}]}{[3\text{-phosphoglycerate}]} = K \times \frac{[\text{ATP}]}{[\text{ADP}]} \times \frac{[\text{NADH}]^2}{[\text{NAD}^+]^2}$$

where

$$K = \frac{K_{\text{triose phosphate isomerase}}}{K_{\text{glyceraldehyde phosphate dehydrogenase}} \cdot K_{\alpha\text{-glycerophosphate dehydrogenase}} \cdot K_{3\text{-phosphoglycerate kinase}}}$$

[For the derivation and experimental justification of this equation see Veech *et al.* (1972).] Thus the ratio of [α -glycerophosphate]/[3-phosphoglycerate] is expected to vary as the square of the [NADH]/[NAD⁺] ratio. This was found to be the case in both the clofibrate-treated and normal rats. The maintenance of this relationship explained the large rise of [α -glycerophosphate].

Kähönen *et al.* (1971) found no change in the redox state of the cytoplasmic NAD couple after perfusion of livers from clofibrate-treated rats with ethanol and, in a subsequent study, no increase in [α -glycerophosphate] was found (Kähönen *et al.*, 1972). They implied that their findings were due to an increased activity of the mitochondrial α -glycerophosphate dehydrogenase, which rises 5-fold after clofibrate treatment (Westerfeld *et al.*, 1968). We were unable to confirm these authors' observations *in vivo*. The activities of alcohol dehydrogenase, in

Table 1. Content of intermediary metabolites, redox and phosphorylation states in liver of rats pretreated with clofibrate before and after ethanol injection

The results are mean values (=S.E.M.) expressed as $\mu\text{mol/g}$ of liver, with the number of determinations indicated in parentheses. Ethanol (30 mmol/kg body wt.) was injected intraperitoneally and the livers were freeze-clamped at 40 min. The symbols * and ** indicate statistical significance at the 1 and 5% levels respectively after ethanol injection as compared with the appropriate NaCl-injected controls. For other details see under 'Experimental'.

	Pretreatment Injection	... None NaCl (5)	None Ethanol (7)	Clofibrate NaCl (5)	Clofibrate Ethanol (7)
Glucose		6.79 $\pm$ 0.20	6.45 $\pm$ 0.15	6.04 $\pm$ 0.26	6.89 $\pm$ 0.19*
Glucose 6-phosphate		0.126 $\pm$ 0.008	0.117 $\pm$ 0.008	0.077 $\pm$ 0.005	0.110 $\pm$ 0.005**
Dihydroxyacetone phosphate		0.022 $\pm$ 0.002	0.018 $\pm$ 0.001	0.014 $\pm$ 0.001	0.019 $\pm$ 0.002
α -Glycerophosphate		0.111 $\pm$ 0.014	0.490 $\pm$ 0.083**	0.064 $\pm$ 0.003	0.302 $\pm$ 0.055**
3-Phosphoglycerate		0.301 $\pm$ 0.026	0.128 $\pm$ 0.048**	0.321 $\pm$ 0.030	0.145 $\pm$ 0.048**
Phosphoenolpyruvate		0.122 $\pm$ 0.006	0.053 $\pm$ 0.026	0.156 $\pm$ 0.012	0.055 $\pm$ 0.018**
Pyruvate		0.071 $\pm$ 0.023	0.016 $\pm$ 0.001**	0.085 $\pm$ 0.015	0.033 $\pm$ 0.011**
Lactate		0.342 $\pm$ 0.074	0.425 $\pm$ 0.055	0.454 $\pm$ 0.099	0.775 $\pm$ 0.058*
Citrate		0.474 $\pm$ 0.062	0.492 $\pm$ 0.099	0.334 $\pm$ 0.015	0.257 $\pm$ 0.030*
α -Oxoglutarate		0.308 $\pm$ 0.039	0.217 $\pm$ 0.022	0.366 $\pm$ 0.036	0.189 $\pm$ 0.024*
Glutamate		3.88 $\pm$ 0.33	3.75 $\pm$ 0.21	4.26 $\pm$ 0.41	3.47 $\pm$ 0.16*
NH ₄ ⁺		0.340 $\pm$ 0.032	0.332 $\pm$ 0.015	0.361 $\pm$ 0.014	0.360 $\pm$ 0.060
Malate		0.272 $\pm$ 0.036	0.654 $\pm$ 0.089**	0.305 $\pm$ 0.033	0.386 $\pm$ 0.037
ATP		2.74 $\pm$ 0.05	2.64 $\pm$ 0.08	2.71 $\pm$ 0.17	2.84 $\pm$ 0.1
ADP		1.01 $\pm$ 0.05	1.07 $\pm$ 0.06	1.21 $\pm$ 0.14	1.26 $\pm$ 0.04
P _i		2.79 $\pm$ 0.08	2.82 $\pm$ 0.09	3.33 $\pm$ 0.18	3.52 $\pm$ 0.09
$\frac{[\text{ATP}]}{[\text{ADP}][\text{H}_2\text{PO}_4^{2-}]} \text{ (M}^{-1}\text{)}$		1650 $\pm$ 120	1520 $\pm$ 150	1250 $\pm$ 260	1090 $\pm$ 70
[NAD ⁺]/[NADH] for lactate dehydrogenase (cytoplasmic)		1740 $\pm$ 180	405 $\pm$ 96**	1770 $\pm$ 210	366 $\pm$ 116**
[NAD ⁺]/[NADH] for glutamate dehydrogenase (mitochondrial)		7.04 $\pm$ 1.01	4.80 $\pm$ 0.56*	8.23 $\pm$ 1.03	4.75 $\pm$ 0.57*
[NADP ⁺]/[NADPH] for isocitrate dehydrogenase (cytoplasmic)		0.02 $\pm$ 0.16	0.007 $\pm$ 0.09**	0.003 $\pm$ 0.27	0.011 $\pm$ 0.05**

Table 2. *Rate of ethanol removal from blood of clofibrate-treated rats*

All values are means ($\pm$ S.E.M.) of six individual determinations. The symbol ** indicates statistical significance at the 1% level. The rate of ethanol removal was calculated as described under 'Experimental'.

	Ethanol removal (μ mol/min per 100g body wt.)	Liver weight (g/100g body wt.)	Ethanol removal (μ mol/min per g of liver)
Control	16.8 $\pm$ 1.0	4.68 $\pm$ 0.28	3.59 $\pm$ 0.15
Clofibrate-treated	25.1 $\pm$ 1.3**	6.71 $\pm$ 0.30**	3.71 $\pm$ 0.14

the forward direction, were 4 ± 0.3 and 5 ± 0.4 μ mol/min per g in normal and clofibrate-treated rats respectively (the activities in the reverse direction were 27 ± 2 and 30 ± 3 μ mol/min per g respectively). Since the activity of cytoplasmic α -glycerophosphate dehydrogenase is about 32 μ mol/min per g (Freedland, 1965) and that of mitochondrial α -glycerophosphate dehydrogenase is between 0.2 and 0.7 μ mol/min per g (Lee & Lardy, 1965; Westerfeld *et al.*, 1968), a 5-fold increase in mitochondrial α -glycerophosphate dehydrogenase activity should hardly be sufficient to remove α -glycerophosphate quickly enough to keep its concentration at control values.

The rate of ethanol removal from blood of clofibrate-treated rats was greater (by 50%) when expressed per 100g body wt. (Table 2). Clofibrate, however, in addition to increasing specific enzyme activities in liver also increases liver size (Fallon *et al.*, 1971; Hess *et al.*, 1965; Kurup *et al.*, 1970). Since ethanol is cleared from the blood almost exclusively by the liver (Leloir & Muñoz, 1938) the data were calculated on the basis of liver weight. When expressed in this way, there was no difference between the two groups. Therefore the increased rate of ethanol removal could be accounted for solely on the basis of increased liver size. No other difference between clofibrate-treated and normal rats needs to be postulated to explain the increased rate of ethanol removal from blood.

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