Biochemical Pharmacology, Vol. 30, No. 8, pp. 825–831, 1981.
Printed in Great Britain.

0006-2952/81/080825-07 $02.00/0

OXIDATION OF ISOPROPANOL BY RAT LIVER MICROSOMES

POSSIBLE ROLE OF HYDROXYL RADICALS*

ARTHUR I. CEDERBAUM,† AZIZ QURESHI and PHILIP MESSENGER‡
Department of Biochemistry, Mount Sinai School of Medicine of the City University of New York, New York, NY 10029, U.S.A.

(*Received* 31 *March* 1980; *accepted* 15 *August* 1980)

Abstract—Isopropanol, a branched chain alcohol, served as a substrate for the microsomal alcohol oxidizing system. Isopropanol oxidation required NADPH; H_2O_2 or an H_2O_2-generating system did not effectively support isopropanol oxidation, indicating that isopropanol was not a substrate for the peroxidatic activity of catalase. The addition of azide by itself or H_2O_2 (in the presence of azide and NADPH) stimulated isopropanol oxidation, suggesting a pivotal, indirect role for H_2O_2 in the system. H_2O_2 may serve as a precursor of hydroxyl radicals. Accordingly, the oxidation of isopropanol was inhibited by hydroxyl radical scavenging agents, namely dimethylsulfoxide, mannitol, benzoate and 2-keto-4-thiomethylbutyric acid. Fe–EDTA, which is known to increase hydroxyl radical generation, stimulated the oxidation of isopropanol. The stimulation by Fe–EDTA was blocked by competing hydroxyl radical scavengers. Model hydroxyl radical generating systems such as the coupled oxidation of xanthine by xanthine oxidase, especially in the presence of Fe–EDTA, or the autoxidation of ascorbate in the presence of Fe–EDTA could also oxidize isopropanol. These results indicate that (a) rat liver microsomes are capable of oxidizing branched chain alcohols, and (b) hydroxyl radicals or a species with the oxidizing power of the hydroxyl radical, generated from microsomal electron transfer, may play a role in isopropanol oxidation.

Isolated rat liver microsomes are capable of oxidizing a series of straight chain aliphatic alcohols, such as methanol, ethanol, propanol and butanol, to the corresponding aldehydes [1, 2]. In the presence of an H_2O_2-generating system, virtually no aldehydes were produced with propanol and butanol as substrates [1, 2]. These longer chain alcohols function poorly as substrates for the peroxidatic activity of catalase [3–5]. A role for a cytochrome P-450 dependent pathway in the oxidation of alcohols by microsomes has been demonstrated [6–9]. Recent experiments from our laboratory indicated that the NADPH-dependent oxidation of ethanol by rat liver microsomes was inhibited by a series of hydroxyl radical (·OH)§ scavengers in the presence of azide, an inhibitor of catalase [10–12]. These ·OH scavengers had no effect on microsomal electron transfer or drug metabolism [10–12]. Three chemical probes were used to detect ·OH production by liver microsomes; microsomes generated ethylene gas from methional and KTBA, and methane gas from Me_2SO [12, 13]. These experiments suggested that the oxidation of ethanol by microsomes reflects the interaction of ethanol with ·OH generated from microsomal electron transfer [10–13]. Similar results were obtained in reconstitution experiments using cytochrome P-450 purified from either phenobarbital- or ethanol-treated rats [14, 15].

In this paper, the ability of microsomes to oxidize isopropanol was evaluated. As will be shown, isopropanol does not serve as an effective substrate for the peroxidatic activity of catalase. Therefore, studies with isopropanol may offer an advantage over studies with ethanol in trying to understand the biochemical pathway by which microsomes oxidize alcohols. In this regard, isopropanol is an effective ·OH scavenging agent [16, 17]. In view of the use of isopropanol to assess the kinetics and control of alcohol oxidation *in vivo*, as well as the finding of isotope effects in the oxidation of isopropanol [18], the possibility that non-alcohol dehydrogenase-dependent pathways, e.g. microsomal pathways, contribute to these observations remains to be explored. The recent observation that acetone excretion is found after administering *t*-butanol to rats [19] offers the possibility that *t*-butanol may be metabolized to isopropanol, which is then oxidized to acetone. In view of the above considerations, and the absence of any reports that branched chain alcohols can serve as substrates for the microsomal alcohol oxidizing system, the studies described below were undertaken.

MATERIALS AND METHODS

Hepatic microsomes were prepared from male

*These studies were supported by USPHS Grants AA-03312 and AA-04413 and by Research Scientist Career Development Award 5K02-AA-00003 (A. I. C.).

† Author to whom all correspondence should be addressed: Department of Biochemistry, Mt. Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029, U.S.A.

‡ Mount Sinai (1979) volunteer.

§ Abbreviations: ·OH, hydroxyl radical; KTBA, 2-keto-4-thiomethylbutyric acid; and Me_2SO, dimethylsulfoxide.

Sprague–Dawley rats (200–300 g) as described previously [20]. The microsomes were washed once and suspended in 125 mM KCl. The oxidation of isopropanol was assayed at 37° in center-well flasks containing 0.6 ml of 15 mM semicarbazide in 180 mM potassium phosphate, pH 7.4, in the center well. The reaction mixture contained 100 mM potassium phosphate, pH 7.4, 10 mM potassium pyrophosphate, 10 mM $MgCl_2$, 10 mM glucose-6-phosphate, 7 units of glucose-6-phosphate dehydrogenase, 0.4 mM $NADP^+$, 0.1 mM EDTA, and about 5 mg of microsomal protein in a final volume of 3.0 ml. The final concentration of isopropanol in most experiments was 44 mM. Azide, when added, was present at a final concentration of 1.0 mM. The reaction was initiated with glucose-6-phosphate plus glucose-6-phosphate dehydrogenase and was terminated by the addition of trichloroacetic acid (final concentration, 4.5%). After an overnight diffusion period, an aliquot from the center well was diluted with H_2O to 3.0 ml, and the optical density of the acetone–semicarbazone complex was determined at 224 nm. Standard curves containing known amounts of acetone were carried out in an identical manner. Maximum absorption of the acetone–semicarbazone complex was found at a wavelength of 224 nm, and an extinction coefficient of 9.41 $mM^{-1}\,cm^{-1}$ was calculated for the complex. In later experiments, activity was calculated using this extinction coefficient. Blanks included zero time controls, boiled microsomes, and flasks lacking either microsomes, isopropanol, or the NADPH-generating system.

Two model systems were used to generate hydroxyl radicals in the absence of microsomes. Experiments were carried out in center-well flasks containing semicarbazide in the center well. One system consisted of autoxidizing ascorbic acid in the presence of iron. The reaction mixture contained 100 mM potassium phosphate, pH 7.4, either ethanol, butanol, isopropanol, or no alcohol, 0.167 mM ferrous ammonium sulfate, and 0.33 mM EDTA in a final volume of 3.0 ml. The reaction was initiated by the addition of ascorbate (final concentration, 1.0 mM) and was quenched after 5 min at 37° by the addition of trichloroacetic acid. After an overnight incubation, aliquots of the center well were assayed as described above. The generation of ·OH by this system has been described previously [12, 21, 22]. The second system was the coupled oxidation of xanthine by xanthine oxidase. The reaction mixture consisted of 100 mM potassium phosphate, pH 7.4, either ethanol, butanol, isopropanol, or no alcohol, 0.1 mM EDTA, and 0.10 units of xanthine oxidase in a final volume of 3.0 ml. In a second set of experiments, Fe–EDTA (83 μM iron–167 μM EDTA) was included in the reaction mixture. The reaction was initiated by the addition of xanthine (final concentration, 1.0 mM) and after 30 min was terminated and assayed as described above for the ascorbate system. The generation of ·OH by this system has been described previously [23, 24].

All chemicals were of the highest grade from commercial sources. All buffers and solutions were prepared from water passed through a Millipore system and subsequently glass distilled. The buffers and solutions (except $MgCl_2$) were passed through a Chelex-100 column (35 × 3 CM, Kontes). An iron–EDTA solution was prepared by dissolving 5 mM ferrous ammonium sulfate in 10 mM EDTA. Formaldehyde production was assayed with the Nash reagent.

All values refer to means ± S.E.M. Statistical analysis was performed by Student's *t*-test.

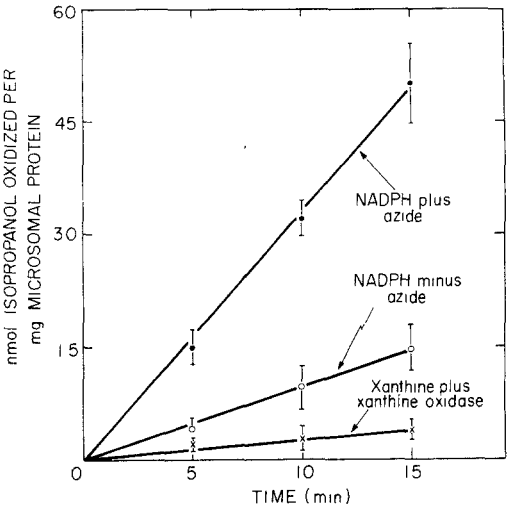

Fig. 1. Time course of the oxidation of isopropanol by rat hepatic microsomes. The oxidation of isopropanol (final concentration, 44 mM) was assayed under three reaction conditions: (a) an NADPH-generating system in the absence of azide; (b) an NADPH-generating system in the presence of 1.0 mM azide; and (c) 1.0 mM xanthine plus 0.02 units xanthine oxidase. Results (± S.E.M.) are from four experiments.

RESULTS

Oxidation of isopropanol by rat liver microsomes. The oxidation of isopropanol was assayed either in the presence of an NADPH-generating system or an H_2O_2-generating system (xanthine plus xanthine oxidase).* Time courses for the oxidation of isopropanol by rat hepatic microsomes are shown in Fig. 1. The rate of H_2O_2-mediated oxidation of isopropanol was considerably lower than the NADPH-mediated rate, and was similar to the rate exhibited by xanthine plus xanthine oxidase alone in the absence of microsomes (see below). A low rate of isopropanol oxidation was also found in two experiments where the concentration of xanthine was varied from 0.1 to 1.0 mM, thereby ruling out the possibility of substrate (xanthine) inhibition of xanthine oxidase. Methanol, ethanol, butanol, and isopropanol were oxidized at rates of 9.52, 10.13, 0.56, and 0.37 (N = 2) $nmoles \cdot min^{-1} \cdot (mg\ microsomal\ protein)^{-1}$, respectively, when xanthine oxidase served

* The oxidation of xanthine by xanthine oxidase produces $O_2^{\cdot-}$ which undergoes dismutation spontaneously to form H_2O_2; ·OH can be produced from this system as well (Materials and Methods, Refs. 23 and 24). The amount of ·OH produced, however, is much lower than the amount of H_2O_2 produced. Therefore, xanthine oxidase can be used as an H_2O_2-generating system and, to a lesser extent, as an ·OH-generating system.

as the H_2O_2-generating system for microsomal catalase. Ethanol and methanol are effective substrates for the peroxidatic activity of catalase, whereas butanol is not [3–5]. These data suggest the following: (a) H_2O_2 does not serve directly to oxidize isopropanol to acetone; (b) isopropanol does not act as an effective substrate for the peroxidatic activity of catalase under these conditions; and (c) the ability of NADPH to support isopropanol oxidation appears to reflect a catalase-independent mechanism.

To further rule out the possibility of a catalase-dependent oxidation of isopropanol, experiments with azide, an inhibitor of catalase, were carried out. At a concentration of 1.0 mM, azide decreased H_2O_2-mediated oxidation of ethanol or methanol by > 90 per cent. However, the NADPH-dependent oxidation of isopropanol was increased 3- to 4-fold in the presence of 1.0 mM azide (Fig. 1). Previous experiments indicated that azide doubled the rate of NADPH-dependent oxidation of butanol by rat hepatic microsomes [10]. Consequently, the oxidation of two alcohols (isopropanol and butanol) that do not serve as effective substrates for microsomal catalase was increased when catalase was inhibited by azide. Cyanide, which was used as an alternative inhibitor of microsomal catalase, also stimulated the rate of isopropanol oxidation, although to a lesser extent than azide did. Rates of isopropanol oxidation [nmoles·min^{-1}·(mg protein)$^{-1}$] were 2.95 ± 0.31 and 1.92 ± 0.22 in the presence of 1.0 mM azide or 1.0 mM cyanide, respectively, while the control rate (absence of azide or cyanide) was 1.31 ± 0.20 (N = 4). The stimulation by azide ($P < 0.003$) and cyanide ($P < 0.05$) was significant. All further microsomal experiments were carried out in the presence of 1.0 mM azide.

Control experiments indicated that there was no production of acetone when any of the following were omitted from the reaction mixture: microsomes, isopropanol, glucose-6-phosphate, or glucose-6-phosphate dehydrogenase. No activity was observed when the microsomes were boiled for 10 min. Isopropanol oxidation was increased in the presence of EDTA; the rate in the absence of EDTA was 1.84 ± 0.19 nmoles·min^{-1}·(mg protein)$^{-1}$, while the rate in the presence of 0.10 mM EDTA was 2.95 ± 0.31 nmoles·min^{-1}·(mg protein)$^{-1}$ ($P < 0.02$, N = 4). EDTA was, therefore, included in all subsequent experiments.

In a separate set of experiments, the formation of acetone from isopropanol was confirmed by a second assay method, gas chromatography. The reaction was carried out in a manner identical to that when center-well flasks were used except that the flasks were sealed with tight serum caps. The reactions were terminated by injection of acid through the caps. The flasks were incubated at 60° and samples (1.0 ml) from the head space were taken with a gas-tight syringe and injected directly into a Hewlett–Packard model 5750 gas chromatograph for measurement of acetone. The gas chromatograph was equipped with a 6-ft column of Poropak N (50–80 mesh) and a flame ionization detector. Operating conditions were similar to those described previously [12] except that inlet temperature was 130°. Under these conditions the retention time for acetone was 11.5 min. Using this method of assay, microsomes oxidized isopropanol to acetone at a rate of 3.35 nmoles·min^{-1}·(mg protein)$^{-1}$. No acetone was produced in the absence of microsomes, isopropanol or components of the NADPH generation system.

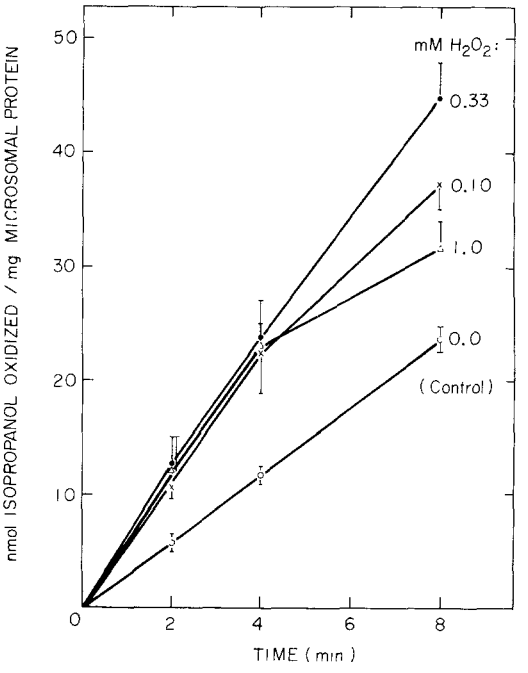

Fig. 2. Effect of H_2O_2 on the oxidation of isopropanol by rat hepatic microsomes. The oxidation of isopropanol (final concentration, 44 mM) was assayed in the presence of 3 mM azide and the indicated concentrations of H_2O_2. Results (± S.E.M.) are from three experiments.

Effect of hydrogen peroxide on microsomal oxidation of isopropanol. One consequence of the inhibition of catalase by azide is the accumulation of H_2O_2, and H_2O_2 may serve as a precursor of ·OH (see Discussion). To evaluate a role (indirect) for H_2O_2 in isopropanol oxidation, the effect of externally added H_2O_2 on isopropanol oxidation was determined. Azide (3 mM) was present to inhibit catalase and prevent the catalatic decomposition of H_2O_2. Microsomal oxidation of isopropanol was increased in the presence of H_2O_2 (0.10 to 1.0 mM) (Fig. 2). However, the addition of H_2O_2 in the absence of the NADPH-generating system (in the absence or presence of azide) resulted in very low levels of isopropanol oxidation [< 0.2 nmole·min^{-1}·(mg protein)$^{-1}$]. Therefore, the mere presence of H_2O_2 is an insufficient condition for the oxidation of isopropanol; electron transfer from NADPH is required. The biphasic concentration effect of H_2O_2 at long time periods, i.e. the greater extent of stimulation by 0.10 and 0.33 mM H_2O_2 than by 1.0 mM H_2O_2, may reflect damage to microsomal membranes which masks the stimulation found at the earlier time periods. Similar results were noted previously in studies on the effect of H_2O_2 on microsomal oxidation of ethanol and l-butanol [10].

Effect of hydroxyl radical scavenging agents on isopropanol oxidation by rat liver microsomes. The experiments with azide and externally added H_2O_2

Table 1. Effect of hydroxyl radical scavenging agents on microsomal oxidation of isopropanol*

Addition	Concn (mM)	Isopropanol oxidation [nmoles·min^{-1}·(mg protein)$^{-1}$]	Effect (%)	P
Control (11)†		2.25 ± 0.21		
Me_2SO (11)	23.5	1.28 ± 0.17	−43	< 0.002
Me_2SO (11)	47	1.16 ± 0.17	−48	< 0.001
Control (8)		2.36 ± 0.27		
Mannitol (8)	33	1.75 ± 0.18	−26	0.10 > P > 0.05
Mannitol (8)	67	1.33 ± 0.23	−44	< 0.02
Control (6)		2.20 ± 0.18		
KTBA (6)	10	1.54 ± 0.24	−30	< 0.05
KTBA (6)	33	1.35 ± 0.19	−39	< 0.02

* NADPH-dependent microsomal oxidation of isopropanol (final concentrations, 44 mM) was assayed in the presence of 1.0 mM azide.

† Results are from six to eleven experiments.

point to a pivotal, indirect role for H_2O_2 in isopropanol oxidation. H_2O_2 serves as a precursor of ·OH, and isopropanol is an effective ·OH scavenging agent [16, 17]. The possibility that microsomal oxidation of isopropanol may reflect the ability of isopropanol to react with ·OH generated from microsomal electron transfer was evaluated by studying the effect of several ·OH scavenging agents on isopropanol oxidation in the presence of 1.0 mM azide. Mannitol, Me_2SO, and KTBA were employed in these experiments since these compounds were previously shown to be effective inhibitors of microsomal oxidation of ethanol in the presence of azide [10, 12]. All three scavengers inhibited microsomal oxidation of isopropanol (Table 1). The scavengers had no effect on the NADPH-generating system (activity of glucose-6-phosphate dehydrogenase), nor did they interfere with recoveries of known amounts of acetone.

The effects of variations in substrate concentration are shown in Table 2. The oxidation of isopropanol increased as the concentration of isopropanol was increased. The extent of inhibition of isopropanol oxidation by Me_2SO, mannitol, or benzoate varied with the concentration of isopropanol; greater inhibition was found at the lower concentration of isopropanol (Table 2), suggesting that the scavengers may compete with isopropanol for oxidation by the microsomal system.

Effect of Fe–EDTA *on microsomal oxidation of isopropanol.* In model systems, e.g. the xanthine–xanthine oxidase reaction, Fe–EDTA was shown to increase ·OH generation [25–27]. Many investigators have commented on the possibility that, in biological systems, iron may stimulate ·OH generation by a modified Haber–Weiss reaction or the Fenton reaction [28, 29]. Since the above experiments appear to be consistent with a role for ·OH in microsomal oxidation of isopropanol, the effect of Fe–EDTA on isopropanol oxidation was determined. The oxidation of isopropanol was increased 100, 221 and 394 per cent in the presence of 8.3, 16.67 and 50 μM Fe–EDTA respectively (Table 3, top line). To confirm that this increase by Fe–EDTA involved, at least in part ·OH, the effect of ·OH scavenging agents was

Table 2. Effect of isopropanol concentration on the inhibition of microsomal oxidation of isopropanol by hydroxyl radical scavenging agents*

	Concentration of isopropanol (mM)					
	22		44		88	
Addition	Isopropanol oxidation†	Effect of scavenger‡	Isopropanol oxidation†	Effect of scavenger‡	Isopropanol oxidation†	Effect of scavenger‡
Control (7)§	1.84 ± 0.34		2.35 ± 0.30		2.70 ± 0.47	
Me_2SO, 23.5 mM (7)	0.87 ± 0.24	−53	1.40 ± 0.16	−40	1.67 ± 0.22	−39
Me_2SO, 47 mM (7)	0.69 ± 0.18	−63	1.22 ± 0.15	−48	1.63 ± 0.24	−40
Control (6)	2.01 ± 0.31		2.46 ± 0.31		2.71 ± 0.44	
Mannitol, 33 mM (6)	1.15 ± 0.19	−43	1.87 ± 0.26	−24	2.18 ± 0.44	−20
Mannitol, 67 mM (6)	0.93 ± 0.19	−54	1.55 ± 0.18	−37	1.86 ± 0.31	−31
Control (3)	2.49 ± 0.39		2.97 ± 0.46		3.15 ± 0.50	
Benzoate, 33 mM (3)	1.53 ± 0.26	−39	2.49 ± 0.16	−16	2.95 ± 0.50	−6
Benzoate, 67 mM (3)	1.52 ± 0.18	−39	2.17 ± 0.14	−27	2.71 ± 0.16	−14

* NADPH-dependent microsomal oxidation of isopropanol was assayed in the presence of 1.0 mM azide and the indicated concentrations of isopropanol and hydroxyl radical scavenging agent.

† Specific activity refers to nmoles isopropanol oxidized per min per mg of microsomal protein.

‡ Percent effect on appropriate control.

§ Number of experiments.

Table 3. Effect of Fe–EDTA on microsomal oxidation of isopropanol*

Addition	Concentration of Fe–EDTA (μM) 0 Isopropanol oxidation†	0 Effect of scavenger‡	8.3 Isopropanol oxidation†	8.3 Effect of scavenger‡	16.67 Isopropanol oxidation†	16.67 Effect of scavenger‡	50 Isopropanol oxidation†	50 Effect of scavenger‡
Control	2.07 ± 0.30		4.14 ± 0.59		6.65 ± 0.71		10.22 ± 1.37	
Me_2SO, 23.5 mM	1.07 ± 0.36	−48	2.11 ± 0.34	−49	3.40 ± 0.46	−49	4.86 ± 0.77	−52
Me_2SO, 47 mM	1.18 ± 0.31	−43	1.73 ± 0.25	−58	2.58 ± 0.36	−61	3.48 ± 0.46	−66
KTBA, 10 mM	1.56 ± 0.34	−25	2.87 ± 0.47	−31	4.44 ± 0.74	−33	7.51 ± 1.70	−27
KTBA, 33 mM	1.29 ± 0.12	−38	2.34 ± 0.40	−43	3.28 ± 0.82	−51	3.44 ± 0.60	−66
Mannitol, 33 mM	1.54 ± 0.29	−26	2.85 ± 0.45	−31	4.74 ± 0.79	−29	6.99 ± 0.97	−32
mannitol, 67 mM	1.01 ± 0.34	−51	2.11 ± 0.32	−49	3.89 ± 0.33	−42	5.27 ± 0.80	−48

* Microsomal oxidation of isopropanol was assayed in the presence of 1.0 mM azide and the indicated concentrations of Fe–EDTA. Results are from four experiments.
† Specific activity refers to nmoles isopropanol oxidized per min per mg of microsomal protein.
‡ Percent effect on appropriate control (0, 8.3, 16.67, or 50 μM Fe–EDTA).

evaluated. The control rate of isopropanol oxidation, as well as the increased rate of isopropanol oxidation found in the presence of Fe–EDTA, was inhibited by Me_2SO, KTBA and mannitol (Table 3). Most of the increase produced by Fe–EDTA was eliminated in the presence of the competing ·OH scavengers, e.g. 50 μM Fe–EDTA increased isopropanol oxidation from 2.07 to 10.22 nmoles·min^{-1}·mg^{-1} (+ 8.15), whereas in the presence of 47 mM Me_2SO or 33 mM KTBA the increase in isopropanol oxidation was only 2.3 or 2.15 nmoles·min^{-1}·mg^{-1} respectively (3.48 to 1.18 for Me_2SO; 3.44 to 1.29 for KTBA) (Table 3).

Isopropanol oxidation by model ·OH *generating systems.* There is a considerable difference between the specific activities of ethanol, butanol, and isopropanol oxidation by rat hepatic microsomes. In general, values for the NADPH-dependent oxidation of ethanol, butanol, and isopropanol by rat hepatic microsomes in the presence of azide are about 7–10, 5–7, and 2–3 nmoles·min^{-1}·(mg protein)$^{-1}$ respectively—i.e. ethanol > butanol > isopropanol. To determine if these differences are specific to the microsomal system, the oxidation of the alcohols in two model ·OH generating systems, in the absence of microsomes, was studied. The systems consisted of autoxidizing ascorbate in the presence of Fe–EDTA, and of xanthine plus xanthine oxidase in the absence or presence of Fe–EDTA (see Materials and Methods). Ethanol, butanol, and isopropanol were oxidized to acetaldehyde, butyraldehyde, and acetone by both model systems (Table 4). The order of oxidation of the alcohols was ethanol > butanol > isopropanol, the same as found for the microsomal system. Whether the greater rate of oxidation of ethanol reflects a chain mechanism or different steric effects by the alcohols is not clear. In the microsomal system, possible differences in binding of the alcohols may also contribute to the different rates of oxidation. It should be noted that the low rate of isopropanol oxidation by the model xanthine oxidase system (Table 4) was similar to the low rate found when this system served to produce H_2O_2 for microsomal catalase (Fig. 1). Consequently, the activity found with microsomes and xanthine oxidase (Fig. 1) probably reflects this low rate of ·OH formation produced during the coupled oxidation of xanthine by xanthine oxidase.

DISCUSSION

The microsomal alcohol oxidizing system, which is known to oxidize a series of straight chain aliphatic alcohols, has now been found to be capable of oxidizing isopropanol, a secondary alcohol. A mechanism that appears to involve ·OH at least in part, plays a role in isopropanol oxidation. Isopropanol oxidation is inhibited by four effective scavengers of ·OH, namely Me_2SO, mannitol, benzoate, and KTBA (Tables 1 and 2). These compounds were previously shown to have no effect on (a) NADH- or NADPH–cytochrome *c* reductase activity, (b) NADH- or NADPH-dependent oxygen consumption, (c) the metabolism of aniline or aminopyrine by liver microsomes, and (d) the metabolism of benzphetamine and aminopyrine by a reconstituted

Table 4. Oxidation of alcohols in model systems*

System	Substrate	Product	Activity (nmoles/min) Expt. 1	Expt. 2
Xanthine + xanthine oxidase	Ethanol	Acetaldehyde	3.28	3.24
	Butanol	Butyraldehyde	1.35	1.89
	Isopropanol	Acetone	0.44	0.50
Xanthine + xanthine oxidase + Fe–EDTA	Ethanol	Acetaldehyde	38.42	42.93
	Butanol	Butyraldehyde	9.43	10.33
	Isopropanol	Acetone	4.81	7.10
Ascorbate + Fe–EDTA	Ethanol	Acetaldehyde	171	141
	Butanol	Butyraldehyde	85.4	128
	Isopropanol	Acetone	34.8	70

* Oxidation of ethanol (51 mM), butanol (45 mM), and isopropanol (44 mM) by three model systems [ascorbate plus Fe–EDTA (167 μM), xanthine plus xanthine oxidase, or xanthine plus xanthine oxidase in the presence of Fe–EDTA (83 μM)] was carried out as described in Materials and Methods. Results are from two experiments carried out in triplicate.

system containing cytochrome P-450 purified from phenobarbital-treated rats [10–14]. Hence, the effect of the ·OH scavengers with regard to inhibiting isopropanol oxidation is somewhat specific. Fe–EDTA, which is known to stimulate ·OH generation by the coupled xanthine–xanthine oxidase reactions [25–27] and by microsomes [30, 31], stimulated the oxidation of isopropanol (Table 3). Moreover, the increase produced by Fe–EDTA was sensitive to ·OH scavenging agents. Isopropanol is also oxidized by two model systems known to generate ·OH: the coupled oxidation of xanthine by xanthine oxidase and the autoxidation of ascorbate in the presence of Fe–EDTA (Table 4). The rank order of effectiveness of alcohols in the model systems (ethanol > butanol > isopropanol) is the same as that found with microsomes.

The extent of inhibition of isopropanol oxidation by competing ·OH scavengers is similar to the extent of inhibition of ethanol oxidation, but it is greater than that found with *n*-butanol oxidation, e.g. 23.5 mM Me_2SO inhibited the oxidation of ethanol, isopropanol, and *n*-butanol by 46, 40–43, and 26 per cent respectively [10] (Tables 1 and 2); 33 and 67 mM mannitol inhibited the oxidation of ethanol 31 and 45 per cent respectively [10], whereas the oxidation of isopropanol was inhibited 26 and 44 per cent (Table 1) respectively. Consequently, the extent of inhibition of microsomal oxidation of alcohols by competing ·OH scavengers correlates with the rate constant for the interaction of the alcohols with ·OH, i.e. ethanol and isopropanol, which have similar rate constants [17], are equally sensitive to Me_2SO and mannitol, whereas *n*-butanol, which has a rate constant about twice that of ethanol and isopropanol [17], is less sensitive.

Isopropanol does not serve as an effective substrate for the peroxidatic activity of catalase since direct addition of H_2O_2 or generation of H_2O_2 via xanthine oxidase activity results in only low levels of isopropanol oxidation (Fig. 1). In this regard, the oxidation of isopropanol represents a less complicated system than the oxidation of ethanol and may be useful in helping to understand the biochemical pathway for microsomal oxidation of alcohols in the absence of a contribution by catalase. H_2O_2 appears to play an indirect, although a major pivotal role in isopropanol oxidation, however, since (a) the addition of azide, which inhibits catalase and permits H_2O_2 to accumulate, stimulated isopropanol oxidation (Fig. 1), and (b) the addition of H_2O_2 augmented the NADPH-dependent oxidation of isopropanol (Fig. 2). H_2O_2 may serve as a precursor of ·OH either via a Fenton reaction mechanism or a modified, i.e. iron-catalyzed, Haber–Weiss reaction mechanism [32, 33]. Isopropanol oxidation was not inhibited by the addition of superoxide dismutase; actually a slight stimulation was observed (+ 15 per cent at 60 μg/ml, 180 units, of superoxide dismutase). As discussed elsewhere [10, 31], however, the effects of superoxide dismutase in complex systems such as microsomes are difficult to evaluate, most notably with regard to the ability of superoxide dismutase to penetrate to the active site of radical generation. It is possible that another species with oxidizing properties similar to those of the hydroxyl radical,

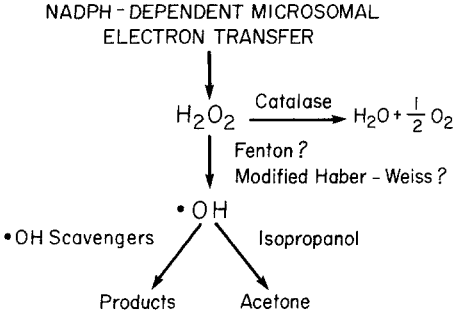

Fig. 3. Scheme for the oxidation of isopropanol by rat liver microsomes. NADPH-dependent microsomal electron transfer generates H_2O_2. Azide, by inhibiting catalase, permits H_2O_2 to accumulate and thereby serve as a source of ·OH. Externally added H_2O_2 also serves as a source of ·OH. Increased generation of ·OH results in increased oxidation of isopropanol. The ·OH is assumed to be bound and not "free" in solution.

and derived from peroxide (e.g. a ferryl radical), may serve as the actual oxidizing agent.

Figure 3 presents a working scheme that may be useful in explaining aspects of the data. NADPH-dependent microsomal electron transfer is known to generate H_2O_2 [34–36]. Azide, by inhibiting catalase, permits H_2O_2 to accumulate. H_2O_2 then serves to generate ·OH. External addition of H_2O_2 also serves to generate ·OH (see below). In view of the stereospecificity shown by the microsomal ethanol oxidizing system [37, 38], the ·OH is assumed to be bound, and not free in solution or released into the medium. The increased generation of ·OH in the presence of azide or azide plus H_2O_2 results in increased oxidation of isopropanol. Inhibition of isopropanol oxidation by ·OH scavengers such as Me_2SO, KTBA, mannitol, or benzoate reflects competition between these scavengers and isopropanol for ·OH. Stimulation of isopropanol oxidation by EDTA may reflect removal of contaminating metals, protection of microsomes from peroxidative damage, or perhaps interaction with endogenous microsomal iron to produce a microsomal Fe–EDTA complex. Augmentation of isopropanol oxidation by Fe–EDTA (Table 3) reflects iron-catalyzed stimulation of ·OH generation [25–27]. It should be emphasized that this system does not merely represent the interaction of H_2O_2 with iron present as a contaminant, since added H_2O_2 alone (in the absence of NADPH) does not result in isopropanol oxidation, and the buffers and solutions were treated with Chelex-100 resin. In this regard, the role of microsomal iron, expecially iron chelates such as cytochrome P-450, in binding ·OH or H_2O_2, or in formation of the actual oxidizing species, remains to be evaluated.

Acknowledgement—We thank Dr. Gerald Cohen, Department of Neurology, for performing the gas chromatography experiment and for valuable discussion.

REFERENCES

1. R. Teschke, Y. Hasumura and C. S. Lieber, *Biochem. biophys. Res. Commun.* **60**, 851 (1974).
2. R. Teschke, Y. Hasumura and C. S. Lieber, *J. biol. Chem.* **250**, 7397 (1975).
3. B. Chance, *Acta chem. scand.* **1**, 236 (1947).
4. D. Keilin and E. F. Hartree, *Biochem. J.* **39**, 293 (1945).
5. T. R. Tephley, G. J. Mannering and R. E. Parks, *J. Pharmac. exp. Ther.* **134**, 77 (1961).
6. R. Teschke, S. Matsuzaki, K. Ohnishi, L. M. DeCarli and C. S. Lieber, *Alcoholism: Clin. expl Res.* **1**, 7 (1977).
7. J. G. Joly, J. P. Villeneuve and P. Mavier, *Alcoholism: Clin. expl Res.* **1**, 17 (1977).
8. K. Ohnishi and C. S. Lieber, *J. biol. Chem.* **252**, 7124 (1977).
9. G. T. Miwa, W. Levin, P. E. Thomas and A. Y. H. Lu, *Archs Biochem. Biophys.* **187**, 464 (1978).
10. A. I. Cederbaum, E. Dicker and G. Cohen, *Biochemistry* **17**, 3058 (1978).
11. A. I. Cederbaum, E. Dicker, E. Rubin and G. Cohen, *Biochemistry*, **18**, 1187 (1979).
12. G. Cohen and A. I. Cederbaum, *Archs Biochem. Biophys.* **199**, 438 (1980).
13. G. Cohen and A. I. Cederbaum, *Science* **204**, 66 (1979).
14. A. I. Cederbaum, G. Miwa, G. Cohen and A. Y. H. Lu, *Biochem. biophys. Res Commun.* **91**, 747 (1979).
15. K. Ohnishi and C. S. Lieber, *Archs Biochem. Biophys.* **191**, 798 (1978).
16. M. Anbar and P. Neta, *Int. J. appl. Radiat. Isotopes* **18**, 493 (1967).
17. L. M. Dorfman and G. E. Adams, *Reactivity of the Hydroxyl Radical in Aqueous Solutions*, p. 46. NSRRS, National Bureau of Standards, Washington, DC (1973).
18. W. S. Chen and B. V. Plapp, in *Third International Symposium on Alcohol and Aldehyde Metabolizing Systems* (Ed. R. G. Thurman), p. 18. Addiction Research Federation, Toronto, Canada (1979).
19. R. C. Baker, S. M. Sorenson, S. R. Eby and R. A. Dietrich, in *Third International Symposium on Alcohol and Aldehyde Metabolizing Systems* (Ed. R. G. Thurman) p. 19. Addiction Research Federation, Toronto, Canada (1979).
20. A. I. Cederbaum, F. F. Becker and E. Rubin, *J. biol. Chem.* **251**, 5366 (1976).
21. G. Cohen and R. E. Heikkila, *J. biol. Chem.* **249**, 2447 (1974).
22. G. Cohen, *J. Neurochem.* **29**, 761 (1977).
23. C. Beauchamp and I. Fridovich, *J. biol. Chem.* **245**, 4641 (1970).
24. G. Cohen, in *Alcohol and Aldehyde Metabolizing Systems* (Eds. R. G. Thurman, J. R. Williamson, H. R. Drott and B. Chance), Vol. 2, pp. 403–12. Academic Press, New York (1977).
25. J. M. McCord and E. D. Day, *Fedn Eur. Biochem. Soc. Lett.* **86**, 139 (1978).
26. B. Halliwell, *Fedn Eur. Biochem. Soc. Lett.* **92**, 321 (1978).
27. B. Halliwell, *Fedn Eur. Biochem. Soc. Lett.* **96**, 238 (1978).
28. G. Cohen, in *Superoxide and Superoxide Dismutase* (Eds. A. M. Michelson, J. M. McCord and I. Fridovich), pp. 317–20. Academic Press, New York (1977).
29. A. M. Mitchelson, J. M. McCord and I. Fridovich (Eds.), in Editors Footnote, *Superoxide and Superoxide Dismutase*, p. 320. Academic Press, New York (1977).
30. C. S. Lai and L. H. Piette, *Archs Biochem. Biophys.* **190**, 27 (1978).
31. A. I. Cederbaum, E. Dicker and G. Cohen, *Biochemistry*, **19**, 3698 (1980).
32. C. Walling, *Accts Chem. Res.* **8**, 125 (1975).
33. F. Haber and J. Weiss, *Proc. R. Soc.* **147**, 332 (1934).
34. R. G. Thurman, H. G. Ley and R. Scholz, *Eur. J. Biochem.* **25**, 420 (1972).
35. A. G. Hildebrandt and I. Roots, *Archs Biochem. Biophys.* **171**, 385 (1975).
36. G. D. Nordblom and M. J. Coon, *Archs Biochem. Biophys.* **180**, 343 (1977).
37. H. Gang, A. I. Cederbaum and E. Rubin, *Biochem. biophys. Res. Commun.* **54**, 264 (1973).
38. R. J. M. Corrall, C. L. Yu, B. A. Rosner, J. M. Margolis, H. M. Rodman, W. Kam and B. R. Landau, *Biochem. Pharmac.* **24**, 1825 (1975).