Inhibition of catalase-dependent ethanol metabolism in alcohol dehydrogenase-deficient deermice by fructose

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Hepatic microsomal fractions from ADH (alcohol dehydrogenase)-negative deermice incubated with an NADPH-generating system metabolized butanol and ethanol at rates around 10 nmol/min per mg. In contrast, cytosolic catalase from ADH-negative deermouse liver oxidized ethanol, but not butanol, when incubated with an H_2O_2 -generating system. Thus butanol is oxidized by cytochrome *P*-450 in microsomal fractions, but not by cytosolic catalase, in tissues from ADH-negative deermice. In perfused livers from ADH-negative deermice, rates of ethanol uptake at low concentrations of ethanol (1.5 mM) were about 60 $\mu\text{mol/h}$ per g, yet butanol (1.5 mM) uptake was undetectable ($< 4 \mu\text{mol/h}$ per g). At higher concentrations of alcohol (25–30 mM), rates of ethanol uptake were about 80 $\mu\text{mol/h}$ per g, whereas rates of butanol uptake were only about 9 $\mu\text{mol/h}$ per g. Because rates of butanol metabolism via cytochrome *P*-450 in deermice were more than an order of magnitude lower than rates of ethanol uptake in livers from ADH-negative deermice, it is concluded that ethanol uptake by perfused livers from ADH-negative deermice is catalysed predominantly via catalase- H_2O_2 . In support of this conclusion, rates of H_2O_2 generation, which are rate-limiting for the peroxidation of ethanol by catalase, were about 65 $\mu\text{mol/h}$ per g in livers from ADH-negative deermice, values similar to rates of ethanol uptake of about 60 $\mu\text{mol/h}$ per g measured under identical conditions. Rates of ethanol uptake by perfused livers from ADH-positive, but not from ADH-negative, deermice were increased by about 50% by infusion of fructose. Thus it is concluded that the stimulation of hepatic ethanol uptake by fructose is dependent on the presence of ADH. Unexpectedly, fructose decreased rates of ethanol metabolism and H_2O_2 generation by about 60% in perfused livers from ADH-negative deermice, probably by decreasing activation of fatty acids and thus diminishing rates of peroxisomal β -oxidation.

INTRODUCTION

Previous studies on hepatic pathways of ethanol oxidation have employed inhibitors of alcohol dehydrogenase (ADH) and catalase; however, many inhibitors have non-specific effects (Kato, 1967; Rubin *et al.*, 1971; Handler *et al.*, 1986). Therefore, to quantify pathways of ethanol metabolism clearly, studies avoiding the use of metabolic inhibitors are necessary. The establishment of a strain of deermouse, *Peromyscus maniculatus*, which is genetically deficient in ADH (Burnett & Felder, 1978), provides an opportunity to study pathways of ethanol metabolism in the absence of ADH without the use of inhibitors. In this strain, ethanol must be metabolized via catalase- H_2O_2 , cytochrome *P*-450, or be eliminated by excretion and respiration. ADH-negative deermice eliminate ethanol *in vivo* at rates 30–70% of those of their ADH-positive counterpart (Burnett & Felder, 1980; Shigeta *et al.*, 1984; Glassman *et al.*, 1985; Handler *et al.*, 1986). The purpose of the present experiments was therefore to study catalase- and cytochrome *P*-450-dependent ethanol metabolism under controlled conditions without the complications present *in vivo*. The data demonstrate that ethanol metabolism occurs predominantly via catalase- H_2O_2 in the ADH-negative deermouse. Preliminary accounts of this work have appeared elsewhere (Handler *et al.*, 1985).

METHODS AND MATERIALS

Animals

Male and female deermice (*Peromyscus maniculatus*), weighing 16–20 g were obtained from a colony maintained at the University of North Carolina at Chapel Hill, established from breeding stock kindly provided by Dr. M. R. Felder of the University of South Carolina. Female Sprague–Dawley rats (150–200 g) and deermice had access to a chow diet and water *ad libitum*.

Haemoglobin-free liver perfusion

The perfusion technique for rat livers has been described elsewhere (Scholz *et al.*, 1973). All perfusions were performed between 10:00 and 14:00 h. The perfusion fluid was Krebs–Henseleit bicarbonate buffer (pH 7.4, 37°C) saturated with O_2/CO_2 (19:1). Deermice were anaesthetized with pentobarbital (80 mg/kg), the abdomen was opened, and the portal vein was cannulated with polyethylene tubing (PE10). The chest was then opened rapidly, the heart was severed, and flow (4–5 ml/min per g of liver) was initiated after a lag which did not exceed 30 s. The liver was removed surgically, and an outflow cannula was secured into the vena cava, using the diaphragm for support. The flow rate was then increased to 6–8 ml/min per g. Deermouse livers were

Table 1. Rates of metabolic processes in perfused livers from rats, ADH-positive and ADH-negative deermice

Livers from female Sprague-Dawley rats and ADH-positive and -negative deermice were perfused in a non-recirculating system. O₂ concentration in the effluent perfusate was determined with a Teflon-shielded Clark-type electrode. Lactate, pyruvate, glucose and LDH were measured enzymically, and 4-nitro catechol formed from the hydroxylation of *p*-nitrophenol (0.06 mM) was measured spectrophotometrically in samples of effluent perfusate as described in the Methods and materials section. Rates were calculated from influent–effluent concentration differences, the flow rate and the liver wet weight: 10 units/h per g reflects the limits of detection of LDH in this study. Data are means $\pm$ S.E.M. for four to nine livers per group: **P* < 0.05, ***P* < 0.01 compared with values for rat livers; †*P* < 0.05 compared with values from ADH-positive deermouse livers.

	LDH release (units/h per g)	Activity (μ mol/h per g)			
		O ₂ uptake	Lactate + pyruvate production	Glucose production	<i>p</i> -Nitrophenol hydroxylation
Rat	< 10	100 $\pm$ 8	88 $\pm$ 5	90 $\pm$ 2	2.1 $\pm$ 0.2
Deermouse					
ADH-positive	< 10	109 $\pm$ 17	18 $\pm$ 4**	67 $\pm$ 15	4.3 $\pm$ 0.4**
ADH-negative	< 10	164 $\pm$ 21*†	19 $\pm$ 3**	61 $\pm$ 18	6.8 $\pm$ 0.6**†

about the size of a 23 mm-diameter coin and weighed 500–800 mg.

In experiments where livers were perfused in a recirculating system (system volume 17 ml), bovine serum albumin (4%, w/v) was included in the perfusate described above. The effluent perfusate was reoxygenated and reheated (37 °C) in an oxygenator described in detail elsewhere (Berry *et al.*, 1974).

Analytical

The effluent perfusate was directed past a Teflon-shielded Clark-type O₂ electrode, and samples were collected every 2 min for analysis of glucose, lactate, pyruvate, ethanol, butanol and lactate dehydrogenase (LDH) by standard enzymic methods (Bergmeyer, 1974). 4-Nitro catechol formed from the hydroxylation of *p*-nitrophenol was measured as described by Chrastil & Wilson (1975). Samples (1 ml) of effluent perfusate were incubated with 250 Fishman units of β -glucuronidase and 20 units of sulphatase for 90 min; 100 ml of 10 M-NaOH was then added to each sample, and the A₅₄₆ was measured immediately (ϵ = 10.28; Reinke & Moyer, 1985). Metabolic rates were calculated from the influent–effluent concentration differences and the constant flow rate and were expressed per g wet wt. of liver.

In experiments where a recirculating perfusion system was used, 0.1 ml samples of perfusate were collected every 30 min and analysed for ethanol and butanol as described above. Rates were calculated from concentration differences per unit time, the volume of the system and the liver wet weight, and were corrected for vaporization from the system, which was less than 10%.

Viability of perfused deermouse liver

When photomicrographs of a typical perfused deermouse liver were studied, it was observed that sinusoids were not distended and hepatocytes had well-defined nuclei. Hence the hepatic architecture of perfused livers from ADH-negative deermice appeared normal. Further, Trypan Blue was excluded from perfused deermouse livers, indicating that plasma membranes of hepatocytes were intact (results not shown).

O₂ uptake, carbohydrate output, mixed-function oxi-

dation and effluent perfusate LDH activity were also monitored to assess viability (Table 1). LDH released from livers from ADH-positive and -negative deermice was below the limit of detectability (< 10 units/h per g; Table 1). Basal rates of O₂ uptake were about 100 μ mol/h per g in livers from rats and ADH-positive deermice; however, rates were significantly higher by about 50% in livers from ADH-negative deermice (see below). Rates of glycolysis (lactate + pyruvate production) were similar in the two strains of deermice studied, but were significantly lower than in livers from rats (Table 1). In contrast, rates of glucose output were comparable in livers from all three groups studied. Livers from rats hydroxylated *p*-nitrophenol at rates of about 2 μ mol/h per g, but rates were 2–3-fold higher in livers from deermice (Table 1), most likely because of higher amounts of cytochrome P-450 in the ADH-negative deermouse, as reported by Shigeta *et al.* (1984). Taken together, these morphological and biochemical data indicate that deermice livers are viable during perfusion for up to 2 h.

Histological

After fixation by perfusion with 1% paraformaldehyde for 10 min, cubes (approx. 0.5 cm) of liver were taken from the left lateral lobe, sectioned, stained with haematoxylin and eosin. In some experiments, Trypan Blue (0.2 mM), a dye excluded by cells with intact plasma membranes, was infused 6 min before livers were fixed. Under these conditions, sections were stained with eosin only to facilitate observation of the dye.

Oxidation of ethanol and butanol by microsomes and cytosol from ADH-negative deermice

Microsomes (microsomal fractions) and cytosol were isolated by differential centrifugation of homogenates (1:5, w/v) of livers from ADH-negative deermice prepared in 0.25 M-sucrose, pH 7.4. Microsomes were washed twice with 0.1 M-pyrophosphate containing 1 mM-EDTA, pH 7.4 (Koop *et al.*, 1984). The oxidation of ethanol by microsomes was measured as described elsewhere (Lieber & DeCarli, 1970). The oxidation of butanol (100 mM) was measured in a similar fashion, with butyraldehyde as the standard.

For the oxidation of alcohols by cytosol, 1–2 mg of cytosolic protein was incubated for 10 min in the buffer

used for microsomal assays described above containing 100 mM-ethanol or -butanol in capped vessels at 37 °C. Reactions were initiated by the addition of 0.1 ml of an H_2O_2 -generating system, consisting of 200 munits of glucose oxidase/ml and 10 mM-glucose, and were terminated after 10 min by the addition of 0.2 ml of 40% (w/v) trichloroacetic acid. Acetaldehyde and butyraldehyde were determined as described above.

Rates of H_2O_2 generation by perfused deermouse livers

Rates of H_2O_2 generation by perfused livers from ADH-positive and -negative deermice were measured as described by Oshino *et al.* (1973a) for rat liver. The steady-state content of catalase- H_2O_2 compound I was measured spectrophotometrically (660–640 nm) through a lobe of the liver (Sies & Chance, 1970), and the concentration of methanol required to diminish the content by 50% in deermouse livers was measured in the presence and absence of fructose (5 mM). The hepatic content of catalase haem was estimated as described previously for rat livers (Oshino *et al.*, 1975). Catalase haem concentrations were calculated from increases in absorbance after the addition of cyanide by using an absorption coefficient of $4.0 \text{ cm}^{-1} \cdot \text{mM}^{-1}$ as reported by Sies *et al.* (1973).

The maximal saturation of deermouse catalase haem was determined in perfused livers from ADH-positive and -negative deermice as described previously for rat liver catalase (Sies *et al.*, 1973). Glycollate (1 mM), a substrate for H_2O_2 generation, was infused to saturate catalase haem maximally as monitored by changes in the steady-state amount of catalase- H_2O_2 ($A_{660-640}$). Methanol was subsequently infused in increasing concentration steps to decrease $A_{660-640}$, followed by stepwise infusion of NaCN (final concn. 300–400 μM) to form the catalase-cyanide complex. Maximal fractional haem saturation was calculated by comparing the absorbance in the presence of glycollate with the maximal increase caused by cyanide in the presence of methanol, as described by Sies *et al.* (1973). Under these conditions, maximal saturation of catalase haem in perfused livers from ADH-positive and ADH-negative deermice with H_2O_2 was 40% (Table 3). Rates of H_2O_2 generation by perfused livers were calculated from the concentration of methanol required to decrease the steady-state amount of catalase- H_2O_2 by 50%, the concentration of catalase haem and kinetic constants for catalase (Oshino *et al.*, 1973a).

Materials

p-Nitrophenol, butyraldehyde and paraformaldehyde were purchased from Aldrich Chemical Co., Milwaukee, WI, U.S.A., and enzymes and cofactors were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Statistics

Comparisons between groups were made by Student's *t* test (Steel & Torrie, 1980).

RESULTS

Ethanol and butanol metabolism by perfused deermouse livers

Rates of O_2 uptake by livers from ADH-positive deermice of around $100 \mu\text{mol/h per g}$ were not altered significantly by infusion of ethanol (1.5 mM; Fig. 1a).

Fig. 1. Effect of fructose on ethanol uptake, O_2 uptake and glycolysis in a perfused liver from an ADH-positive deermouse liver

The liver from an ADH-positive deermouse was perfused in a non-recirculating system. Ethanol (1.5 mM) and fructose (5 mM) were infused as indicated by the vertical arrows and horizontal bars. O_2 uptake, ethanol uptake and production of lactate+pyruvate (glycolysis) were measured as described in the Methods and materials section. Results of a typical experiment are shown.

Infusion of fructose (5 mM) caused a large increase in O_2 uptake, to around $140 \mu\text{mol/h per g}$. Basal rates of lactate plus pyruvate production of $10\text{--}20 \mu\text{mol/h per g}$ were also not affected by ethanol, but were increased significantly to around $60 \mu\text{mol/h per g}$ by fructose (Fig. 1b). Basal rates of ethanol uptake in livers from ADH-positive deermice of about $90 \mu\text{mol/h per g}$ were also increased significantly, to $110\text{--}120 \mu\text{mol/h per g}$, by fructose (Fig. 1; Table 2).

In a liver from an ADH-negative deermouse, basal rates of O_2 uptake were around $140 \mu\text{mol/h per g}$ (Fig. 2), values significantly higher than in livers from ADH-positive deermice (Table 1). Ethanol did not affect O_2 uptake, but fructose caused a rapid increase of about $20 \mu\text{mol/h per g}$, which reached a new steady-state value in about 6 min. As in the ADH-positive strain, lactate plus pyruvate production was not affected by ethanol, but was increased significantly to about $60 \mu\text{mol/h per g}$ by fructose (Fig. 2b). Basal rates of ethanol uptake at

Table 2. Effect of fructose on uptake of ethanol and butanol by perfused livers from ADH-positive and ADH-negative deermice

Livers from ADH-positive and -negative deermice were perfused as described in the Methods and materials section. Rates of ethanol and butanol uptake were measured in a non-recirculating system by employing the experimental design depicted in Figs. 1 and 2. Ethanol or butanol (1.5 mM) was infused for 20 min, followed by fructose (5 mM) for 20 min concomitant with the alcohol. Ethanol and butanol were measured enzymically in samples of effluent perfusate, and rates of alcohol uptake were calculated from the influent–effluent concentration differences, the flow rate and the liver wet weight. Livers were also perfused in a recirculating system containing 4% bovine serum albumin. Ethanol or butanol (25–30 mM) was added to the system and samples of perfusate were collected after 30 min. Fructose (10 mM) was then added and samples of perfusate were collected after an additional 30 min of perfusion. Rates of ethanol and butanol uptake were calculated from concentration differences per unit time, the liver wet weight and the volume of perfusate (17 ml). All rates were corrected for vaporization, which was 5–10%. Data represents means $\pm$ S.E.M. for four to seven livers per group: * $P < 0.05$, ** $P < 0.01$ compared with control values.

Alcohol	Concn. (mM)	Deermouse strain	Alcohol uptake ($\mu\text{mol/h per g}$)	
			Control	Fructose
Ethanol	1.5	ADH-positive	88 $\pm$ 9	115 $\pm$ 2*
	25–30	ADH-negative	106 $\pm$ 10	26 $\pm$ 9**
Butanol	1.5	ADH-negative	62 $\pm$ 11	24 $\pm$ 3**
	25–30	ADH-negative	78 $\pm$ 3	11 $\pm$ 4*
Butanol	1.5	ADH-negative	4 $\pm$ 3	17 $\pm$ 2*
	25–30	ADH-negative	9 $\pm$ 1	17 $\pm$ 2*

low ethanol concentrations (1.5 mM) in livers from ADH-negative deermice were $62 \pm 11 \mu\text{mol/h per g}$; at high ethanol concentrations (25–30 mM), rates were $78 \pm 3 \mu\text{mol/h per g}$ (Table 2). Rates were decreased unexpectedly by about 60% by infusion of fructose, to 26 ± 9 and $24 \pm 3 \mu\text{mol/h per g}$ at low and high concentrations of ethanol respectively (Fig. 2, Table 2).

Basal rates of butanol uptake by livers from ADH-negative deermice were about an order of magnitude lower than rates of ethanol uptake. Values were $4 \pm 3 \mu\text{mol/h per g}$ at 1.5 mM-butanol and $9 \pm 1 \mu\text{mol/h per g}$ at 25–30 mM-butanol (Table 2). In the presence of fructose, rates were increased slightly, to 11 ± 4 and $17 \pm 2 \mu\text{mol/h per g}$, at low and high concentrations of butanol respectively.

Effect of fructose on rates of H_2O_2 generation by perfused liver

In order to determine rates of H_2O_2 generation in livers perfused in a non-recirculating system, it is necessary to know the hepatic content of catalase haem. This, calculated from measurements of the catalase–cyanide complex at 660–640 nm, was about 20% greater in livers from ADH-positive than in ADH-negative deermice (Table 3), consistent with the report that catalase activity is higher in livers of the ADH-positive

Fig. 2. Effects of fructose on ethanol uptake, O_2 uptake and glycolysis in a perfused liver from an ADH-negative deermouse

Conditions were as in Fig. 1, except that the liver was from an ADH-negative deermouse. Results of a typical experiment are shown.

strain (Shigeta *et al.*, 1984). However, the concentration of methanol necessary to decrease the steady-state amount of catalase– H_2O_2 by 50% was almost 4 times greater in livers from ADH-negative than from ADH-positive deermice (Table 3). Rates of H_2O_2 generation calculated from these values as described in the Methods and materials section were $22 \pm 2 \mu\text{mol/h per g}$ in livers from ADH-positive and $63 \pm 5 \mu\text{mol/h per g}$ in livers from ADH-negative deermice (Table 3). Fructose (5 mM) decreased rates of H_2O_2 generation in livers from ADH-negative deermice to about $26 \mu\text{mol/h per g}$ (Table 3).

Metabolism of ethanol and butanol by cytosol and microsomes from ADH-negative deermice

NADPH-dependent oxidation of butanol and ethanol by microsomes from ADH-negative deermice was 9.6 ± 0.5 and $10.1 \pm 0.6 \text{ nmol/min per mg}$ of protein respectively (Table 4). Cytosol isolated from homogenates of deermouse liver incubated with an H_2O_2 -generating system oxidized ethanol at rates of $22 \pm 2 \text{ nmol/min per mg}$. Under similar conditions, butanol metabolism was below the limits of detection (Table 4).

Table 3. Catalase content and parameters related to H_2O_2 generation in perfused livers from ADH-positive and ADH-negative deermice

Livers from ADH-positive and -negative deermice were perfused briefly to remove blood and were homogenized in 0.1 M-phosphate buffer, pH 7.4, containing 1 % sodium deoxycholate, 1 % Triton X-100 and 20 mM-methanol. Catalase haem content was determined as described in the Methods and materials section. The steady-state amount of catalase- H_2O_2 was measured spectrophotometrically (660–640 nm) through a lobe of the liver. Maximal fractional saturation of catalase haem with H_2O_2 and a_1 values were determined by infusion of glycolate (1 mM) into perfused livers, followed by infusion of increasing concentrations of methanol. When there was no further change in $A_{660-640}$ with increasing concentrations of methanol, NaCN (10–400 μ M) was infused to form the cyanide-catalase complex, and absorbance at 660–640 nm was recorded. Rates of H_2O_2 generation were calculated as described in the Methods and materials section. Data represent means $\pm$ S.E.M. for four livers per group.

	Catalase haem content (nmol/g wet wt.)	a_1 (mM)	Maximal fractional saturation of catalase haem with H_2O_2	H_2O_2 generation (μ mol/h per g)
ADH-positive	107 $\pm$ 4	0.11 $\pm$ 0.01	0.4	22 $\pm$ 2
ADH-negative	79 $\pm$ 3	0.42 $\pm$ 0.09	0.4	63 $\pm$ 5
+ Fructose (5 mM)		0.17 $\pm$ 0.05		26 $\pm$ 4

DISCUSSION

Stimulation of ethanol metabolism by fructose requires ADH

It is well known that fructose stimulates ethanol metabolism in isolated hepatocytes (Berry & Kun, 1978), in perfused livers (Scholz & Nohl, 1976) and in man (Carpenter & Lee, 1937; Crownover *et al.*, 1986). The first step in this sequence of events is the phosphorylation of fructose by fructokinase. This increases ADP supply, which accelerates electron flux through the respiratory chain and leads to increased rates of reoxidation of NADH (Scholz & Nohl, 1976). Ultimately, the increase in NADH reoxidation elevates the rate of oxidation of ethanol via ADH. In addition, fructose increases oxidation of NADH by cytoplasmic dehydrogenases and increases components of the glycerol-3-phosphate shuttle (Berry & Kun, 1978). In the present study, fructose stimulated ethanol metabolism in livers from ADH-positive, but not from ADH-negative, deermice (Figs. 1 and 2; Table 2). Therefore it is concluded that the stimulation of ethanol oxidation by fructose has an absolute requirement for ADH. This conclusion is

consistent with the observation that the stimulation of ethanol metabolism by fructose was blocked by 4-methylpyrazole, an inhibitor of ADH (Berry & Kun, 1978).

Pathways of ethanol metabolism in ADH-negative deermice

Others (Shigeta *et al.*, 1984; Takagi *et al.*, 1986) have concluded that ethanol metabolism in ADH-negative deermice is catalysed predominantly by cytochrome P-450. These conclusions were based on observations that pretreatment for 3–6 h with the catalase inhibitor aminotriazole did not decrease rates of ethanol elimination in ADH-negative deermice *in vivo*. In contrast, when catalase- H_2O_2 was monitored spectrophotometrically as a control for the efficacy of aminotriazole, Handler *et al.* (1986) observed that a brief (1.5 h) pretreatment with aminotriazole blocked changes in catalase- H_2O_2 completely. Under these conditions, the peroxidation of ethanol by catalase and ethanol elimination *in vivo* were depressed nearly totally. Peroxidatic activity of catalase and rates of ethanol elimination returned to basal values 6 h after aminotriazole administration. Thus time of treatment with aminotriazole appears to be an important experimental variable. It is therefore concluded that caution must be employed when using metabolic inhibitors to study pathways of ethanol oxidation.

In this study, ethanol was metabolized in perfused livers from ADH-negative deermice at rates about two-thirds as fast as in livers from the ADH-positive strain at both low and high concentrations of ethanol (Table 2), in general agreement with values from previous studies *in vivo* (Burnett & Felder, 1980; Shigeta *et al.*, 1984; Glassman *et al.*, 1985; Handler *et al.*, 1986).

To evaluate pathways of ethanol elimination in perfused livers from ADH-negative deermice, butanol was used as a tool. In this strain, ethanol was oxidized by both cytosolic catalase and microsomal cytochrome P-450, whereas butanol was metabolized by cytochrome P-450, in microsomes, but not by cytosolic catalase (Table 4), consistent with previous studies in the rat (Chance, 1947; Teschke *et al.*, 1975). Therefore butanol is a selective substrate for cytochrome P-450 and is a useful tool with which to differentiate between cytochrome

Table 4. Oxidation of ethanol and butanol by subcellular fractions from ADH-negative deermice

ADH-negative deermice were decapitated, livers were homogenized and cytosolic and microsomal fractions were prepared by differential centrifugation as described in the Methods and materials section. Ethanol and butanol oxidation were measured as described in the Methods and materials section by trapping the aldehydes formed as semicarbazones. Data represent means $\pm$ S.E.M. for four livers per group.

	Oxidation (nmol/min per mg of protein)	
	Ethanol	Butanol
Cytosol	21.8 $\pm$ 1.7	< 0.4
Microsomes	10.1 $\pm$ 0.6	9.6 $\pm$ 0.5

P-450- and catalase-dependent pathways. Perfused livers from ADH-negative deermice metabolized butanol at rates of 4–9 $\mu\text{mol/h}$ per g, values at least an order of magnitude lower than rates of ethanol uptake, of 62–80 $\mu\text{mol/h}$ per g (Table 2). Since deermouse cytochrome *P*-450 has the same affinity for ethanol and butanol (Table 4), it is concluded that rates of ethanol metabolism via cytochrome *P*-450 are insufficient to account for rates of ethanol uptake in perfused livers from ADH-negative deermice. Because ethanol can only be metabolized by catalase– H_2O_2 and cytochrome *P*-450 in perfused livers from ADH-negative deermice, it follows that ethanol oxidation is catalysed predominantly via catalase– H_2O_2 in this mutant.

H_2O_2 generation in perfused deermouse livers

Although hepatic catalase content is higher in ADH-positive than in ADH-negative deermice, rates of H_2O_2 generation were about 3-fold higher in ADH-negative than in ADH-positive deermice (Table 3). Since 1 mol of O_2 is required for the formation of 1 mol of H_2O_2 , much more O_2 must be taken up in livers from ADH-negative deermice to support high rates of H_2O_2 generation. Indeed, O_2 uptake was about 50% higher in livers from ADH-negative compared with ADH-positive deermice (Table 1). Since H_2O_2 can be generated in perfused rat livers at high rates (up to 80 $\mu\text{mol/h}$ per g) from fatty acids via peroxisomal β -oxidation (Handler & Thurman, 1987), it is possible that more rapid rates of peroxisomal β -oxidation of fatty acids in livers from ADH-negative than from ADH-positive deermice account for the higher rates of H_2O_2 generation observed.

There is a nearly 1:1 stoichiometry between rates of H_2O_2 generation and rates of peroxidation of ethanol via catalase– H_2O_2 (Oshino *et al.*, 1973*b*). Rates of H_2O_2 generation in livers from ADH-negative deermice of about 65 $\mu\text{mol/h}$ per g measured in a non-recirculating system agreed well with rates of ethanol uptake of about 60 $\mu\text{mol/h}$ per g measured under identical experimental conditions (Tables 2 and 3), supporting the hypothesis that ethanol oxidation by ADH-negative deermice is mediated predominantly via catalase– H_2O_2 . Similar results were obtained in perfused rat livers in the presence of fatty acids (Handler & Thurman, 1987).

Rates of ethanol uptake at high (25–35 mM) concentrations of ethanol were about 25% faster than at low (1.5 mM) concentrations (Table 2). These data agree with a previous report that rates of ethanol oxidation by isolated hepatocytes from ADH-negative deermice were increased at higher concentrations of ethanol (Takagi *et al.*, 1986). The increase in rates of ethanol oxidation by perfused livers at elevated ethanol concentrations is most probably not due to increased rates of ethanol oxidation via cytochrome *P*-450, since rates of butanol oxidation increased by only 5 $\mu\text{mol/h}$ per g when the concentration of butanol was increased, but rates of ethanol oxidation were elevated by about 20 $\mu\text{mol/h}$ per g when ethanol concentrations were raised (Table 2). Thus increased oxidation of ethanol by cytochrome *P*-450 most probably accounts for only a small portion of the increase in rates of ethanol uptake at high compared with low ethanol concentrations. A more likely explanation of the higher rates of ethanol uptake at elevated ethanol concentrations is that rates of H_2O_2 generation were higher in the recirculating perfusion system utilized in experiments where ethanol uptake was

measured at high ethanol concentrations. Since bovine serum albumin was used in the latter experiments, it is feasible that fatty acids bound to the albumin were taken up by the perfused livers and metabolized via peroxisomal β -oxidation, with the concomitant formation of H_2O_2 (Handler & Thurman, 1987).

Fructose decreases catalase-dependent ethanol metabolism in perfused livers from ADH-negative deermice

Unexpectedly, fructose decreased rates of ethanol metabolism in livers from ADH-negative deermice (Fig. 2, Table 2). Since ethanol metabolism in perfused livers from ADH-negative deermice is mediated predominantly via catalase– H_2O_2 , fructose should affect either the catalase enzyme or the supply of H_2O_2 , or both. Fructose did not affect the peroxidatic activity of catalase incubated with a H_2O_2 -generating system (results not shown); however, fructose decreased rates of H_2O_2 generation in livers from ADH-negative deermice by about 60% (Table 3). Since H_2O_2 supply is rate-limiting for the peroxidation of ethanol by catalase (Oshino *et al.*, 1973*b*), decreases in H_2O_2 generation should cause a concomitant decline in rates of ethanol uptake in a system dependent on catalase– H_2O_2 . In fact, rates of ethanol uptake were decreased from 60 to 26 $\mu\text{mol/h}$ per g by fructose (Table 2). Thus fructose decreases ethanol uptake by ADH-negative deermice by decreasing H_2O_2 supply for the peroxidation of ethanol via catalase– H_2O_2 . In contrast, butanol oxidation, which is catalysed by cytochrome *P*-450 and not catalase, was not decreased by fructose in perfused deermouse livers; rather, fructose increased butanol oxidation slightly, by about 5 $\mu\text{mol/h}$ per g (Table 2). This increase is most probably due to an increase in the supply of NADPH for cytochrome *P*-450, owing to increased flux of carbohydrate through the pentose phosphate cycle (Kauffman *et al.*, 1979).

How, then, does fructose decrease the supply of H_2O_2 for catalase-dependent ethanol metabolism? It has been demonstrated that fatty acids, which generate H_2O_2 via peroxisomal β -oxidation (Lazarow & de Duve, 1976), stimulate catalase-dependent ethanol metabolism in perfused rat liver (Handler & Thurman, 1985, 1987). To be metabolized by the peroxisome, fatty acids must first be activated to acyl-CoA compounds in a reaction which requires ATP. Because fructose depletes ATP (Ravio *et al.*, 1969; Woods *et al.*, 1970), it is hypothesized that fructose decreases the energy-dependent activation of fatty acids, thereby decreasing rates of H_2O_2 generation via peroxisomal β -oxidation. Thus, in the presence of fructose, ethanol should be metabolized nearly exclusively via ADH in normal animals, because of concomitant inhibition of peroxidation of ethanol via catalase– H_2O_2 .

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