METHANOL METABOLISM IN THE RAT[1]

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The controversy as to which enzyme system is primarily responsible for the first step in the oxidation of methanol by mammals can be said to have originated with the isolation of crystalline horse liver alcohol dehydrogenase by Bonnichsen (1950). Until then it was widely believed that alcohol dehydrogenase (ADH) mediated the oxidation of both ethanol and methanol as follows:

$$RCH_2OH + DPN \overset{ADH}{\rightleftharpoons} RCHO + DPNH + H^+.$$

Lutwak-Mann (1938), who performed the first extensive studies on ADH, did much to encourage this view when she showed that the partially purified enzyme, which required DPN as a cofactor, reacted with both ethanol and methanol. Ethanol was oxidized about 9 times more rapidly than methanol. It came somewhat as a surprise, then, that the pure enzyme in combination with DPN, oxidized ethanol and other aliphatic alcohols, but not methanol (Theorell and Bonnichsen, 1951). This observation redirected attention to the peroxidative system of Keilin and Hartree (1936, 1945) which had been proposed earlier as a mechanism for the oxidation of alcohols. More recently, it has been shown that when the substrate concentration is very high, ADH will oxidize methanol slowly (Kini and Cooper, 1961), which is not totally unexpected in view of the previous knowledge that ADH will react with formaldehyde to produce methanol (Theorell and Chance, 1951; Winer, 1958). However, the relatively low methanol oxidizing capacity and the highly unfavorable Michaelis constants for methanol (1.7×10^{-2} M at pH 9.6 and 9.2×10^{-2} at pH 7.4) of monkey liver ADH (Kini and Cooper, 1961) are not those that encourage the view that ADH plays a primary role in the physiological oxidation of methanol.

Keilin and Hartree showed that catalase catalyzed the oxidation of alcohols to their aldehydes when hydrogen peroxide was supplied slowly in low concentrations, such as could be provided in living tissues through the action of certain flavin enzymes on their substrates (*e.g.*, xanthine oxidase, amine oxidase, D-amino acid oxidase). They presented arguments to support their view that catalase is not present in the tissues to protect against peroxide intoxication, as was generally contended, but rather to carry out coupled (peroxidative) oxidations. Employing techniques that permitted very rapid spectral determinations, Chance (1947) conducted an exhaustive study of the catalase reaction in which he identified the intermediate complexes and analyzed the kinetics of the component reactions given in the following scheme:

$$\underset{\text{(very rapid combination)}}{\text{Catalase} + H_2O_2} \rightarrow \underset{\text{(rapid second order reaction)}}{\text{Catalase}(H_2O_2) + RCH_2OH} \rightarrow$$

$$\underset{\text{(relatively unstable)}}{\text{Catalase}(H_2O_2)(RCH_2OH)} \rightarrow \text{Catalase} + 2H_2O + RCHO.$$

Chance considered the reactivity of the system to be more than adequate to account for the disappearance of methanol from the blood of rabbits. On the basis of the work of Keilin and Hartree and of Chance, and from their own studies with pure ADH, Theorell and Bonnichsen (1951) concluded that in all probability, ethanol was oxidized by ADH, and methanol by catalase.

Roe's (1943) suggestion that ethanol inhibits the oxidation of methanol has been confirmed experimentally *in vivo* with rabbits (Agner and Belfrage, 1947; Koivusalo, 1956), rats (Bartlett,

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1950b; Aebi *et al.*, 1957), and man (Zatman, 1946), and *in vitro* with slices or homogenates of liver (Bartlett, 1950a; Koivusalo, 1956, 1959; Aebi *et al.*, 1957; Smith, 1961). Koivusalo concluded that the inhibition exhibited by ethanol is most likely of a competitive nature. Jacobsen (1952a,b) offered the generalization that all of the methanol and about one-fifth of the ethanol were oxidized by the peroxidative system, the remaining ethanol metabolism going by way of ADH.

A direct mean of studying the role of the catalase peroxidative system in the metabolism of methanol was provided when Heim *et al.* (1955) showed that the intraperitoneal injection of 3-amino-1,2,4-triazole (AT) caused about a 90% reduction in the hepatic and renal catalase activity in rats. Mannering and Parks (1957) found that the AT-induced inhibition of hepatic catalase was accompanied by a 70% reduction of the methanol-oxidizing capacity of rat liver homogenates. The addition of crystalline beef liver catalase to these homogenates restored methanol oxidation to normal. Aebi and coworkers (1957) administered AT to guinea pigs 2 and 4 hours after they had received C^{14}-methanol and noted an immediate drop in $C^{14}O_2$ production. A similar reduction in expired $C^{14}O_2$ was also noted after the feeding of isopropylallylacetureide, a known inhibitor of hepatic catalase synthesis. Liver slices from animals fed this compound also showed a greatly reduced capacity to oxidize methanol. Smith (1961) confirmed the finding of concurrent inhibition of catalase and methanol metabolism by AT using rat liver slices.

Reduction of the H_2O_2 component of the catalase·H_2O_2 complex reduces the methanol oxidizing capacity of liver homogenates. Higgins *et al.* (1956) found a marked depletion of the xanthine oxidase activity of liver homogenates from rats fed sodium tungstate. It would appear that other enzymes responsible for peroxide generation are also seriously affected because liver homogenates from tungstate poisoned rats were essentially devoid of methanol-oxidizing capacity (Tephly *et al.*, 1961a). The addition of purified xanthine oxidase to these homogenates completely restored methanol metabolism.

The preponderance of evidence from *in vitro* experiments favors the catalase peroxidative pathway over the ADH pathway for methanol oxidation in the rat. However, results obtained *in vivo* presented a seeming contradiction since treatment with AT, tungstate or ethanol had no measurable effect on the rate of disappearance of blood methanol (Mannering and Parks, 1957; Tephly *et al.*, 1961a). As will be seen later, when renal and pulmonary routes of methanol excretion are considered, this apparent discrepancy between results obtained *in vivo* and *in vitro* does not present a serious objection to concepts favoring the peroxidative oxidation of methanol. The problems that arise when attempts are made to interpret results from studies based on the disappearance of blood methanol dictated the approach employed in the present study, namely, the direct measurement of the metabolism and excretion of C^{14}-methanol (Tephly *et al.*, 1961a).

The work to be reported here was an attempt to execute quantitative experiments *in vivo* in a manner amenable to interpretation at the enzyme level. This was accomplished through the use of radioactive methanol or ethanol and three experimental approaches: (1) the "apparent *in vivo* Michaelis constants (K_m)"[5] were determined and compared with the established *in vitro* K_m's of methanol for the catalase·H_2O_2 and ADH systems; (2) the effects *in vivo* of 1-butanol and ethanol on methanol oxidation and of 1-butanol and methanol on ethanol oxidation were studied with reference to what is already known about the relative effectiveness of these alcohols as substrates for catalase·H_2O_2 and ADH systems; and (3) the effects of AT on the oxidation of methanol and ethanol by the intact rat were studied.

A preliminary report of this work has been presented (Tephly *et al.*, 1962).

METHODS AND MATERIALS. *Labeled compounds.* The specific activity of C^{14}-methanol was determined by oxidation to carbon dioxide with chromic acid in an apparatus described by Van Slyke *et al.* (1941). Ethanol was oxidized to $C^{14}O_2$ with the wet combustion mixture suggested by Lindenbaum *et al.* (1948). $BaC^{14}O_2$ plates were prepared and analyzed for their radioactivity using a gas-flow counter equipped with an end window. All labeled

[5] The "apparent *in vivo* K_m" is defined as the dose of methanol in mol/l of body water at which methanol is oxidized to CO_2 at one-half the maximal rate (V_{max}).

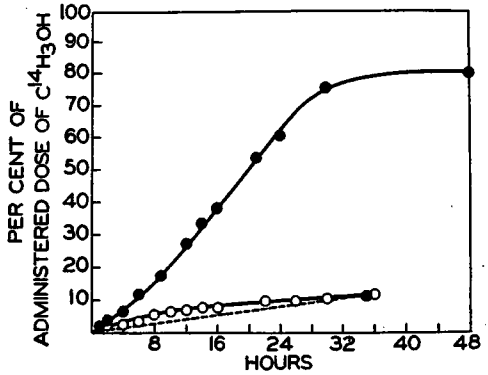

FIG. 1. C^{14}-methanol metabolism in the rat *in vivo*.

●——●, Accumulated pulmonary $C^{14}O_2$; ○——○, accumulated pulmonary methanol; ●- - - -●, fecal and urinary excretion of methanol (determined at the 36-hour period only). Methanol-C^{14} was injected intraperitoneally at a dose level of 1 g/kg as a 10% solution in saline (1.94×10^3 cpm/mg). Each point represents the average of 4 rats.

materials were purchased from New England Nuclear Corporation.

3-Amino-1,2,4-triazole (*AT*). AT was generously supplied by the American Cyanamid Company and was purified as described previously (Tephly *et al.*, 1961a).

In vivo experiments. Male albino rats of the Holtzman strain weighing 250 to 350 g and maintained on a stock diet were injected with various amounts of 10% (w/v) C^{14}-methanol and/or 14.4% (w/v) 1-C^{14}-ethanol in saline. When the 6-g/kg dose level of methanol was employed, a 60% methanol solution was used. The specific activity of the C^{14}-methanol was 1.94×10^3 cpm/mg and that of the 1-C^{14}-ethanol, either 1.4×10^3 or 3.1×10^2 cpm/mg. AT was injected at a dose level of 1 g/kg of body weight 1 hour before the administration of an alcohol. All injections were made intraperitoneally.

After administration of the radioactive material, the animal was placed in a metabolism chamber of the design described by Weinhouse and Friedmann (1951). A vacuum pump was used to draw air through the apparatus. Air entering the chamber was passed through a saturated solution of sodium hydroxide to remove carbon dioxide and through a column of Drierite to adsorb moisture. Respired air was passed first through about 10 g of magnesium perchlorate to adsorb methanol and then through two 3 N sodium hydroxide solutions (100 ml each) placed in series to collect the $C^{14}O_2$. To test the efficiency of magnesium perchlorate in trapping methanol, 1 ml of a standard solution of methanol in water (26 mg/ml) was placed on filter paper in the empty chamber and air was passed through the apparatus for 3 hours. Recoveries averaged 98% when 5 g of magnesium perchlorate were employed. No measurable amount of $C^{14}O_2$ was recovered from the magnesium perchlorate. Depending upon the amount of alcohol administered and the duration of the experiment the perchlorate was replaced every 2, 4 or 6 hours.

At various time intervals the sodium hydroxide solutions were removed, combined and analyzed for their $C^{14}O_2$ content. Urine and feces were collected periodically, combined and stored at 10°C until analyzed for their methanol contents. The magnesium perchlorate, urine and fecal samples were steam distilled and the resulting distillates were analyzed for their methanol content by the method of Agner and Belfrage (1947). $C^{14}O_2$ was precipitated as $BaC^{14}O_3$, collected as described by Aronoff (1956) and analyzed for radioactivity using a gas-flow counter equipped with an end window employing the usual correction for self-absorption.

RESULTS. *Oxidation and excretion of methanol in vivo*. The rate of oxidation of a single dose of C^{14}-labeled methanol (1 g/kg) injected into rats intraperitoneally as measured by $C^{14}O_2$ collection over a 48-hour period is plotted in figure 1. The rate of the pulmonary excretion of unmetabolized methanol and the total urinary and fecal output of the alcohol are also shown. Methanol was oxidized at a constant rate of 24 mg/kg/hr during the first 28 hours following administration. Bartlett (1950a) observed an oxidation rate of 25 mg/kg/hr during this same time period in rats receiving the same dose of methanol. By the end of 36 hours, 77% of the methanol had been converted to $C^{14}O_2$ and 24% of the dose was excreted unchanged. About equal quantities of methanol were eliminated by the pulmonary and renal plus fecal routes.

The effect of the dose on the rate of methanol oxidation was studied employing doses ranging between 0.05 and 3.0 g/kg. The dose-oxidation rate curve (fig. 2) is plotted by the Lineweaver-Burk method (1934) which is employed frequently in kinetic studies of isolated enzyme systems for the determination of Michaelis constants (K_m) and maximum velocities (V_{max}). The "apparent *in vivo* K_m"[5] for methanol oxidation derived from this graph is 7.0×10^{-3} mol/l of body water and the apparent V_{max} is 30 mg or about 1 mmol of methanol oxidized/kg of body

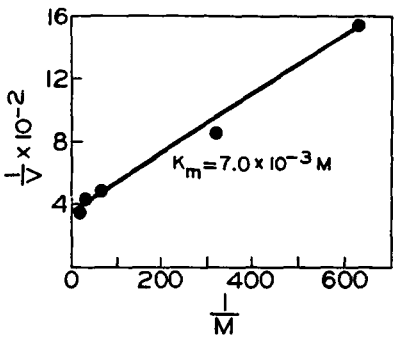

FIG. 2. "Apparent *in vivo* K_m" for C^{14}-methanol oxidation in the rat.

v = the rate of C^{14}-methanol oxidation to $C^{14}O_2$ oxidized/kg of body weight/hour. Rates were determined over the first 4 hours following intraperitoneal injection of C^{14}-methanol during which time $C^{14}O_2$ formation was linear. M = concentration of C^{14}-methanol in mol/l of body water. V_{max} = 30 mg of $C^{14}H_3OH$ oxidized/kg of body weight/hour. Each point represents the average of 3 rats. Injected doses varied from 0.05 to 3.0 g of $C^{14}H_3OH$/kg of body weight and metabolic rates ranged from 6.5 to 27.3 mg of $C^{14}H_3OH$ oxidized/kg of body weight/hour. The K_m value was determined using least squares regression line analysis (Dixon and Massey, 1957).

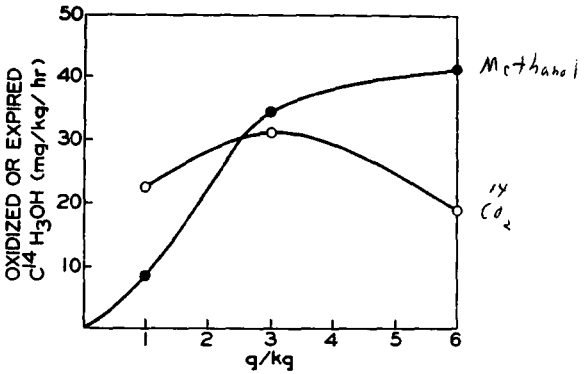

FIG. 3. The effect of dosage on the pulmonary excretion of C^{14}-methanol in the rat.

●——●, Expired methanol; ○——○, pulmonary $C^{14}O_2$. C^{14}-methanol was injected intraperitoneally as a 10 or 60% (w/v) solution in saline (1.94×10^3 cpm/mg). Pulmonary $C^{14}O_2$ and methanol were collected periodically over an 8-hour time period following intraperitoneal injection. The rates of elimination of both substances were linear throughout this time interval. Each point represents the average of 4 rats.

weight/hr. The "apparent *in vivo* K_m" was expressed in terms of body water on the basis of the assumption that body water is represented by about 70% of the body weight and the observation that methanol distributes rapidly and evenly throughout the total body water (Yant and Schrenk, 1937). In contrast to methanol and ethanol, "apparent *in vivo* K_m" values of most other substances would not be equatable with K_m's obtained with isolated enzyme systems because equilibrium and distribution factors in the whole animal differ greatly from those that occur *in vitro*. The "apparent *in vivo* K_m" for methanol of 7.0×10^{-3} M should be considered as the upper limit of the true value since it assumes instantaneous equilibration of methanol throughout body water and the maintenance of the zero time concentration through the 4-hour period of measurement. The average concentration of methanol in the body water during this period would be significantly lower than the hypothetical zero-time concentration. It is of considerable interest to note that this "apparent *in vivo* K_m" ($<7.0 \times 10^{-3}$ M) and the K_m determined in the isolated catalase peroxidative system for methanol (1.5×10^{-3} M: Tephly *et al.*, 1961a) are of a similar order of magnitude. On the other hand the "apparent *in vivo* K_m" is considerably lower than the K_m of methanol with the ADH system ($1.7\text{–}9.2 \times 10^{-2}$ M: Kini and Cooper, 1961). Lundquist and Wolthers (1958) have calculated "apparent *in vivo* K_m" values for the overall oxidation of ethanol in humans.

A number of investigators (Agner and Belfrage, 1947; Koivusalo, 1956; Gilger *et al.*, 1959; Mannering and Parks, 1957; Kini and Cooper, 1961) have studied the disappearance of methanol from the blood as a measure of the rate of methanol oxidation without regard for the possible contribution that pulmonary and renal excretion may have been making to this disappearance. The slow rate of methanol oxidation should permit appreciable amounts of methanol to escape by way of the kidneys and lungs. Figure 3 shows the effect of increasing doses of methanol on the rate of pulmonary excretion of methanol. The rate of pulmonary excretion at the 6-g/kg dose level is much less than one would have predicted from Henry's Law which governs the partition of methanol between blood and alveolar air. The rats receiving this very high dose of methanol were deeply sedated and their respiration was observed to be depressed greatly, which would account for the leveling off of the pulmonary excretion of methanol. The rate of

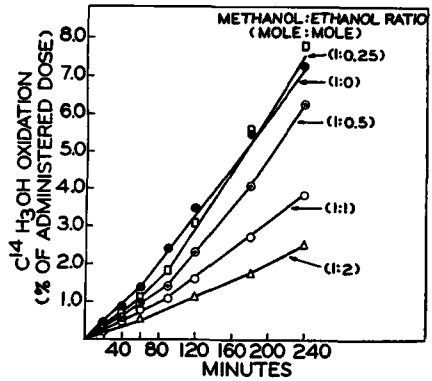

FIG. 4. The effect of ethanol on C^{14}-methanol oxidation in the rat *in vivo*.

C^{14}-methanol oxidation was measured by collecting pulmonary $C^{14}O_2$. C^{14}-methanol (31.2 mmol/kg) and various doses of ethanol were administered simultaneously by the intraperitoneal route. Each point represents the mean value of 3 rats.

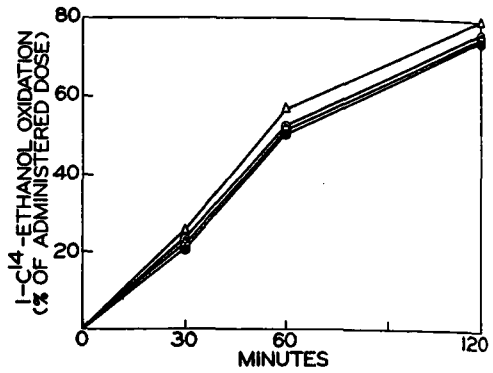

FIG. 5. The effect of methanol on 1-C^{14}-ethanol metabolism in the rat *in vivo*.

1-C^{14}-ethanol oxidation was measured by collecting pulmonary $C^{14}O_2$. ○, 1-C^{14}-ethanol alone. △, 1-C^{14}-ethanol–methanol, 1:1. ●, 1-C^{14}-ethanol–methanol, 1:4. ⊙, 1-C^{14}-ethanol–methanol, 1:8. Ethanol-1-C^{14} (3.12 mmol/kg) was injected intraperitoneally alone or simultaneously with various doses of methanol. Each point represents the average of 4 rats.

methanol oxidation at the 6-g/kg level was significantly less than that seen at the 3-g/kg level ($P < .001$). The observed sedation and respiratory depression could account for this decline in methanol oxidation.

Effects of ethanol on C^{14}-methanol oxidation and of methanol on C^{14}-ethanol oxidation in vivo. Ethanol and methanol have approximately equal K_m's with the isolated catalase peroxidative system (Chance, 1947; Tephly *et al.*, 1961a) whereas with the purified ADH system the K_m of ethanol (2×10^{-3} M: Theorell and Bonnichsen, 1951) is about 10- to 50-fold lower than the K_m of methanol (depending upon the pH) (Kini and Cooper, 1961). Therefore, an equimolar amount of ethanol should inhibit methanol oxidation by about 50% if the peroxidative system is the primary pathway and by more than 90% if the ADH system predominates.

Varying amounts of ethanol were injected with a constant dose of C^{14}-methanol (1 g/kg) and $C^{14}O_2$ was collected at intervals during 4-hour periods (fig. 4). Because of the rapid disappearance of ethanol, particularly at the lower levels of administration, the values obtained during the first 90 minutes are the most meaningful. It can be seen that the degree of inhibition of methanol oxidation increased as the molar ratios of methanol–ethanol were decreased from 1:0.25 to 1:2. When equimolar amounts of ethanol and C^{14}-methanol were given, a 55% reduction of $C^{14}O_2$ collection was observed at the 90-minute time interval. This is in good agreement with results obtained when ethanol was employed as an inhibitor of methanol metabolism in studies using liver slices (Bartlett, 1950b; Smith, 1961), liver homogenates, and the purified peroxidative system (Tephly *et al.*, 1961a).

Since the oxidation of methanol is inhibited about 55% by an equimolar dose of ethanol, if both alcohols follow a common major pathway for conversion to their respective aldehydes, one should expect an equimolar dose of methanol to inhibit the oxidation of C^{14}-ethanol by about 50%. However, methanol administration had no discernible effect on the rate of oxidation of ethanol even when the methanol–ethanol molar dose ratio was as high as 8:1 (fig. 5). The conclusion is inescapable that in the intact rat the major pathway for ethanol oxidation plays at best an insignificant role in the oxidation of methanol, and, conversely, that the principal step for the oxidation of methanol to formaldehyde is a minor one for the conversion of ethanol to acetaldehyde. Smith (1961) has shown that the oxidation of 1-C^{14}-ethanol by rat liver slices is not affected by the presence of methanol in equimolar concentration.

Effects of 1-butanol on the oxidation of C^{14}-methanol and 1-C^{14}-ethanol in vivo. With the purified ADH system butanol and ethanol display a similar maximum velocity (Theorell and Bonnichsen, 1951), but the affinity of butanol

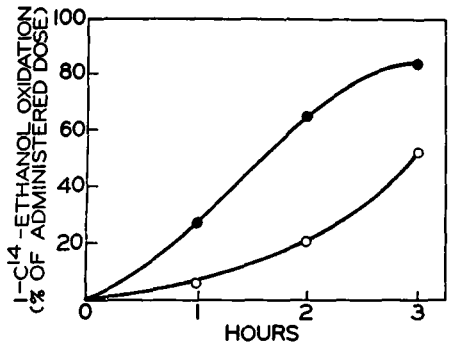

FIG. 6. The effect of 1-butanol on 1-C^{14}-ethanol metabolism in the rat *in vivo*.

1-C^{14}-ethanol oxidation was measured by collecting pulmonary $C^{14}O_2$. ●——●, 1-C^{14}-ethanol alone (15.6 mmol/kg). ○——○, 1-C^{14}-ethanol (15.6 mmol/kg) + 1-butanol (7.8 mmol/kg). Ethanol-1-C^{14} was injected intraperitoneally alone or simultaneously with 1-butanol. Each point represents the average of 4 rats.

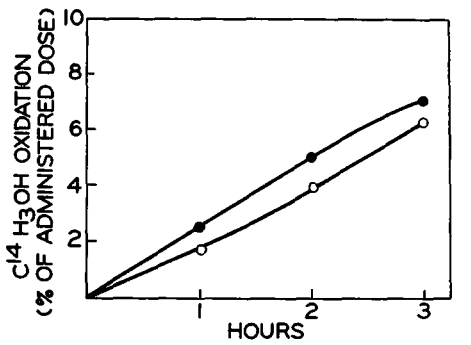

FIG. 7. The effect of 1-butanol on C^{14}-methanol metabolism in the rat *in vivo*.

C^{14}-methanol oxidation was measured by collecting pulmonary $C^{14}O_2$. ●——●, C^{14}-methanol alone (15.6 mmol/kg). ○——○, C^{14}-methanol (15.6 mmol/kg) + 1-butanol (7.8 mmol/kg). C^{14}-methanol was injected intraperitoneally alone or simultaneously with 1-butanol. Each point represents the average of 4 rats.

for ADH is about ten times that for ethanol (1-butanol: $K_m = 2.2 \times 10^{-4}$ M; ethanol: $K_m = 2 \times 10^{-3}$ M). On the other hand, 1-butanol is much less reactive with the peroxidative system than is methanol or ethanol (Chance, 1947). In keeping with the line of reasoning employed above one would predict that 1-butanol would be a potent inhibitor of ethanol oxidation *in vivo*, but a poor inhibitor of methanol metabolism. That this is actually the case is seen in figures 6 and 7. The slight inhibition of methanol oxidation by 1-butanol is expected because 1-butanol is known to be acted upon slowly by the catalase-peroxide system (Chance, 1947). Aebi *et al.* (1957) have reported a similar moderate inhibitory effect of 1-butanol on methanol oxidation in the guinea pig.

Effects of 3-amino-1,2,4-triazole (AT) inhibition of hepatic catalase on the oxidation of C^{14}-methanol and 1-C^{14}-ethanol in vivo. The rate of methanol metabolism in rats that had received the catalase inhibitor, AT, is illustrated in figure 8. A comparison of methanol oxidation by AT-treated (fig. 8) and nontreated (fig. 1) rats within the first 16 hours after methanol administration, during which time the rates were linear in both groups, shows that AT effects a decrease in oxidation of about 50%. Pulmonary and urinary plus fecal excretion of methanol were not affected. From the dose-oxidation rate curve as plotted by the Lineweaver-Burk method (fig. 9) an "apparent *in vivo* K_m" of 5.0×10^{-2} mol/l

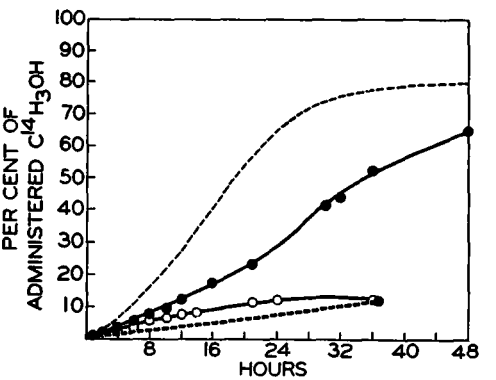

FIG. 8. The effect of 3-amino-1,2,4-triazole (AT) on the oxidation of C^{14}-methanol in the rat *in vivo*.

●——●, Accumulated pulmonary $C^{14}O_2$; ○——○, accumulated pulmonary methanol; ●----●, fecal and urinary excretion of methanol (determined at the 36-hour period only). ——, Accumulated $C^{14}O_2$ from nontreated rats (taken from fig. 1). Methanol-C^{14} was injected intraperitoneally at a dose level of 1 g/kg as a 10% (w/v) solution in saline (1.94×10^5 cpm/mg). AT (1 g/kg) was administered intraperitoneally 1 hour before methanol administration. Each point represents the average of 4 rats.

was obtained. The K_m of methanol for the isolated ADH system is in this concentration range (Kini and Cooper, 1961). Therefore, the possibility must be considered that the ADH system could account for the methanol oxidation that continues after the hepatic catalase peroxidative system has been inhibited with AT.

Aebi *et al.* (1957) noted a reduction in methanol

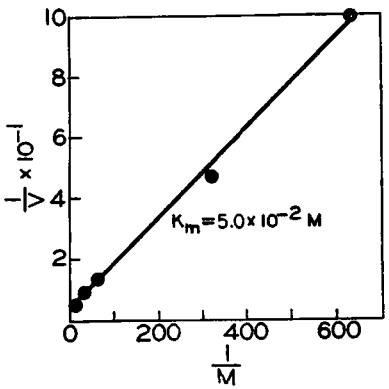

FIG. 9. The effect of AT on the "apparent *in vivo* K_m" for C^{14}-methanol oxidation in the rat.

v = the rate of C^{14}-methanol oxidation to $C^{14}O_2$ recorded as mg of $C^{14}H_3OH$ oxidized/kg of body weight/hour. Rates were determined over the first 4 hours following intraperitoneal injection of C^{14}-methanol during which time $C^{14}O_2$ formation was linear. M = concentration of C^{14}-methanol in mol/l of body water. V_{max} = 25 mg of $C^{14}H_3OH$ oxidized/kg of body weight/hour. AT (1 g/kg) was injected intraperitoneally 1 hour prior to C^{14}-methanol administration. Each point represents the average of 3 rats. Injected doses of methanol varied from 0.05 to 3.0 g/kg of body weight and metabolic rates ranged from 0.99 to 18.7 mg/kg/hr. The K_m value was determined using least squares regression line analysis (Dixon and Massey, 1957).

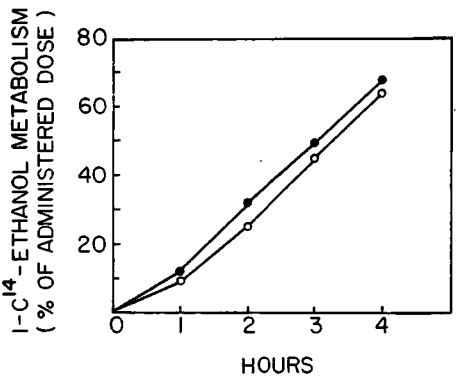

FIG. 10. The effect of AT on the oxidation of 1-C^{14}-ethanol in the rat *in vivo*.

1-C^{14}-ethanol oxidation was measured by collecting pulmonary $C^{14}O_2$. ○——○, C^{14}-ethanol only (1 g/kg); ●——●, C^{14}-ethanol (1 g/kg) 1 hour after AT administration (1 g/kg). AT and ethanol were administered intraperitoneally. Each point represents the average of 2 rats.

metabolism in rats and guinea pigs after single injections of AT. They also administered isopropylallylacetureide, a known inhibitor of hepatic catalase synthesis, for 4 days to rats and observed a reduction in the rate of methanol oxidation. The hepatic catalase activity was reduced to about 30% of normal in these animals.

In keeping with expectations, AT had no effect on ethanol oxidation (fig. 10). Nelson *et al.* (1957) had previously shown that ethanol disappearance from the blood of dogs was not affected by AT administration.

DISCUSSION. These studies show that in the intact rat the catalase-peroxide system plays a major role in the oxidation of methanol and that this system is not primarily responsible for the oxidation of ethanol. However, it must be emphasized that this conclusion applies only to the whole rat and may not necessarily hold for other species or for individual tissues. Indeed the high toxicity of methanol for primates and the selective damage to the human retina indicate that marked species differences in methanol metabolism do exist.

The conclusions drawn from this study are valid only if the first step in the overall oxidation of methanol to carbon dioxide is rate limiting. In the series of reactions leading to the formation of CO_2 from methanol AT would necessarily inhibit at the rate limiting step. In a study that will be reported later in detail, AT (1 g/kg) had no effect on C^{14}-formate oxidation to $C^{14}O_2$ over a wide dose range (10 to 500 mg/kg). It follows that either the oxidation of methanol to formaldehyde or of formaldehyde to formate must be rate limiting. Because formaldehyde does not accumulate *in vivo*, its oxidation is not thought to be the rate limiting step.

In previous reports from this laboratory (Mannering and Parks, 1957; Tephly *et al.*, 1961a) some doubt was cast upon the *in vivo* role of the catalase peroxidative mechanism because of the failure of AT to effect a measurable decrease in the rate of methanol disappearance from the blood of rats. The present studies employing labeled methanol make possible a more precise interpretation of the earlier work. It is apparent from figure 3 that any effect of AT, tungstate or ethanol on the overall rate of blood methanol disappearance was obscured by the large percentage of methanol that was eliminated by the pulmonary and renal routes. With the methanol dose of 3 g/kg used in the earlier work, methanol disappeared at a rate of about 600 μmol/hr in 200-g rats (Tephly *et al.*, 1961a). The above studies indicate that only 200 μmol/rat/hr could have been oxidized to CO_2 and that about two-thirds of the total methanol

disappearance occurred by renal and pulmonary elimination. A further discrepancy in the prior reports remains to be explained. Unsupplemented rat liver homogenates oxidize methanol to formaldehyde at a rate of about 6 μmol/g of wet weight of liver/hr (Mannering and Parks, 1957; Tephly *et al.*, 1961a). Since the liver of a 200-g rat weighs about 10 g, this rate can account for about 60 μmol of methanol oxidized/hr, far short of the 200 μmol/hr demonstrated in the whole animal. In a recent study conducted in this laboratory methanol oxidation by rat liver homogenates was increased from 6 to 26 μmol/g/hr by the addition of a hydrogen peroxide generating system (purified glucose oxidase and glucose). It can be expected that the catalase peroxidative mechanism functions more efficiently in the intact liver than in the unsupplemented homogenate, where, in the latter case, the peroxide generating enzymes and their substrates may be diluted considerably. Therefore it is possible that in the intact rat weighing 200 g the hepatic catalase peroxidative system functions at a rate with an upper limit of 260 μmol/hr. This is adequate to account for the rate of oxidation observed in the intact rat.

The methanol oxidizing capacity of intact rats that remains after AT treatment is characterized by a 7-fold higher "apparent *in vivo* K_m" value than seen with untreated rats. This suggests the existence of a secondary metabolic pathway for methanol oxidation. Thus, the role of ADH in the oxidation of methanol has not been entirely eliminated by this study. The possibility must not be ignored that rat liver may contain an alcohol dehydrogenase(s) that differs from the horse and monkey liver enzymes in that it reacts equally well with both ethanol and methanol. A second alcohol dehydrogenase has been identified recently in horse liver (Treble, 1962). The prominant role assigned to ADH in the metabolism of methanol in the monkey by Kini and Cooper (1961) resulted from an unfortunate misinterpretation of data (Mannering *et al.*, 1962). Whether or not the catalase peroxidative mechanism functions in the oxidation of methanol in the monkey remains to be determined. It should be noted that erythrocytes have a high content of catalase and that this enzyme is not affected by AT as employed in these studies. Earlier this laboratory reported (Tephly *et al.*, 1961b; Parks *et al.*, 1961) that erythrocytes which normally do not react with alcohols can be induced to oxidize methanol to formaldehyde at significant rates by various measures that cause the production of hydrogen peroxide, *e.g.*, incubation with ascorbic acid or methylene blue and glucose. It is conceivable that after the hepatic and renal catalase systems become inactivated, hydrogen peroxide diffuses into the erythrocytes and induces the oxidation of methanol by these cells. Experiments are planned to explore this possibility.

Bartlett (1952), in a critical analysis of information available at the time, concluded that the role of catalase in coupled peroxidations, including any that might involve methanol, was highly unlikely. Several of his arguments against the catalase peroxidative mechanism, which still summarize much of the current thinking of those who oppose the concept, should be reviewed in light of current findings: (1) It was argued that because catalase·H_2O_2 oxidizes methanol and ethanol at the same rate *in vitro*, ethanol should be oxidized at the same rate as methanol if the peroxidative system is implicated, not 7 times more rapidly as is the case. This argument ignores the possibility that ethanol may be oxidized by two different pathways, one proceeding relatively rapidly (as with the ADH system) and the other quite slowly (as with the peroxidative system), while methanol may be oxidized slowly by one or both pathways. (2) Bartlett contended that because ethanol inhibits the oxidation of methanol in a competitive manner, a mutual site of oxidation of both alcohols is involved. Also, since ethanol is already known to be oxidized through ADH, it was argued that methanol must be oxidized through ADH. Again, there is a failure to recognize the existence of two pathways, one of which acts preferentially on ethanol. (3) Based on the observed linearity of the oxidation rates of both methanol and ethanol *in vivo* over the wide range of alcohol concentrations that obtain in the animal as the alcohols are metabolized (Bartlett, 1950a), it was argued that, being independent of substrate concentration, the oxidation rate of methanol *in vivo* fits the kinetics for ethanol metabolism by ADH as derived from *in vitro* studies rather than the first order kinetics established for methanol oxidation by Chance (1947) in his studies with the isolated peroxidative system. Actually, the oxidation of methanol *in vivo* obeys first order kinetics when doses of methanol lower than 1 g/kg are employed (fig. 2).

SUMMARY

Rats received labeled methanol and ethanol, and $C^{14}O_2$ was collected as a measure of the rates of oxidation of these alcohols under a variety of conditions. It was concluded from these studies that the peroxidative system involving hepatic catalase plays a major role in the oxidation of methanol in the rat and that this system is not primarily responsible for the oxidation of ethanol. These conclusions were based on the following observations:

1. The upper limit of the "apparent *in vivo* Michaelis constant (K_m)" for C^{14}-methanol oxidation was found to be 7.0×10^{-3} mol/l of body water, a lower value than would have been expected if alcohol dehydrogenase were functioning as the major catalyst for methanol oxidation.

2. Ethanol and methanol are known to be equally reactive in the isolated peroxidative system. When equimolar amounts of ethanol and C^{14}-methanol were given to the rat, the oxidation of methanol was reduced about 50%.

3. Hepatic alcohol dehydrogenase (ADH) is known to react readily with ethanol, but poorly with methanol. Methanol was found to have no effect on the rate of oxidation of 1-C^{14}-ethanol *in vivo*, even when the methanol–ethanol molar dose ratio was as high as 8:1.

4. The reactivity of the isolated peroxidative system with 1-butanol is known to be relatively poor. 1-Butanol was found to be a very weak inhibitor of C^{14}-methanol oxidation *in vivo*.

5. The affinity of butanol for the isolated ADH system is known to be greater than that of ethanol ($K_m = 2 \times 10^{-3}$ M; 1-butanol; $K_m = 2.2 \times 10^{-4}$ M). 1-Butanol was found to be a potent inhibitor of 1-C^{14}-ethanol oxidation *in vivo*.

6. The rate of methanol metabolism was reduced about 50% in rats which had received the potent hepatic catalase inhibitor, 3-amino-1,2,4-triazole (AT). AT effected a shift in the "apparent *in vivo* K_m" for methanol oxidation from 7.0×10^{-3} mol/l to 5.0×10^{-2} mol/l, which may indicate a secondary metabolic pathway for methanol oxidation.

7. AT had no effect on 1-C^{14}-ethanol oxidation.

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