Methanol Metabolism in the Monkey

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SUMMARY

The peroxidative system involving hepatic catalase plays a major role in the oxidation of methanol in the rat (1), but in the monkey the peroxidative mechanism does not appear to be important. This conclusion is based on the following observations: (a) ethanol and methanol were about equally reactive with the peroxidative system, but ethanol was much more reactive with the alcohol dehydrogenase system than methanol. Ethanol was a much more effective inhibitor of methanol oxidation in the intact monkey than it was in the rat, which is what would be expected if methanol is oxidized by the alcohol dehydrogenase system in the monkey, but by the peroxidative system in the rat. (b) By similar reasoning, 1-butanol was a stronger inhibitor of methanol oxidation in the monkey than it should have been if the peroxidative system was involved. (c) 3-Amino-1,2,4-triazole, a potent inhibitor of hepatic catalase, greatly reduced methanol oxidation in the rat, but had no measurable effect on methanol oxidation in the monkey. (d) Ethylene glycol stimulated the rate of methanol oxidation in the rat, probably as a result of an increased H_2O_2 production that occurs when glycolic acid, a metabolite of ethylene glycol, is oxidized to glyoxylic acid (6, 7); no such stimulation was seen in the monkey. Studies *in vitro* which measured the methanol-oxidizing activity of hepatic alcohol dehydrogenase isolated from monkeys also support the view that this enzyme is largely responsible for methanol oxidation in this species.

INTRODUCTION

The question as to which enzyme system is primarily responsible for the first step in the oxidation of methanol has been resolved in the case of the rat, where the peroxidative system involving catalase was shown to play a major role (1). At one time it was widely believed that methanol was oxidized through the action of hepatic alcohol dehydrogenase (alcohol: NAD oxidoreductase, EC 1.1.1.1), but the report by Bonnichsen (2) that the crystalline enzyme from horse liver would not react with this alcohol did much to discredit this concept. More recently, Kini and Cooper (3) showed that methanol will react with alcohol dehydrogenase of both horse and monkey when high substrate concentrations are present. These investigators performed kinetic studies on monkey liver alcohol dehydrogenase, and from the disappearance of methanol from the blood of the monkey, they concluded that this enzyme was responsible for the oxidation of methanol *in vivo* in this species. However, Mannering *et al.* (4) re-evaluated the data of Kini and Cooper and concluded that the amount of alcohol dehydrogenase reported as being present in the liver of the monkey was inadequate to account for the rate of methanol disappearance from the blood. Thus, it seemed pertinent to reinvestigate

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methanol metabolism in the monkey to determine whether the peroxidative system functions in this species as it does in the rat.

The approaches used previously in the study of methanol metabolism in the rat *in vivo* (1) and in the isolated perfused rat liver (5) were applied to the monkey: (a) the relative abilities of ethanol and 1-butanol to inhibit methanol oxidation *in vivo* were compared with the known reactivities of the three alcohols with the peroxidative and alcohol dehydrogenase systems *in vitro;* (b) methanol oxidation was studied in animals that had been treated with the potent hepatic catalase inhibitor 3-amino-1,2,4-triazole; and (c) the "apparent *in vivo* Michaelis constant (K_m)" for methanol oxidation was determined for comparison with the Michaelis constants of methanol oxidation of the catalase–H_2O_2 and alcohol dehydrogenase systems determined *in vitro*. A fourth approach was based on studies by Van Harken, Tephly, and Mannering (6, 7), which showed that ethylene glycol stimulates the activity of the peroxidative system in the intact rat and in the isolated, perfused liver.

MATERIALS AND METHODS

Labeled alcohols. The specific activities of methanol-^{14}C and ethanol-1-^{14}C were determined as described previously (1). Both compounds were purchased from New England Nuclear Corporation.

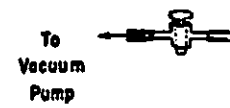
3-Amino-1,2,4-triazole. AT[3] was generously supplied by the American Cyanamid Company and was purified as described previously (8).

Experiments in vivo. Young male rhesus monkeys (1.5–3.5 kg) were employed. Six monkeys were used repeatedly throughout the study. They were rested between experiments for at least 1 week, except after the administration of very small amounts of the alcohols, when occasionally 3-day rest periods were used. Immediately after the intraperitoneal injection of the alcohols (10 or 20% solutions) the monkeys were placed in a metabolism chamber (Fig. 1). The chamber was made by bisecting a 5-gal glass bottle which had one hole drilled in the bottom and another near the spout. A circular stainless steel screen (0.5-inch mesh) served as a floor for the monkey, and this was held firmly in place by a bolt which passed through a rubber stopper placed in the spout. When urine was collected, this stopper was removed and the urine was rinsed into a beaker. The new edges of the bottle were covered with pressure-sensitive tape,[4] and the monkey was sealed in the chamber by binding the two halves of the botttle together with the same tape. Air pulled through the chamber (about 3.5 liters/min) was dried by passing it through a column of calcium chloride. Respired air was pulled first through 30–50 g of magnesium perchlorate contained in three to five absorption tubes to collect methanol and then through four 3 N NaOH solutions (100 ml each) to collect $^{14}CO_2$. Collected methanol and $^{14}CO_2$ were measured as described previously (1). Measured quantities of $^{14}CO_2$ introduced into the chamber were trapped quantitatively within 5 min.

Alcohol dehydrogenase preparations. Treble (9) demonstrated the existence of two alcohol dehydrogenases in horse liver. The first was precipitated between 30 and 42% ammonium sulfate saturation and was distinguished by its ability to catalyze the oxidation of 2-fluoroethanol to fluoroacetaldehyde. The second, which precipitated between 50 and 80% ammonium sulfate saturation, was apparently the familiar alcohol dehydrogenase first isolated in crystalline form by Bonnichsen and Wassen (10). It was inhibited competitively by 2-fluoroethanol. By means of Treble's procedure, the two liver fractions were prepared from two monkey livers processed individually, from two batches of five livers from adult male Sprague-Dawley rats, also processed separately, and from the liver of a freshly killed horse

[3] The abbreviation used is: AT, 3-amino-1,2,4-triazole.

[4] Scotch Brand No. 471 (width, 2 inches) Minnesota Mining and Manufacturing Co., St. Paul, Minnesota.



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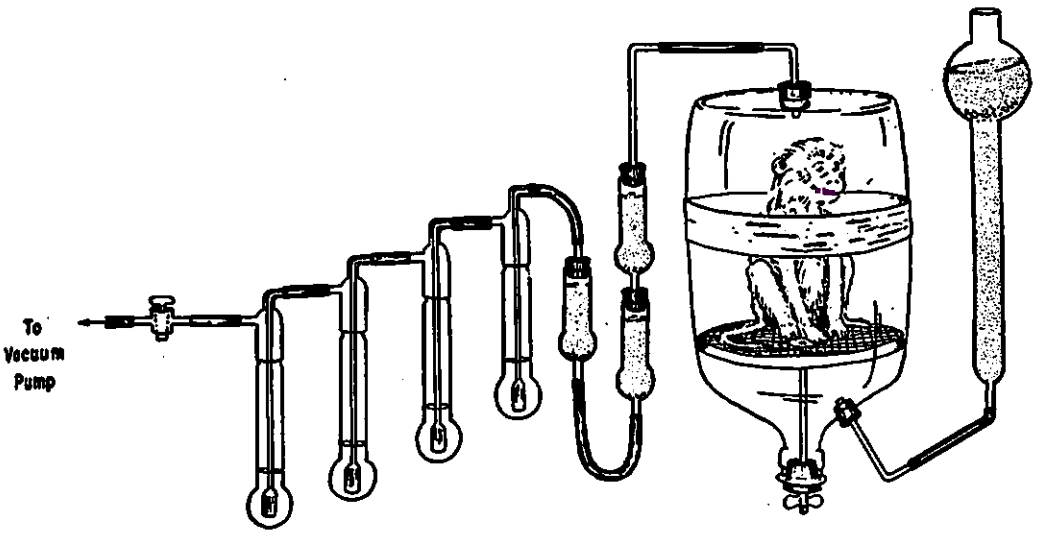

FIG. 1. *Metabolism chamber for the collection of respired methanol and $^{14}CO_2$*

The fresh liver was minced and stirred with ice-cold water (500 g of liver per liter) for 1 hr at 2° and filtered through muslin. The volume was reconstituted by adding cold water to the washed mince, and the mixture was homogenized in a Waring Blendor for 2 min. The homogenate was brought to 52° within 5 min and held at that temperature for 15 min before cooling rapidly to 2°. It was then centrifuged at 3000 *g* for 30 min at 0°. The precipitate was discarded, and a saturated solution of ammonium sulfate containing ammonium hydroxide[5] was added to the supernatant to bring saturation to 30%. After centrifugation at 3000 *g* for 30 min at 0°, the precipitate was discarded and more saturated ammonium sulfate solution was added gradually to bring saturation to 50%. The mixture was allowed to stand for 30 min at 0° and recentrifuged. The supernatant (supernatant A) was used later for the preparation of the second alcohol dehydrogenase fraction. The precipitate was dissolved in a small volume of freshly boiled, double glass-distilled water and dialyzed overnight at 0° against two changes of 20 volumes of water. The preparation was refractionated in the same way except that the limits of saturation with ammonium sulfate were 30 and 42% rather than 30 and 50%. The final preparation was stored at —15° until assayed. Supernatant A was saturated with the ammonium sulfate solution to 80%. Following centrifugation, the precipitate was dissolved in a small volume of freshly boiled, double glass-distilled water, dialyzed, and stored at —15°.

Evaluation of alcohol dehydrogenase activities of liver preparations. Reaction rates were determined by measuring the reduction of DPN at 340 mμ in a Beckman model DB recording spectrophotometer. The reaction mixture (3 ml) contained 1 mg of DPN, 1.4 ml of 0.1 M glycine-NaOH buffer (pH 10.0), 0.1 ml of alcohol dehydrogenase preparation, and 1.0 ml of methanol, ethanol, or 2-fluoroethanol solution, which was added at zero time. Various concentrations of the alcohols were employed (ethanol and 2-fluoroethanol, 1–10 mM; methanol, 10–100 mM), and rates were recorded while they were proceeding linearly. The Michaelis constants and maximum velocities (V_{max}) of the reactions were determined by the Lineweaver-Burk method (11). The data employed to derive the kinetic constants were submitted to statistical analysis (12) with calculations performed by a digital computer accord-

[5] Ammonium hydroxide was added in an amount such that when the solution was diluted 100 times, its pH was 6.5.

ing to a FORTRAN program written by Cleland (13).

Evaluation of catalase activities of liver homogenates. Liver homogenates were assayed for catalase activity by the method of Feinstein (14), and values were expressed in Kat. f. units as defined by von Euler and Josephson (15).

RESULTS

Rate of methanol metabolism. The rates of oxidation of two doses of methanol-^{14}C (1 and 6 g/kg) injected intraperitoneally are plotted in Fig. 2. The pulmonary excretion and urinary output of unmetabolized methanol by monkeys receiving the higher dose are also shown. At the 1 g/kg dose, methanol-^{14}C was oxidized at the rate of 37 mg/kg of monkey per hour between the first and fourth hours, when the rate of $^{14}CO_2$ formation was linear. The animals receiving 6 g of labeled methanol per kilogram oxidized the alcohol at the rate of 47 mg/kg/hr during the same time interval. The rates of the two dose levels are significantly different ($p < .05$). In animals receiving the high dose of methanol, 49% of the methanol disappeared as a result of oxidation, 35% by means of pulmonary excretion, and 16% by way of the kidneys.

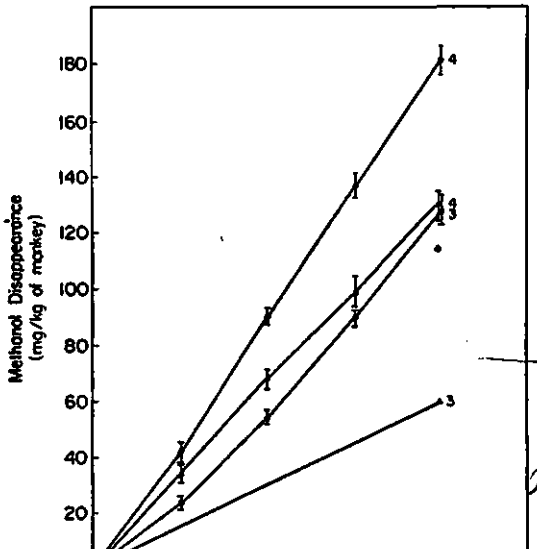

FIG. 2. *Disappearance of methanol-^{14}C from the monkey by oxidation to $^{14}CO_2$ and by urinary and pulmonary excretion*

○——○, △——△, and ▲——▲, loss of methanol-^{14}C by oxidation to $^{14}CO_2$, by pulmonary excretion, and by renal excretion, respectively, when the dose was 6 g of methanol-^{14}C/kg; ●——●, loss of methanol-^{14}C by oxidation to $^{14}CO_2$ when the dose was 1 g of methanol-^{14}C/kg. Figures at termini of curves represent the number of animals. Vertical bars denote ±standard error.

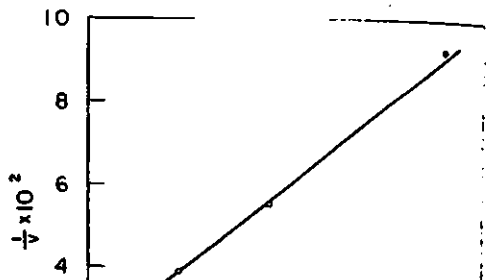

FIG. 3. *Lineweaver-Burk plot of methanol-^{14}C oxidation in the monkey in vivo*

v = the rate of methanol-^{14}C oxidation to $^{14}CO_2$ in milligrams per kilogram per hour. M = concentration of methanol (moles per liter of body water, assuming that water constitutes 70% of the body weight). Each point represents the mean of three monkeys. The apparent *in vivo* V_{max} = 48 mg of methanol-^{14}C per kilogram per hour; the apparent *in vivo* K_m = 8.7 mmoles of methanol-^{14}C per liter of body water.

The effect of the dose on the rate of methanol oxidation was studied with doses ranging between 0.05 and 1.0 g/kg of methanol-^{14}C. The dose-oxidation rate curve (Fig. 3), plotted by the Lineweaver-Burk method (11), yielded an "apparent *in vivo* K_m"[6] of 8.7 mM and an "apparent

[6] The apparent *in vivo* K_m is defined as the concentration of methanol-^{14}C in moles per liter of body water at which methanol-^{14}C is oxidized to $^{14}CO_2$ at one-half the rate calculated to occur at infinite substrate concentration.

in vivo V_{max}"[1] of 48 mg of methanol oxidized per kilogram of body weight per hour. The apparent *in vivo* K_m was calculated on the basis of total body water content on the assumption that body water is represented by 70% of the body weight and that methanol distributes rapidly and evenly throughout the total body water (16). The apparent *in vivo* K_m is about half that reported by Kini and Cooper (3) for the oxidation of methanol by monkey liver alcohol dehydrogenase (17 mM at pH 7.4). The rate of methanol oxidation when 6 g of methanol per kilogram were injected, 47 mg/kg/hr (Fig. 2), is virtually equal to the apparent *in vivo* V_{max}, 48 mg/kg/hr. A dose of 6 g of methanol per kilogram would provide a methanol concentration in the body water about 30 times the apparent *in vivo* K_m concentration, and it is thus to be expected that at this very high level of methanol administration the calculated apparent *in vivo* K_m would closely approximate the maximum rate of methanol oxidation observed directly.

Effect of ethanol on methanol-^{14}C oxidation and of methanol on ethanol-1-^{14}C oxidation. Ethanol and methanol are about equally reactive with the isolated catalase peroxidative system (17), whereas with the purified horse alcohol dehydrogenase system the K_m of ethanol, 2 mM (18), is about 10- to 50-fold lower (depending upon the pH at which the reaction is conducted) than the K_m of methanol for the monkey enzyme (3). If horse and monkey alcohol dehydrogenases possess similar reactivities with methanol and ethanol, then an equimolar amount of ethanol should inhibit methanol oxidation by about 50% if the peroxidative system is the primary pathway involved, and by more than 90% if the alcohol dehydrogenase system predominates.

Varying amounts of ethanol were injected with a constant dose of methanol-^{14}C (0.5 g/kg), and $^{14}CO_2$ was collected at intervals during 4-hr experimental periods (Fig. 4). When equimolar quantities of ethanol

[redacted], and even when the molar ratio of methanol to ethanol [redacted]

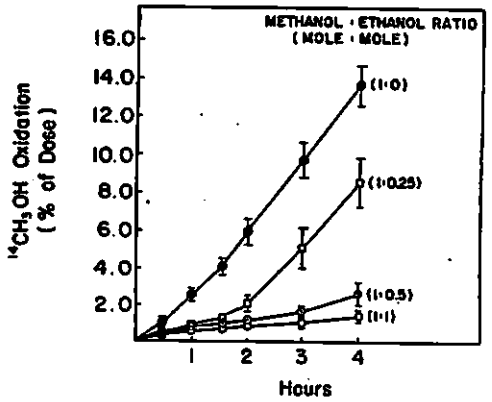

FIG. 4. *Effect of ethanol on methanol-^{14}C oxidation in the monkey in vivo*

●——●, Methanol-^{14}C (31.2 mmoles/kg), three monkeys; ○——○, methanol-^{14}C (31.2 mmoles/kg) simultaneously with ethanol (31.2 mmoles/kg), four monkeys. Rates of $^{14}CO_2$ production are significantly different from control rates at each time ($p < .01$). ⊙——⊙, Methanol-^{14}C (31.2 mmoles/kg) simultaneously with ethanol, (15.6 mmoles/kg), four monkeys. Rates of $^{14}CO_2$ production are significantly different from control rates at each time interval ($p < .01$). □——□, Methanol-^{14}C (31.2 mmoles/kg) simultaneously with ethanol, (7.8 mmoles/kg), six monkeys. Rates of $^{14}CO_2$ production are significantly different from control rates at each time interval up to 2 hr: $p < .01$ for time intervals up to 90 min, and $p < .05$ for the time interval from 90 min to 2 hr. All injections were made intraperitoneally. Vertical bars denote ±standard error.

[redacted] These findings clearly favor the view that the alcohol dehydrogenase system, or some system other than the peroxidative mechanism, is responsible for methanol oxidation in the monkey.

With ethanol being 10–50 times more reactive with alcohol dehydrogenase than methanol, a very high ratio of methanol to ethanol would be required for methanol to inhibit ethanol oxidation. Ratios as high as 8:1 produced no significant change in the rate of ethanol-1-^{14}C oxidation (Fig.

[1] The apparent *in vivo* V_{max} is defined as the calculated rate of methanol-^{14}C oxidation at infinite substrate concentration.

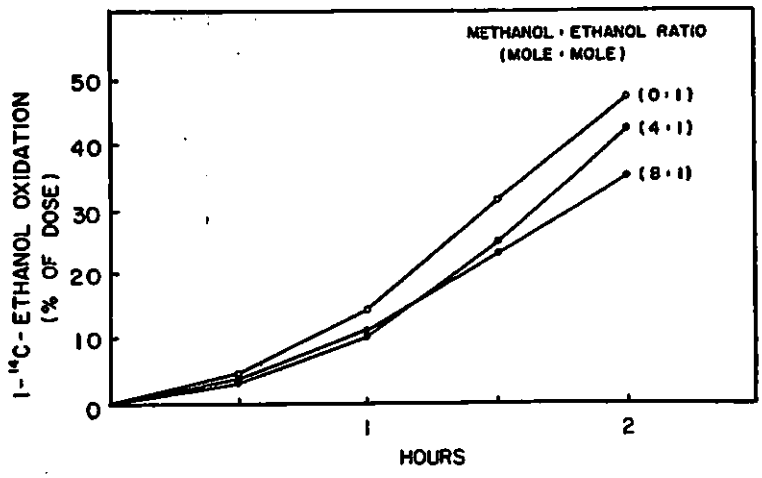

FIG. 5. *Effect of methanol on ethanol-1-^{14}C oxidation in the monkey in vivo*

○——○, Ethanol-1-^{14}C (15.6 mmoles/kg), three monkeys. ⊙——⊙, ethanol-1-^{14}C (15.6 mmoles/kg) simultaneously with methanol (62.4 mmoles/kg), three monkeys. Rates of $^{14}CO_2$ production are not significantly different from control rates at any time interval ($p > .05$). ●——●, Ethanol-1-^{14}C (15.6 mmoles/kg) simultaneously with methanol (124.8 mmoles/kg), three monkeys. Rates of $^{14}CO_2$ production are not significantly different from control rates at any time interval ($p > .05$). All injections were made intraperitoneally.

5). At the level of ethanol-1-^{14}C used, higher ratios could not be employed because of the acute toxicity that resulted when large quantities of methanol were used. The ratio could not be increased by decreasing the dose of ethanol-1-^{14}C because of the rapid disappearance of small doses of ethanol during the time interval deemed necessary for accurate measurement of $^{14}CO_2$.

Effect of 1-butanol on the oxidation of methanol-^{14}C. The reactivity of 1-butanol ($K_m = 0.22$ mM) is greater than that of ethanol ($K_m = 2$ mM) for the alcohol dehydrogenase system (18). On the other hand, 1-butanol is much less reactive with the peroxidative system than is methanol or ethanol (17). Thus, if methanol is oxidized peroxidatively in the monkey, 1-butanol should have little effect on its rate of oxidation, whereas a profound depression of methanol oxidation would be expected if the oxidation of methanol is mediated through alcohol dehydrogenase. With a molar ratio of methanol-^{14}C to 1-butanol of 1:0.5 the oxidation of methanol was inhibited 63% during the first 90 min after administration of the alcohols (Fig. 6). 1-Butanol exerted little inhibitory effect after 120 min, presumably because its concentration in the animal had been greatly reduced by oxidation. The inhibitory effect of 1-butanol on ethanol-^{14}C metabolism was similar to its effect on methanol-^{14}C metabolism (Fig. 7). Again the view is favored that in the monkey the alcohol dehydrogenase system, or some system other than that involving catalase, is responsible for methanol oxidation.

Effect of 3-amino-1,2,4-triazole inhibition of hepatic catalase on the oxidation of methanol-^{14}C. In the rat, AT reduced hepatic catalase activity by 90% or more, with a concomitant 50% reduction of methanol oxidation *in vivo* (1). This observation was employed with other evidence to establish the role of the catalase-H_2O_2 system in the oxidation of methanol in the rat.

Three monkeys received 1 g of AT per kilogram 1 hr before the administration of methanol-^{14}C (1 g/kg). Two other monkeys received the same dose of AT 3 hr before receiving labeled methanol. All injections were made intraperitoneally. The rate of methanol-^{14}C oxidation in the five monkeys between the first and fourth hours following injection of the methanol

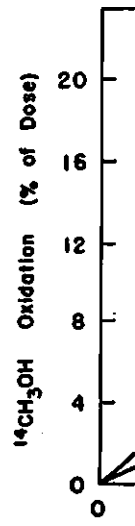

FIG. 6. *Effect of ... tion in the monkey i*

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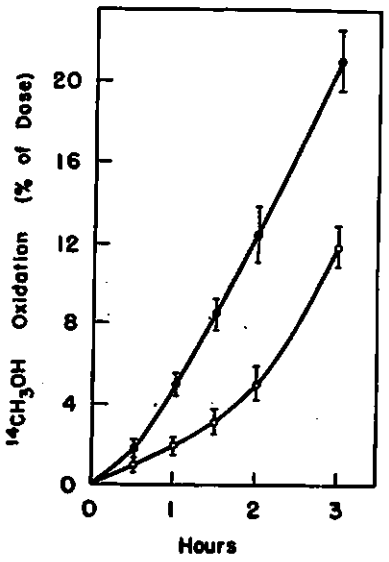

FIG. 6. *Effect of 1-butanol on methanol-^{14}C oxidation in the monkey in vivo*

●——●, Methanol-^{14}C (15.6 mmoles/kg), three monkeys; O——O, methanol-^{14}C (15.6 mmoles/kg) simultaneously with 1-butanol (7.8 mmoles/kg), three monkeys. Rates of $^{14}CO_2$ production are significantly different from control rates at each time interval between 30 min and 3 hr: $p < .01$ for time intervals between 30 min and 2 hr, and $p < .05$ for the time intervals between 2 and 3 hr. All injections were made intraperitoneally. Vertical bars denote ±standard error.

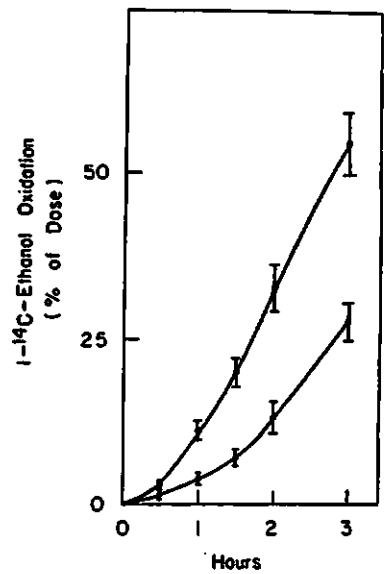

FIG. 7. *Effect of 1-butanol on ethanol-1-^{14}C oxidation in the monkey in vivo*

●——●, Ethanol-1-^{14}C (15.6 mmoles/kg), three monkeys; O——O, ethanol-1-^{14}C (15.6 mmoles/kg) simultaneously with 1-butanol (7.8 mmoles/kg), three monkeys. Rates of $^{14}CO_2$ production are significantly different from control rates at each time interval up to 3 hr: $p < .01$ for time intervals between 30 and 90 min, and $p < .05$ for time intervals between 0 and 30 min and between 90 min and 3 hr. All injections were made intraperitoneally. Vertical bars denote ±standard error.

ranged from 33 to 37 mg/kg/hr (Fig. 8). The average rate of methanol oxidation in control animals during the same time period (37 mg/kg/hr, Fig. 2) was not significantly different ($p > .05$) from the rates observed in AT-treated animals.

The observation that AT had no effect on the rate of methanol oxidation suggested the possibility that, in contrast to the rat, hepatic catalase is not inhibited by AT in the monkey. To test this possibility, hepatic catalase activity was determined in tissue obtained by biopsy. Two monkeys were anesthetized with pentobarbital sodium (50 mg/kg intraperitoneally) and laparotomies were performed. Liver specimens removed at this time contained 3975 and 4260 Kat. f. units of catalase activity per gram of tissue. AT (1 g/kg) was then introduced into the peritoneal cavity, and liver biopsies were performed 1 and 3 hr later. At 1 hr the livers showed catalase activities of 85 and 260 Kat. f. units/g, and at 3 hr, 57 and 147 Kat. f. units/g. Thus, AT is as effective an inhibitor of hepatic catalase in the monkey as it is in the rat.

The experiments with AT support the view that the peroxidative system is important in the oxidation of methanol in the rat, but of little consequence in the monkey.

Effect of ethylene glycol on methanol-^{14}C oxidation. Ethylene glycol and certain of its metabolites were found almost to double the rate of methanol oxidation in the intact rat and in the perfused liver of this species (6, 7). Experimental evidence suggested that the effect was due to H_2O_2 produced during the oxidation of glycolic acid, a metabolite of ethylene glycol (6,

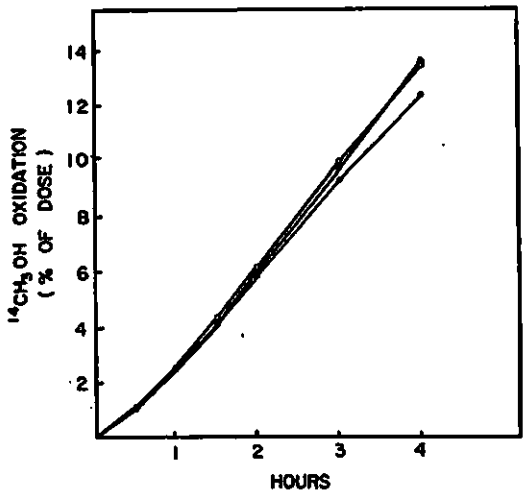

FIG. 8. *Effect of 3-amino-1,2,4-triazole on methanol-^{14}C oxidation in the monkey in vivo*

●———●, Methanol-^{14}C (1 g/kg), three monkeys; ○———○, methanol-^{14}C (1 g/kg) 1 hr after the administration of AT (1 g/kg), three monkeys. Rates of $^{14}CO_2$ production are not significantly different from control rates at any time interval ($p > .05$). ◑———◑, Methanol-^{14}C (1 g/kg) 3 hr after the administration of AT (1 g/kg), two monkeys. Rates of $^{14}CO_2$ production are not significantly different from control rates at any time interval ($p > .05$). All injections were made intraperitoneally.

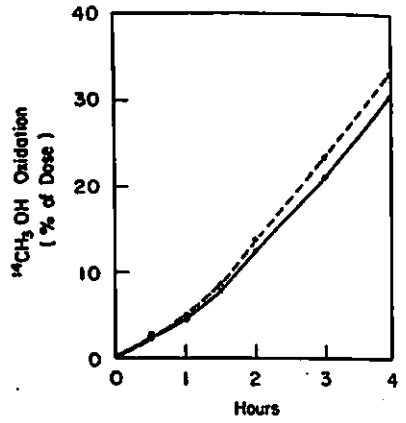

FIG. 9. *Effect of ethylene glycol on methanol-^{14}C oxidation in vivo*

●·····●, Methanol-^{14}C (31.2 mmoles/kg), three monkeys; ○———○, methanol-^{14}C (31.2 mmoles/kg) simultaneously with ethylene glycol (15.6 mmoles/kg), three monkeys. Rates of $^{14}CO_2$ production are not significantly different from control rates at any time interval ($p > .05$). All injections were made intraperitoneally.

7). Glycolic acid and molecular oxygen react through the action of the flavin enzyme, glycolic acid oxidase, to form glyoxylic acid and H_2O_2 (19). Since it is the catalase–H_2O_2 complex rather than catalase per se that is in short supply *in vivo*, this additional synthesis of H_2O_2 makes possible an increased rate of formation of the complex, and hence an increased rate of methanol oxidation. In view of the studies that had already been completed, it was to be expected that ethylene glycol would have no such stimulatory effect on methanol oxidation in the monkey, and this proved to be the case.

Three monkeys received simultaneous injections (i.p.) of 960 mg of ethylene glycol per kilogram and 1 g of methanol-^{14}C (molar dose ratio, 0.5:1). In these doses, ethylene glycol stimulated the rate of methanol-^{14}C oxidation in the rat by about 40% (6). The average rates of methanol-^{14}C oxidation in control and ethylene glycol-treated monkeys during the first 4-hr period after injections were 41 and 38 mg/kg/hr, respectively (Fig. 9). These rates are not statistically different.

Ethylene glycol is known to react with the alcohol dehydrogenase system (20) and could conceivably have inhibited the oxidation of methanol by competing with it for the enzyme. This did not appear to occur, conceivably because the ethylene glycol to methanol ratio at the metabolic site was not sufficiently high. However, the possibility must be considered that ethylene glycol may have inhibited methanol oxidation by the alcohol dehydrogenase system to almost exactly the same degree that it stimulated the peroxidative oxidation of methanol. In any event, a clear difference is seen in the action of ethylene glycol on methanol metabolism in the rat and monkey, and this strengthens the view that the peroxidative system is of minimal importance in the oxidation of methanol in the monkey.

Studies in vitro. The studies *in vitro*, which used partially purified hepatic alcohol dehydrogenase from the monkey, rat,

TABLE 1

Reaction kinetics of partially purified alcohol dehydrogenases isolated from the livers of the monkey, rat, and horse

The reaction mixture (3 ml) contained 1 mg of DPN, 1 ml of 0.1 M glycine-NaOH buffer (pH 10), 0.5 ml of alcohol dehydrogenase preparation, and 1.0 ml of solution containing the substrate. Substrate concentrations were 1 – 10 mM when ethanol and 2-fluoroethanol were used, and 10 – 100 mM when methanol was employed. The incubation temperature was 23°. K_m values are expressed in mM. V_{max} values are expressed as micromoles of substrate oxidized per equivalent of 1 g of liver per hour.

	Fraction I[a]						Fraction II[b]					
	Monkey[c]		Rat[d]		Horse[e]		Monkey[c]		Rat[d]		Horse[e]	
Substrate	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
Methanol	20	1.1	ND[f]	ND	5.0	5.0	15	20	31	4.0	60	12
	12	1.4	ND	ND	5.3	5.6	17	26	15	1.5	50	16
Ethanol	1.0	1.9	2.5	5.0	1.4	122	2.0	72	1.6	9.0	2.1	320
	2.1	3.7	1.4	4.2	1.5	135	2.1	89	2.0	11.5	1.9	360
2-Fluoroethanol	ND	ND	ND	ND	3.3	25	3.2	15	ND	ND	ND	ND
	ND	ND	ND	ND	—	—	4.1	19	ND	ND	—	—

[a] Precipitated between 30 and 42% ammonium sulfate saturation.
[b] Precipitated between 50 and 80% ammonium sulfate saturation.
[c] Fractions were prepared from two monkey livers processed individually.
[d] Fractions were prepared from two pools of rat livers processed individually.
[e] Fractions were processed individually from a single horse liver.
[f] ND = no reaction detected.

and horse, are summarized in Table 1. The separation of a horse liver homogenate into fractions containing alcohol dehydrogenases with different substrate specificities, as first described by Treble (9), was demonstrated. In accordance with expectations, the dehydrogenase capable of oxidizing both 2-fluoroethanol and ethanol was found in the fraction that precipitated between 30 and 42% ammonium sulfate saturation (fraction I), and the fraction that precipitated between 50 and 80% ammonium sulfate saturation (fraction II) contained a dehydrogenase that was capable of oxidizing ethanol, but not 2-fluoroethanol. Treble found the newly recognized alcohol dehydrogenase to oxidize 2-fluoroethanol at about 80% of the rate of ethanol. However, in the current study, fraction I oxidized 2-fluoroethanol at only about 20% of the rate of ethanol (Table 1), which suggests that separation of the two dehydrogenases may not have been as complete as that obtained by Treble. Not in accordance with expectations, the 2-fluoroethanol-oxidizing activity of monkey liver preparations was found in fraction II rather than in fraction I. Despite this inability to partition the 2-fluoroethanol-oxidizing and the strictly ethanol-oxidizing dehydrogenases between the two fractions from monkey liver, the 2-fluoroethanol-oxidizing activity of fraction II from the monkey, as compared to its ethanol-oxidizing activity, is about the same as that observed with fraction I from the horse, namely, about 20%. This raises some questions as to qualitative differences that may exist between the alcohol dehydrogenases from horse and monkey livers, but in view of the crude enzyme preparations used in this study, it would be wise at this time to withhold speculation. It is also to be noted that whereas about 38% of the alcohol dehydrogenase activity was found in fraction I from horse liver when ethanol was used as a substrate, only about 5% of this activity was found in fraction I from monkey liver.

No reactivity of 2-fluoroethanol with either fraction I or fraction II from rat liver was demonstrable. However, the al-

cohol dehydrogenase activities of both fractions are very low, and the method may not have been sufficiently sensitive to permit the detection of the small amounts of 2-fluoroethanol-oxidizing activity that may have been present.

The total alcohol dehydrogenase activities of the liver preparations from the three species are seen to vary greatly. The combined activities of fractions I and II (micromoles of ethanol oxidized by the equivalent of 1 g of liver per hour) were about 470, 85, and 15 for the horse, monkey, and rat, respectively.

The ratio of ethanol to methanol oxidation by horse liver fractions (rate of ethanol oxidation = 1) was about the same in fractions I and II, namely, about 0.04. This is considerably lower than the 0.14 value reported by Lutwak-Mann (21) and the 0.11 value given by Zatman (22) for crude horse liver alcohol dehydrogenase preparations, or the value of 0.14 seen by Kini and Cooper (3) when crystalline horse liver alcohol dehydrogenase was employed. No explanation for this lower value is offered at this time.

Relative to its reactivity with ethanol, monkey liver alcohol dehydrogenase is more reactive with methanol than is the enzyme from horse liver. The ethanol to methanol oxidation rate was 0.45 for fraction I and 0.35 for fraction II. The latter value compares favorably with the value of 0.33 obtained by Kini and Cooper (3) with their purified monkey preparation. With a value of 0.37, rat liver alcohol dehydrogenase resembles the monkey liver enzyme. These observations again suggest qualitative differences between the alcohol dehydrogenases of the three species.

The K_m values for ethanol oxidation were quite similar regardless of the liver fraction or species employed. The values of 2.0 and 2.1 mM obtained with fraction II from monkey liver compare favorably with the value of 2.7 mM reported by Kini and Cooper (3) for monkey liver alcohol dehydrogenase. The K_m values for methanol oxidation by the monkey liver enzyme (fraction II), 15 and 17 mM, compare very well with the K_m value of methanol for monkey liver alcohol dehydrogenase (17 mM) given by Kini and Cooper (3). The K_m values for methanol oxidation by rat liver preparations (fraction II) were quite similar to those found with the monkey liver preparations, but the values obtained with the horse liver extract were considerably higher.

DISCUSSION

These studies lead to the conclusion that a species difference exists in the manner in which oxidation of methanol occurs in the rat and in the monkey. The peroxidative mechanism provides the major pathway for the primary oxidation of methanol in the rat, but in the monkey it is not involved in methanol oxidation to any significant degree. This conclusion is based on a number of observations, none of which in itself can be considered conclusive; however, when viewed collectively, these observations form a strong basis for opinion.

1. Ethanol and methanol are known to be equally reactive with the isolated peroxidative system involving catalase, but ethanol is oxidized much more readily by alcohol dehydrogenase than is methanol. Thus, ethanol should compete with methanol for its oxidation on an equal basis if the peroxidative system is primarily involved in the oxidation of methanol, and this proved to be the case in the intact rat (1). On the other hand, if the alcohol dehydrogenase system is important in the oxidation of methanol, lesser amounts of ethanol would be required to inhibit methanol oxidation than if the peroxidative system were strongly implicated. This proved to be the case when ethanol was used as an inhibitor of methanol oxidation in the intact monkey.

2. With respect to their reactivities with the peroxidative system and the alcohol dehydrogenase system, 1-butanol and ethanol behave oppositely; 1-butanol is even more reactive with the alcohol dehydrogenase system than is ethanol, and ethanol is more reactive with the peroxidative system than is 1-butanol. Thus, if the peroxidative system is largely responsible for

methanol oxidation, 1-butanol should be a relatively poor inhibitor of methanol oxidation, but if methanol oxidation is mediated through the action of alcohol dehydrogenase, 1-butanol should be a very good inhibitor of methanol oxidation. In the rat, 1-butanol was a relatively poor inhibitor of methanol oxidation (1); in the monkey, it was a relatively good inhibitor of methanol oxidation.

3. 3-Amino-1,2,4-triazole, injected intraperitoneally, inhibits hepatic catalase activity by more than 90% in both the rat and the monkey. This caused a 50% reduction in the rate of methanol oxidation in the rat (1), but had no effect on the oxidation of methanol in the monkey.

4. The administration of ethylene glycol doubles the rate of oxidation of methanol in the rat (6, 7). This is thought to be due to the increased production of H_2O_2 that results when glycolic acid, a metabolite of ethylene glycol, is oxidized to glyoxylic acid. Ethylene glycol had no effect on the rate of methanol oxidation in the monkey.

With the peroxidative system eliminated as a likely contributor to the oxidation of methanol in the monkey, attention is directed to the alcohol dehydrogenase system. The studies *in vitro* support the view that the latter system may be important in the oxidation of methanol in the monkey. By employing the mean V_{max} of 2.4×10^{-5} mole of methanol oxidized per gram of liver per hour (Table 1, monkey liver fractions I + II) and the mean weight of the livers of the two monkeys (20 g per kilogram of body weight), and assuming that DPN is not rate-limiting *in vivo*, it can be calculated that alcohol dehydrogenase, as judged from the studies *in vitro*, could account for the oxidation of methanol *in vivo* at the rate of 15.4 mg/kg of monkey per hour. The apparent *in vivo* V_{max} (Fig. 3) was calculated to be 48 mg of methanol oxidized per kilogram of monkey per hour, and this value agreed with the observed rate of methanol oxidation *in vivo* when the 6-g/kg dose of methanol was employed (Fig. 2). Thus, about 32% of the oxidation of methanol observed *in vivo* can be accounted for by the alcohol dehydrogenase activity found in the liver. The values given in Table 1 were obtained at an incubation temperature of 23°. The effect of temperature was studied, and a 60% increase in the V_{max} values given in Table 1 was observed at 37°. If this is taken into account, the "recovery" value of 32% can be raised to 51%. When one takes into consideration the losses in alcohol dehydrogenase activity that probably occurred during the isolation of the enzyme fraction, the ability to account for half of the oxidation of methanol observed *in vivo* from studies *in vitro* provides strong evidence for the role of this enzyme in the metabolism of methanol in the intact monkey.

In a study to be reported later (A. B. Makar and G. J. Mannering) measurements of the disappearance rates of ethanol from the blood of intact monkeys were subjected to calculations provided by Lundquist and Wolthers (23) to obtain an apparent *in vivo* V_{max} of about 320 mg of ethanol oxidized per kilogram of monkey per hour. From the values given in Table 1 and the same kind of arithmetic that was employed to obtain the 51% recovery value for methanol oxidation, 38% of the apparent *in vivo* V_{max} for the oxidation of ethanol in the intact monkey can be accounted for by the studies *in vitro*. The relatively good agreement between the "recovery" values for methanol and for ethanol supports the view that both methanol and ethanol are oxidized by alcohol dehydrogenase in the intact monkey.

The apparent *in vivo* V_{max} for methanol oxidation by the rat is 30 mg/kg/hr (1). From the data in Table 1 and the calculations used previously for the monkey experiments, and taking into account the fact that rat liver constitutes about 4% of the body weight, a recovery value of 10% was obtained when the alcohol dehydrogenase of lowest activity was considered, and 27% when the more active preparation was offered for comparison. Thus it is possible that the alcohol dehydrogenase system in the rat may account for an appreciable amount of methanol oxidation, although considerably less than that which can be

expected to occur in the liver of the monkey.

In studies similar to those performed with the monkey, in which the rate of ethanol disappearance from the blood was used to obtain kinetic values for the oxidation of ethanol *in vivo*, the apparent *in vivo* V_{max} in the rat was shown to be 270 mg of ethanol oxidized per kilogram per hour (A. B. Makar and G. J. Mannering, unpublished data). From this value and the data presented in Table 1 a mean recovery value of 16% can be calculated for ethanol in the rat. The amount of alcohol dehydrogenase in rat liver clearly cannot account for the relatively rapid rate of ethanol oxidation seen in the intact rat, and along with the several factors that might contribute to this discrepancy, including the possibility that the fractionation procedure resulted in poor recoveries of enzyme activity, some consideration should be given to the possibility that ethanol may be oxidized in the rat by some mechanism that does not involve either alcohol dehydrogenase or catalase.

Kini and Cooper (3) measured the disappearance of methanol from the blood of a 3-kg monkey over a 22-hr period. Assuming little pulmonary or renal loss of methanol and ignoring the fact that methanol distributes throughout all body water, not only throughout water contained in the blood, they considered the 3-kg monkey to have oxidized methanol at the rate of 10.45 μmoles/min. This is about 6.7 mg of methanol oxidized per kilogram of monkey per hour, well below the 48 mg/kg/hr reported in the current study when the same dose of methanol (6 g/kg) was administered. When the fact is acknowledged that methanol distributes throughout body water, rather than confining itself to the blood (4), the rate of methanol oxidation in the monkey can be calculated to be about 53 rather than 6.7 mg/kg/hr. However, when a 6-g/kg dose of methanol is administered, about half of the disappearance of methanol from the monkey results from pulmonary and renal excretion (Fig. 2). When this is taken into account, the rate of methanol oxidation in the monkey, as calculated from the data given by Kini and Cooper, becomes about 27 mg/kg/hr, which is still well below the rate observed in the current study. While it is true that the rate of methanol disappearance was determined over a 22-hr period in the study by Kini and Cooper and over only a 4-hr period in the current study, this should not have greatly influenced the results; with a 6-g/kg dose of methanol, the lowering of the concentration of methanol in the body water during the 22-hr period would not have been sufficient to decrease the rate of methanol oxidation greatly during that time period.

In a previous communication (4) it was estimated that the alcohol dehydrogenase activity found in liver preparations from monkeys by Kini and Cooper could only account for about 3.6% of the disappearance of methanol from the intact monkey, assuming that pulmonary and renal losses were negligible. Since the pulmonary and renal excretion of methanol accounts for about half of the methanol disappearance, the 3.6% recovery can be doubled, but this is still much lower than the 51% recovery seen in the current studies. It should be pointed out, however, that the two recovery values were derived quite differently. In their calculations, Kini and Cooper assumed that the kinetic values furnished by Theorell and Bonnichsen (18) from their studies with crystalline horse liver alcohol dehydrogenase could be applied to the relatively crude preparations obtained from monkey liver. This may not be a valid assumption. The lower recovery of enzyme obtained by Kini and Cooper may have been due to the more drastic conditions employed during fractionation: they heated the liver extract at 55° for 30 min, whereas in the current study the extracts were heated at 52° for 15 min.

The catalase activity of monkey liver was found to be about 4000 Kat. f. units per gram of tissue, which is about 4 times that found in rat liver. With a liver size relative to total body weight about half that of the rat, the monkey possesses about twice as much hepatic catalase activity as the rat on a per-kilogram basis. In the rat

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the activity of the peroxidative system involving catalase is limited by the rate of peroxide generation, but when AT is used to inhibit hepatic catalase, the availability of catalase becomes rate-limiting (6). One might also expect this to be the case in the monkey, and, if so, at least some effect of AT on methanol oxidation should have been observed even though the main pathway for methanol oxidation appears to proceed via alcohol dehydrogenase. When a 1-g/kg dose of methanol was given to the rat, AT reduced the oxidation of methanol from 24 to 12 mg/kg/hr. An AT-induced reduction of methanol oxidation of this magnitude would have been detected in the monkey had it occurred. The question must be asked why the hepatic catalase in the monkey is not utilized for the peroxidative oxidation of methanol. Three possibilities may be considered: (a) the peroxide-generating systems in the intact monkey are even more deficient than they are in the rat; (b) the distribution of catalase in the hepatic cell of the monkey is such that it does not have intimate access to the peroxide-generating systems; or (c) the hepatic catalases of the rat and monkey differ such that the peroxidative activity of monkey catalase is less with respect to its catalytic activity than is the case for rat liver catalase. The two latter possibilities were studied and the results are presented in the accompanying publication (24).

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