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Hepatic Microsomal Alcohol-Oxidizing System in Normal and Acatalasemic Mice: Its Dissociation from the Peroxidatic Activity of Catalase-H_2O_2

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SUMMARY

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To assess whether catalase-H_2O_2 is an obligatory component in the microsomal alcohol-oxidizing system, various primary alcohols were incubated with hepatic microsomes of both normal and acatalasemic mice in the presence of an NADPH-generating system. Methanol, ethanol, propanol, butanol, and pentanol were metabolized at striking rates by microsomes of both strains. By contrast, when the NADPH-generating system was replaced by a H_2O_2-producing one, propanol, butanol, and pentanol were not metabolized, indicating that these higher aliphatic alcohols are not substrates for catalase-H_2O_2. Furthermore, mild heat treatment of microsomes of acatalasemic mice resulted in inactivation of contaminating catalase and virtually abolished alcohol peroxidation of methanol and ethanol by catalase-H_2O_2, whereas the rates of the NADPH-mediated alcohol oxidation by the microsomal fraction persisted with methanol, ethanol, propanol, butanol, and pentanol as substrates. In addition, the microsomal alcohol-oxidizing system of both normal and acatalasemic mice was solubilized and isolated from catalase by DEAE-cellulose column chromatography. These findings therefore dissociate the NADPH-dependent microsomal alcohol-oxidizing system from alcohol peroxidation via catalase-H_2O_2 by difference in substrate specificity and rule out an obligatory involvement of catalase-H_2O_2 in the microsomal system.

INTRODUCTION

The capacity of hepatic microsomes to oxidize ethanol to acetaldehyde in a reaction requiring NADPH and molecular oxygen is well documented (1, 2). This microsomal ethanol-oxidizing system was considered to play some role in ethanol metabolism *in vivo* (3) as well as *in vitro* in liver slices (2, 4, 5), perfused liver (6, 7), and isolated parenchymal cells (5, 8). Evidence against an obligatory role of catalase-H_2O_2 in the activity of the NADPH-dependent microsomal alcohol-oxidizing system (2, 9-11) was provided by a variety of studies, including the recent separation of the rat liver microsomal ethanol-oxidizing system from catalase by DEAE-cellulose column chromatography (12-14).

The biochemical nature of the microsomal ethanol-oxidizing system, however, became the subject of lively debate as a re-

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sult of the claim that the alcohol-oxidizing activity may be due exclusively to a catalase-H_2O_2-mediated reaction (15–17). In particular, it was reported that hepatic microsomes of acatalasemic mice displayed no ethanol metabolism following heat inactivation of the thermolabile catalase (17). Under the latter experimental conditions, however, when product formation was assessed (18) rather than substrate disappearance (17), striking rates of microsomal acetaldehyde production were observed (18). In view of these conflicting reports further studies were undertaken to resolve the question whether catalase-H_2O_2 is an obligatory component in the NADPH-dependent microsomal alcohol oxidation in livers from acatalasemic mice. To that effect, we tested the specificity of the microsomal system with respect to various alcohols as substrates, including butanol and pentanol, which have been previously shown not to react peroxidatically with catalase-H_2O_2 (19). Furthermore, experiments were carried out to determine the effect of heat inactivation of contaminating catalase on the activity of the NADPH-dependent microsomal alcohol-oxidizing system.

MATERIALS AND METHODS

Materials. The chemicals and enzymes were obtained from the following sources: NADPH, $NADP^+$ (yeast), isocitrate dehydrogenase, and DL-isocitrate (type I), from Sigma Chemical Company; methanol, 1-propanol, 1-butanol, 1-pentanol, formaldehyde, D-glucose, disodium EDTA, and sodium azide, from Fisher Scientific Company; acetaldehyde, propionaldehyde, butyraldehyde, and valeraldehyde, from Eastman Kodak; ethanol (dehydrated) from US Industrial Chemicals Corporation; and glucose oxidase (type I), from Boehringer/Mannheim.

Preparation of microsomes. The strains of normal (Cs^a) and acatalasemic (Cs^b) mice were derived from breeding stocks kindly donated by Dr. R. N. Feinstein, Division of Biological and Medical Research, Argonne, Ill. After weaning, the animals were fed Purina chow and tap water ad libitum. When they reached body weights of 20–28 g, the mice were decapitated and the livers were excised after removal of the gall bladders. Pools of 4–30 livers of each strain served for the preparation of washed microsomes as described previously (3).

Biochemical determinations. The activity of the NADPH-dependent microsomal alcohol-oxidizing system was determined with washed microsomes (5 mg of protein per flask) which had been incubated with the respective alcohol (methanol, 100 mM; ethanol, 1-propanol, 1-butanol, or 1-pentanol, 50 mM) for 5 min at 37°. The reaction was initiated by adding NADPH (0.4–1.0 mM) or an NADPH-generating system (0.4 mM $NADP^+$, 8 mM sodium isocitrate, and 0.34 unit/ml of isocitrate dehydrogenase). The incubations were carried out in a final volume of 3.0 ml containing 1.0 mM disodium EDTA and 5.0 mM $MgCl_2$ in 0.1 M phosphate buffer (pH 7.4). The reactions were performed in closed 50-ml Erlenmeyer flasks and terminated after 0, 5, and 10 min of incubation time by adding 0.5 ml of 70% (w/v) trichloroacetic acid. Formaldehyde produced upon the oxidation of methanol was measured according to Nash (20). The aldehydes formed by the oxidation of ethanol, propanol, butanol, and pentanol were measured as their semicarbazone derivatives (21), using as standards incubation flasks to which known amounts of the respective aldehyde were added. Product identification and quantitative analysis were performed by absorption spectrophotometry and by gas-liquid chromatography (22).

Peroxidatic activity of catalase was determined under assay conditions similar to those described for the NADPH-dependent microsomal alcohol-oxidizing system except that the NADPH-generating system was replaced by a H_2O_2-producing one, consisting of glucose (10 mM) and glucose oxidase (5 μg/ml of incubation medium). Under these conditions glucose was first incubated with microsomes and ethanol (50 mM), and the reaction was started by addition of glucose oxidase. With each incubation set, experiments were performed in which the microsomes were replaced by 0.1 M phosphate buffer (pH 7.4), and the ob-

served blank values thus obtained were subtracted from the corresponding experimental results. Catalatic activity of catalase was measured by following the disappearance of added H_2O_2 at 240 nm and expressed in units according to Lück (23). Alcohol dehydrogenase activity was assayed by monitoring the reduction of NAD^+ at 340 nm in the presence of 50 mM ethanol (24). Protein concentration was determined according to Lowry *et al* (25). Cytochrome P-450 and b_5 contents were estimated by the method of Omura and Sato (26). The activity of NADPH–cytochrome *c* reductase was measured according to Masters *et al*. (27). NADH–cytochrome b_5 reductase activity was determined by the method of Strittmatter (28), with potassium ferricyanide (0.25 mM) as electron acceptor.

Isolation of microsomal alcohol-oxidizing system. Washed microsomes of both normal and acatalasemic mice were solubilized by ultrasonication and treatment with deoxycholate as described (14). Solubilized microsomes were subjected to DEAE-cellulose column chromatography, and elution was performed by a stepwise increase of the KCl gradient. Fractions recovered with 0.4 M KCl were combined following overnight dialysis against 0.1 M phosphate buffer (pH 7.0) and used for characterization of the isolated microsomal alcohol-oxidizing system (14, 22).

Statistical analysis. Each individual result was compared with the value of its corresponding control; the means and standard errors of individual differences were calculated and their significance was assessed by the paired Student's *t*-test.

RESULTS

Hepatic microsomes of both normal and acatalasemic mice actively oxidized methanol, ethanol, propanol, butanol, and pentanol to their corresponding aldehydes in the presence of an NADPH-generating system and molecular oxygen at similar rates in both strains (Table 1). This finding raises the question of the biochemical nature of this reaction, in particular whether the observed NADPH-dependent alcohol oxidation can be ascribed either to the activities of alcohol dehydrogenase and catalase-H_2O_2 or to a mechanism which operates independently of these two enzymes.

TABLE 1

NADPH-dependent microsomal alcohol-oxidizing system in normal (Cs^a) and acatalasemic (Cs^b) mice

Washed microsomes (5.0 mg of protein per assay) were incubated with the respective alcohol (methanol, 100 mM; ethanol, propanol, butanol, or pentanol, 50 mM) in phosphate buffer (0.1 M, pH 7.4) at 37° for 5 min. The reaction was started by adding the NADPH-generating system. The final incubation volume was 3.0 ml and contained 1 mM EDTA and 5 mM $MgCl_2$. Incubations were carried out for 0, 5, and 10 min. The data are expressed as means ± standard errors of four experiments.

Alcohol	Normal strain	Acatalasemic strain
	nmoles aldehyde/min/mg microsomal protein	
Methanol	11.9 ± 0.6	9.9 ± 1.3
Ethanol	13.0 ± 0.7	13.4 ± 0.6
Propanol	9.3 ± 0.5	10.5 ± 0.7
Butanol	9.0 ± 0.3	8.8 ± 0.8
Pentanol	4.9 ± 0.9	5.3 ± 0.7

Hepatic microsomes of both strains contain small but measurable amounts of catalase, as assayed by its property to decompose H_2O_2 catalatically (Table 2). However, the amount of catalase found in the microsomal fractions represented less than 1% of that in the corresponding homogenate (Table 2). Compared to the normal strain, the activity of catalase in microsomes of the acatalasemic mice was significantly decreased by 33% ($p < 0.01$) when expressed per gram of liver (Table 2). The observed difference of catalase activity in microsomes between the normal and the acatalasemic strain may be explained by the difference in enzyme activity found in the liver homogenates of both strains: catalase activity in homogenates of the acatalasemic strain represented only 47% ($p < 0.001$) of the activity present in homogenates of normal mice (Table 2).

In view of the observed contamination of the microsomal fraction by catalase (Table 2), it became important to test whether alcohols which were metabolized in the presence of NADPH (Table 1) might also be substrates for catalase-H_2O_2. This was

TABLE 2

Catalase activity of liver homogenate and microsomes in normal (Cs^a) and acatalasemic (Cs^b) mice

Catalase activity was measured in aliquots of the 25% liver homogenate as well as in washed microsomes and expressed in units per gram of liver, wet weight, according to Lück (23). The data represent means ± standard errors of seven experiments, in which pools of 4–30 livers of each strain were used.

Preparation	Catalase activity				p^a
	Normal strain		Acatalasemic strain		
	units/g liver	%	*units/g liver*	%	
Homogenate	3649 ± 351	100	1720 ± 180	100	<0.001
Microsomes	22.3 ± 2.0	0.61	14.9 ± 1.0	0.87	<0.01

[a] Comparison between normal and acatalasemic strains.

studied by measuring aldehyde production in the microsomal fraction under experimental conditions in which the NADPH-generating system was replaced by a H_2O_2-producing one. In the normal strain, both methanol and ethanol were metabolized with the H_2O_2-generating system to some extent, whereas the rate of propanol oxidation was negligible (Table 3). This finding indicates that methanol and ethanol are capable of reacting peroxidatically with catalase-H_2O_2 when the rate of H_2O_2 generation is sufficient. By contrast, butanol and pentanol were not at all metabolized in the presence of the H_2O_2-generating system, whereas striking rates could be demonstrated with the NADPH-producing one (Table 3).

Compared to the normal strain, microsomes of acatalasemic mice were found to oxidize methanol and ethanol at much lower rates with the H_2O_2-generating system (Table 3). Concomitantly, no H_2O_2-dependent oxidation of propanol, butanol, or pentanol could be demonstrated in microsomes of acatalasemic mice, a finding similar to that observed in the normal strain (Table 3). However, the NADPH-dependent oxidation of all alcohols persisted in the acatalasemic strain (Table 3), at rates similar to those measured in normal animals (Tables 1 and 3). Thus, under conditions in which the H_2O_2-mediated peroxidation of methanol and ethanol was greatly reduced, the rates of the NADPH-dependent oxidation of various alcohols, including methanol and ethanol, persisted (Table 3).

Of interest was the finding that the rates of the H_2O_2-mediated methanol and ethanol oxidation in microsomes of the mutant acatalasemic strain were diminished by 80–90% compared to those observed in normal animals, whereas catalase activity was decreased by only 30% (Table 3). Thus the peroxidatic activity of catalase, as measured by H_2O_2-mediated oxidation of methanol and ethanol, was much lower in microsomes of the mutant strain than one

TABLE 3

Comparison between rates of NADPH-dependent and H_2O_2-mediated alcohol oxidation in hepatic microsomes from normal (Cs^a) and acatalasemic (Cs^b) mice

Thirty livers of each strain were pooled and served as the source of washed microsomes. Each alcohol (methanol, 100 mM; ethanol, propanol, butanol, or pentanol, 50 mM) was first preincubated with microsomes (5.0 mg of protein per assay) in phosphate buffer (0.1 M, pH 7.4). The reaction was started by adding the NADPH-generating system and was carried out for 0, 5, and 10 min in a final incubation volume of 3.0 ml containing 1 mM EDTA and 5 mM $MgCl_2$. When a H_2O_2-generating system was used, glucose (10 mM) was included in the initial incubation mixture, and the reaction was started by addition of glucose oxidase (5 μg/ml of incubation medium). Catalatic activity in microsomes of normal and acatalasemic mice was 1.3 and 0.9 unit/mg of microsomal protein, respectively.

Alcohol	Normal strain		Acatalasemic strain	
	NADPH	H_2O_2	NADPH	H_2O_2
	nmoles aldehyde/min/mg microsomal protein			
Methanol	11.7	9.8	8.5	1.0
Ethanol	12.9	9.0	14.0	1.5
Propanol	8.5	0.3	10.6	0
Butanol	8.3	0	8.5	0
Pentanol	4.2	0	3.5	0

would have predicted for the observed values of catalatic activity. Since the H_2O_2-generating system used was the same for both microsomal preparations, the low rates of H_2O_2-dependent methanol and ethanol oxidation in the acatalasemic strain (Table 3) might have been caused by inactivation of catalase itself during both the preliminary and final incubations, which lasted for a total of 15 min under the standard assay for H_2O_2-mediated alcohol oxidation. That this was indeed the case was verified by subjecting hepatic microsomes to mild heat treatment at 37° for various lengths of time. When measured by its catalatic property, catalase activity in microsomes of the acatalasemic strain was rapidly inactivated within the first 15 min of treatment (Fig. 1), a finding which explains the low rate of H_2O_2-dependent and catalase-mediated oxidation of methanol and ethanol in this particular strain (Table 3). These results led to the conclusion that catalase activity becomes the rate-limiting enzyme in the H_2O_2-mediated peroxidation of methanol and ethanol during heat treatment of microsomes of acatalasemic mice. In contrast to the acatalasemic strain, the activity of catalase in normal mice remained stable during the 1-hr heat treatment (Fig 1), which is consistent with the much higher rates of H_2O_2-dependent oxidation of methanol and ethanol observed in this particular strain compared to those in acatalasemic mice (Table 3).

Following heat treatment for 1 hr, catalatic activity in microsomes of acatalasemic mice was decreased by approximately 90% compared to values obtained before treatment (Fig. 1). Since microsomal preparations contain less than 1% of the catalase activity of the respective liver homogenate (Table 2), the catalase activity in microsomes of the acatalasemic strain following the 1-hr heat treatment represents less than 0.1% of the values of liver homogenates of the acatalasemic mice and less than 0.05% of those of liver homogenates of the normal strain. It was therefore of interest to study the capacity of the residual catalase to peroxidize alcohols which are known to be substrates for catalase-H_2O_2. Peroxidatic activity of catalase was measured with the H_2O_2-generating system and was found to be virtually abolished in microsomes of acatalasemic mice following heat inactivation of catalase for 60 min, using either ethanol (Table 4) or methanol as substrate for the assay. Under these experimental conditions, however, the rates of the NADPH-dependent microsomal alcohol oxidation persisted (Table 4). Of particular interest was the finding that striking oxidation rates for methanol and ethanol were still observed in the presence of the NADPH-generating system despite the almost complete heat inactivation of catalase (Table 4). Similar rates of alcohol oxidation were found when NADPH (1.0 mM) rather than the NADPH-generating system was used (Table 5). To further assess whether the NADPH-dependent oxidation of lower aliphatic alcohols might be due to trace amounts of catalase remaining in the system, heat-treated microsomes (37° for 60 min) of acatalasemic mice were incubated with ethanol and the catalase inhibitor sodium azide (final concentration, 1.0 mM). Despite the presence of the inhibitor, microsomal ethanol oxidation remained unaffected whether NADPH (1.0 mM) or the NADPH-generating system was employed (Table 5). These

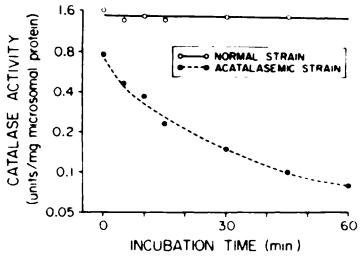

FIG. 1. *Time course of mild heat treatment at 37° of microsomes with respect to catalase activity of normal (Cs^a) and acatalasemic (Cs^b) mice*

Liver microsomes derived from pools of four to six livers of animals in each strain were incubated in a Dubnoff metabolic shaking incubator (100 strokes/min) at 37° in vessels closed with rubber tops. At the indicated time intervals aliquots of the microsomal suspensions were harvested with a needle and syringe through the closed top of the vessel and immediately chilled in ice before the determination of catalatic activity of catalase according to Lück (23). The data are expressed in units per milligram of microsomal protein and represent means of five experiments.

TABLE 4

Effect of mild heat treatment (37° for 60 min) of microsomes on activities of catalase and NADPH-dependent microsomal alcohol-oxidizing system in normal (Cs^a) and acatalasemic (Cs^b) mice

Microsomes were derived from pools of 30 livers from each strain. Catalatic activity was measured by its property to decompose added H_2O_2 and expressed as units per milligram of microsomal protein (23). Peroxidatic activity of catalase was determined by incubating glucose oxidase (5 μg/ml of incubation medium) with microsomes (5 mg of protein per assay) which had been incubated with ethanol (50 mM) and glucose (10 mM) for 5 min at 37°. The incubations were carried out for 0, 5, and 10 min in a final incubation volume of 3.0 ml containing 1 mM EDTA and 5 mM $MgCl_2$ in phosphate buffer (0.1 M, pH 7.4). Peroxidatic activity is expressed as nanomoles of acetaldehyde produced per minute per milligram of microsomal protein. Rates of alcohol oxidation were determined with the NADPH-generating system as described under MATERIALS AND METHODS and expressed as nanomoles of aldehyde produced per minute per milligram of microsomal protein.

Activity	Normal strain		Acatalasemic strain	
	No prior treatment	Heat treatment	No prior treatment	Heat treatment
Catalatic activity	1.4	1.1	1.0	0.1
Peroxidatic activity	8.4	8.7	1.4	0.3
Methanol oxidation	12.5	12.7	8.2	7.8
Ethanol oxidation	12.8	12.8	13.8	10.0
Propanol oxidation	8.5	7.2	10.4	10.4
Butanol oxidation	8.0	5.9	8.6	8.5
Pentanol oxidation	4.0	3.0	3.6	2.9

results therefore clearly show that hepatic microsomes contain a NADPH-dependent alcohol-oxidizing system which operates independently of catalase.

To test whether the activity of the microsomal alcohol-oxidizing system might persist following complete removal of catalase, microsomes of both normal and acatalasemic mice were solubilized and isolated by DEAE-cellulose column chromatography (Table 6). The column fractions exhibiting striking rates of alcohol oxidation in the presence of NADPH were completely devoid of catalatic as well as peroxidatic activity of catalase (Table 6). These fractions contained cytochrome P-450, NADPH–cytochrome *c* reductase, cytochrome b_5, and NADH–cytochrome b_5 reductase.

DISCUSSION

The present study demonstrates the capacity of hepatic microsomes of both normal and acatalasemic mice to metabolize at significant rates various higher primary alcohols with a NADPH-generating system (Table 1) but not with a H_2O_2-producing one (Table 3). Furthermore, inactivation of catalase was associated with a loss of microsomal oxidation of lower primary alcohols in the presence of generated H_2O_2, whereas the NADPH-dependent alcohol oxidation persisted (Table 4). These findings contrast with a previous report (29), in which no NADPH-dependent oxidation of propanol and butanol in porcine hepatic microsomes was detected. This discrepancy between the latter study (29) and the present data may be explained by differences in assay conditions under which aldehydes were determined. Based on the previous failure to demonstrate propanol and butanol oxidation by hepatic microsomes (29), it was concluded by some that the NADPH-dependent microsomal methanol and ethanol oxidation is due exclusively to catalase-H_2O_2 (16, 30), since catalase reacts peroxidatically only with methanol and ethanol (19). Indeed, the present experiments show an extremely low affinity of higher aliphatic alcohols for catalase-H_2O_2 of mouse liver, as demonstrated by the finding that these alcohols are not metabolized in the presence of an active H_2O_2-generating system and catalase (Table 3), thereby confirming other reports (19, 21, 31, 32). By contrast, various primary alcohols, including those with higher aliphatic chains, are metabolized at significant rates by hepatic microsomes of both

TABLE 5

Effect of sodium azide on NADPH-dependent oxidation of ethanol after mild heat treatment (37° for 60 min) of microsomes of acatalasemic (Cs^b) mice

Microsomes were derived from pools of 15 livers of acatalasemic mice and subjected to mild heat treatment at 37° for 60 min prior to assay. Microsomes (3 mg of protein per assay) were first incubated with ethanol (50 mM) and, when indicated, with sodium azide (1.0 mM). The reactions were started by adding either NADPH (1.0 mM) or the NADPH-generating system (0.4 mM $NADP^+$, 8 mM sodium isocitrate, and 0.34 unit/ml of isocitrate dehydrogenase) and carried out for 0, 5, and 10 min in a medium (final volume, 3.0 ml) containing 1.0 mM disodium EDTA, 5.0 mM $MgCl_2$, and 0.1 M phosphate buffer (pH 7.4).

	Sodium azide	NADPH-dependent ethanol oxidation
		nmoles acetaldehyde/min/mg protein
NADPH	−	15.8
	+	15.5
NADPH-generating system	−	14.0
	+	14.6

strains of mice in the presence of NADPH (Tables 1 and 3), which clearly dissociates the NADPH-dependent microsomal alcohol-oxidizing system from the peroxidatic activity of catalase-H_2O_2 by differences in substrate specificity. A similar dissociation has also been described for hepatic microsomes of rat liver (21).

Of some interest was the finding that the rates of NADPH-dependent oxidation of various alcohols, including methanol and ethanol, remained unchanged under conditions which led to almost complete loss of activity of the thermolabile catalase in the acatalasemic strain (Table 4). With respect to ethanol oxidation, these results differ from data published by others (17), who were unable to observe ethanol disappearance under these conditions. The latter finding led to the conclusion that catalase is an obligatory component in NADPH-dependent microsomal ethanol oxidation (17). More recently, however, it has been stated that NADPH-dependent microsomal ethanol oxidation persisted even when as much as 99% of the catalase activity commonly present in the microsomal fraction was inactivated (33). Since microsomes contain less than 1% of the catalase activity present in the homogenate (Table 2), a further 99% reduction of catalase activity in the microsomal fraction itself (33) would result in a catalase contamination of less than 0.01% compared to the respective homogenate. This low catalase activity would hardly be sufficient to react peroxidatically with ethanol, since the over-all rate of catalase-dependent ethanol oxidation in the microsomal

TABLE 6

Components and enzyme activities of microsomal fraction obtained by DEAE-cellulose column chromatography which exhibits microsomal alcohol-oxidizing activity

Microsomes of normal and acatalasemic mice were solubilized and subjected to DEAE-cellulose column chromatography, and the determinations of enzyme activities and components were carried out as described under MATERIALS AND METHODS. The activity of the microsomal alcohol-oxidizing system was determined with NADPH (1.0 mM) in a medium containing 1 mM disodium EDTA, 5 mM $MgCl_2$, and 0.1 M phosphate buffer (pH 7.4). The values are expressed per milligram of protein.

Activity	Normal strain	Acatalasemic strain
Cytochrome P-450 (nmoles/mg)	0.31	0.45
NADPH–cytochrome *c* reductase (μmoles/min/mg)	0.259	0.290
Cytochrome b_5 (nmoles/mg)	0.58	0.66
NADH-cytochrome b_5 reductase (μmoles/min/mg)	5.37	5.34
Microsomal alcohol-oxidizing system (nmoles/min/mg)		
Methanol	4.5	6.3
Ethanol	8.3	8.8
Propanol	6.9	7.9
Butanol	5.1	6.0
Pentanol	2.3	3.0
Alcohol dehydrogenase (nmoles/min/mg)	0	0
Catalatic activity (unit/mg)	0	0
Peroxidatic activity of catalase (nmoles/min/mg)	0	0

fraction may be limited by the concentration of catalase or the small amount of catalase-H_2O_2 complex (11). Thus the observed dissociation between NADPH-dependent alcohol oxidation and catalase activity (33) favors the existence of a catalase-independent pathway for microsomal ethanol oxidation. Further support for this interpretation was provided by other studies, in which NADPH-dependent ethanol oxidation in rat liver microsomes was found to persist despite complete inactivation of contaminating catalase (15). This was achieved by addition of the catalase inhibitor aminotriazole to microsomal suspension *in vitro*, which resulted in the complete failure of an active H_2O_2-generating system to peroxidize any ethanol under these conditions. Similarly, the activity of the NADPH-dependent microsomal ethanol-oxidizing system persisted following complete elimination of catalase by DEAE-cellulose column chromatography (14). These various data therefore fail to support the concept that catalase-H_2O_2 is an obligatory component in NADPH-dependent microsomal alcohol oxidation. However, the present study does not rule out the involvement of peroxidatic activity of some other hemoprotein in microsomal alcohol oxidation.

It has also been proposed by some that microsomal H_2O_2 generation by NADPH oxidase activity is the rate-limiting step in NADPH-dependent microsomal alcohol oxidation (16, 17). However, the latter claim has now been retracted by one group, which pointed out that microsomal H_2O_2 generation by NADPH oxidase is indeed much too low to account for the observed rates of ethanol oxidation (34), a conclusion reached also by others (14). Indeed, the rates of microsomal H_2O_2 generation as measured with the highly specific cytochrome *c* peroxidase method were only 1.5–1.7 nmoles of H_2O_2 produced per minute per milligram of microsomal protein (16, 35), whereas the rate of ethanol oxidation was 8 nmoles/min/mg of protein (16). This was substantiated by the present finding that microsomal preparations devoid of catalase activity actively oxidized ethanol only with NADPH, whereas the H_2O_2-generating system was incapable of peroxidizing ethanol to acetaldehyde in mice (Table 6) and in rats (14). These results were confirmed in a recent collaborative study.[1] Similarly, the finding in the present study that various alcohols are metabolized at significant rates only by NADPH but not in the presence of an active H_2O_2-generating system (Table 3 and 4) casts serious doubts on the claim for a rate-limiting role of microsomal H_2O_2 generation in the overall process of NADPH-dependent microsomal alcohol oxidation (16). We therefore conclude from the present data that hepatic microsomes contain a system capable of oxidizing various alcohols in a reaction requiring NADPH rather than H_2O_2 or catalase-H_2O_2.

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