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# Hepatic ethanol metabolism is mediated predominantly by catalase-$H_2O_2$ in the fasted state

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Methanol and butanol were employed as selective substrates for catalase-$H_2O_2$ and alcohol dehydrogenase (ADH), respectively, in the perfused rat liver. As expected, rates of butanol metabolism accounted for over 85% of overall rates of alcohol oxidation indicating that ADH was the predominant pathway of alcohol metabolism in both the fed or fasted state in the absence of added substrate. In the fasted state, however, addition of oleate (1 mM) diminished butanol oxidation 20–25% yet increased rates of methanol oxidation over 4-fold. Under these conditions, methanol uptake accounted for nearly two-thirds of overall rates of alcohol oxidation. These data demonstrate that catalase-$H_2O_2$ is the predominant pathway of alcohol oxidation in the fasted state in the presence of fatty acids. Accordingly, it is concluded that diet and nutritional state play important roles in the contribution of the ADH and catalase pathways to alcohol oxidation.

Ethanol metabolism; Catalase; Alcohol dehydrogenase; Alcohol oxidation; (Liver)

## 1. INTRODUCTION

Alcohol dehydrogenase (ADH) has long been accepted as the major enzyme involved in ethanol oxidation in mammals. This point of view is based largely on the fact that ethanol elimination is abolished nearly completely by inhibitors of ADH such as alkylpyrazoles [1,2]. However, the selectivity of pyrazole derivatives is questionable since they are also relatively potent inhibitors of cytochrome P-450 [3]. The possibility that other pathways of ethanol metabolism in addition to ADH are important is raised by the fact that a strain of deermouse which lacks ADH is capable of metabolizing ethanol at rates between a half and two-thirds of the values observed in deermice with ADH [4–6]. Indeed, it was demonstrated recently that ADH-negative deermice metabolize ethanol primarily via catalase-$H_2O_2$ [7,8]. Thus, the role of ADH in ethanol metabolism may not be as clear as was thought previously.

It is well established that ADH and catalase-$H_2O_2$ have different affinities for short-chain aliphatic alcohols. In particular, rodent liver ADH oxidizes ethanol, propanol and butanol with increasing velocities [9] but will not metabolize methanol [10,11]. In contrast, purified catalase from rat liver peroxidizes methanol and ethanol at about equal rates but is essentially incapable of metabolizing butanol [12]. The contribution of cytochrome P-450 to alcohol oxidation can be discounted since mixed-function oxidation occurs in perfused rat livers at rates more than an order of magnitude lower than rates of alcohol oxidation [13]. Thus, methanol and butanol can be used as selective substrates for the catalase-$H_2O_2$ and ADH pathways, respectively. Accordingly, we quantitated ADH- and catalase-dependent alcohol metabolism by measuring rates of uptake of

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methanol and butanol in perfused livers from fed and fasted rats.

## 2. MATERIALS AND METHODS

### 2.1. *Materials*

Oleate (sodium salt) and bovine serum albumin were from Sigma, St. Louis, MO. Alcohols and other chemicals were from standard sources.

### 2.2. *Liver perfusion*

Livers from normally fed and fasted female rats were perfused in a recirculating system with Krebs-Henseleit buffer (pH 7.4, 37°C) containing 4% bovine serum albumin as described previously [14]. Oxygen concentration in the perfusate leaving the liver was monitored continuously with a Clark-type $O_2$ electrode to measure viability.

### 2.3. *Measurement of methanol and butanol*

1 ml samples of perfusate were collected every 20 min and incubated in stoppered 25 ml Erlenmeyer flasks at 37°C for 60 min. Samples of headspace (1 ml) were injected onto a carbowax 60/80 column and analyzed for methanol and butanol with a flame ionization detector. Operating parameters were: oven 110°C; detector, 250°C; injection port, 250°C; and carrier gas flow, 40 ml/min. Rates of methanol (25–30 mM) and butanol (15–20 mM) uptake were calculated in the presence and absence of oleate (1 mM) by decreases in alcohol concentration over time, the system volume (65 ml) and liver weights. Rates were corrected for vaporization from the system, which was about 50%.

## 3. RESULTS AND DISCUSSION

In perfused livers from fed rats, overall rates of alcohol metabolism were around 100 $\mu$mol/g per h and butanol metabolism accounted for over 85% of the total (fig.1). Under these conditions, addition of oleate diminished rates of butanol metabolism by about 20% while rates of uptake of methanol were not altered significantly. Thus, these data concur with the accepted viewpoint that ADH is the predominant pathway of ethanol oxidation in the fed state.

In livers from fasted rats, overall rates of alcohol uptake were about 85 $\mu$mol/g per h and were decreased about 25% by infusion of oleate (fig.1). In contrast to data obtained from livers of fed rats, rates of butanol uptake of about 75 $\mu$mol/g per h were diminished by about 60% by infusion of oleate. Concomitantly, rates of methanol uptake were increased about 400% from 10 up to 40 $\mu$mol/g per h. Thus, methanol oxidation accounted for about two-thirds of the total rate of alcohol metabolism in the presence of fatty

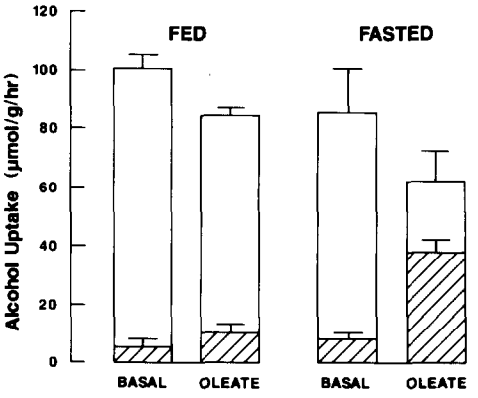


Fig.1. Rates of methanol and butanol uptake by perfused livers from fed and fasted rats in the presence and absence of oleate. Rates of uptake of methanol (hatched) and butanol (open) were measured in perfused livers from fed and fasted rats as described in section 2. Basal rates, 0–20 min; rates in the presence of oleate, 20–40 min of perfusion. Data represent mean ± SE for 4–7 livers per group.

acids in the fasted state. This is consistent with the hypothesis that catalase-$H_2O_2$ is the major pathway of ethanol oxidation under these conditions.

To evaluate this hypothesis, fasted rats were treated with the catalase inhibitor, aminotriazole (1.5 g/kg, i.p.), 1.5 h before liver perfusion. It has been demonstrated previously that the peroxidative reaction of catalase is abolished completely by this treatment [14]. In livers from aminotriazole-treated rats, methanol uptake was below the limits of detection ($<2$ $\mu$mol/g per h) both in the presence and absence of oleate. Thus, we conclude that the increase in rates of methanol uptake observed in livers from fasted rats in the presence of fatty acids is due to increased peroxidation of methanol via catalase-$H_2O_2$. Studies with ADH-negative deermice demonstrating that ethanol can be peroxidized via catalase-$H_2O_2$ at high rates (up to 80 $\mu$mol/g per h) [15] in the absence of ADH are consistent with this conclusion.

It has been demonstrated that $H_2O_2$, the rate-limiting component in the peroxidation of ethanol via catalase $H_2O_2$ [16], can be generated at high rates via the peroxisomal $\beta$-oxidation of fatty acids [17]. Thus, the stimulation of methanol uptake by oleate was due most likely to increased $H_2O_2$ generation by fatty acids via this pathway. Since ethanol ingestion increases circulating as well as

hepatic fatty acid levels [18], substrate for the pathway described above may be provided as a normal consequence of drinking.

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