ROLE OF THE INTRACELLULAR DISTRIBUTION OF HEPATIC CATALASE IN THE PEROXIDATIVE OXIDATION OF METHANOL

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Previous to 1950, when Bonnichsen[1] isolated crystalline alcohol dehydrogenase (ADH) and showed that it did not react with methanol,‡ it was believed that the ADH system was responsible for the oxidation of all primary aliphatic alcohols, including methanol (FIGURE 1). This observation redirected attention to the peroxidative system as a means of oxidizing methanol. As early as 1936, Keilin and Hartree[3] showed that catalase catalyzed the oxidation of alcohols to their aldehydes when hydrogen peroxide was supplied in low concentrations, as might be provided in living cells through the action of flavin and other peroxide-generating enzymes. They presented arguments to support their view that catalase is not present in the tissues to protect against peroxide intoxication, as was widely contended, but rather to carry out coupled (peroxidative) oxidations. Employing techniques that permitted very rapid spectral determinations, Chance[4] identified the intermediate complexes and analyzed the kinetics of the components of the reaction (FIGURE 1).

Ideas were exchanged for several years as to whether or not the peroxidative system participated in the *in vivo* oxidation of methanol and other alcohols. A direct means of examining the question was provided when Heim and coworkers[5] showed that the intraperitoneal injection of 3-amino-1,2,4-triazole (AT) caused a reduction in hepatic and renal catalase activities of 90% or more. Mannering and Parks[6] 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. While these *in vitro* studies pointed to a role of catalase in methanol oxidation, they did little to establish its participation *in vivo*. Because of the complexity of the system, involving as it does the rate of formation of hydrogen peroxide, which in turn depends upon the concentration of substrates available to the peroxide-generating enzymes as well as the availability of hepatic catalase to the hydrogen peroxide produced by these enzymes, it seemed unlikely that *in vitro* studies would provide much information as to what was occurring in the intact animal. This presentation is devoted largely to a review of the evidence that shows that the intact rat oxidizes methanol largely through the peroxidative system, but that ethanol oxidation proceeds differently, probably almost entirely via the ADH system. The evidence is based on *in vitro* studies that employed several approaches: 1) ^{14}C-methanol and 1-^{14}C-ethanol oxidation were studied

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‡ More recently, Kini and Cooper[2] showed methanol oxidation to occur with crystalline horse liver ADH when high concentrations of methanol were employed.

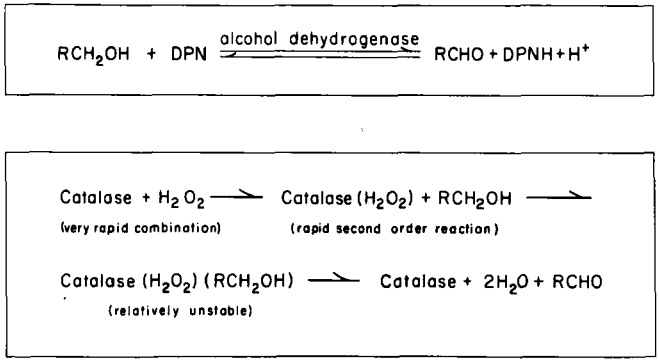

FIGURE 1. Alcohol dehydrogenase (ADH) and peroxidative systems for the oxidation of alcohols.

in rats treated with AT; 2) the relative abilities of ethanol and 1-butanol to inhibit ^{14}C-methanol and of methanol and 1-butanol to inhibit 1-^{14}C-ethanol oxidation were compared with the known reactivities of these three alcohols with the peroxidative and ADH systems *in vitro*; 3) ethylene glycol, which stimulates the production of hydrogen peroxide, was employed in an attempt to increase the rate of methanol oxidation in the rat; 4) pyrazole, a potent inhibitor of ADH, was studied for what effect it might have on the rates of ethanol and methanol oxidation in the rat.

Using approaches similar to those employed with the rat, it was shown that the monkey differs from the rat in that the peroxidative system does not play an important role in the oxidation of methanol. Both methanol and ethanol appear to be oxidized by the ADH system in this species. Because the monkey has large amounts of catalase in its liver, it became necessary to explain why catalase was not functioning peroxidatively. Studies of hepatic catalase activities in the rat, mouse, guinea pig, and monkey showed that not only did the total catalase activity vary greatly from species to species, but the distribution of catalase between soluble and particulate fractions of liver homogenates also varied widely. A direct correlation was shown between the *in vivo* oxidation of methanol and particulate catalase activity in the rat, mouse, and guinea pig; no such correlation was seen in the monkey or with soluble catalase activity in any of the species. The failure of catalase to function peroxidatively in the oxidation of methanol in the monkey is explained in several ways: (a) catalase is distributed in the hepatic cell so that little is accessible to peroxide-generating systems; (b) monkey hepatic catalase is less active peroxidatively than rodent hepatic catalase; (c) there is less peroxide-generating activity in the peroxisomes from monkey liver than in peroxisomes from rat liver.

Effect of 3-Amino-1,2,4-Triazole (AT) on the In Vivo *Oxidation of Methanol*

AT is a potent inhibitor of hepatic and renal catalase *in vivo*.[5] It is an *in vivo* inhibitor of catalase only when H_2O_2 is present, which suggests that AT inhibits by combining with the catalase-H_2O_2 complex (FIGURE 1) rather than with catalase itself.[7] FIGURE 2 shows the effect of AT on ^{14}C-methanol oxidation in the rat as determined by the amount of pulmonary $^{14}CO_2$ collected after the intraperitoneal injection of a saturating dose of the alcohol. It is seen that during

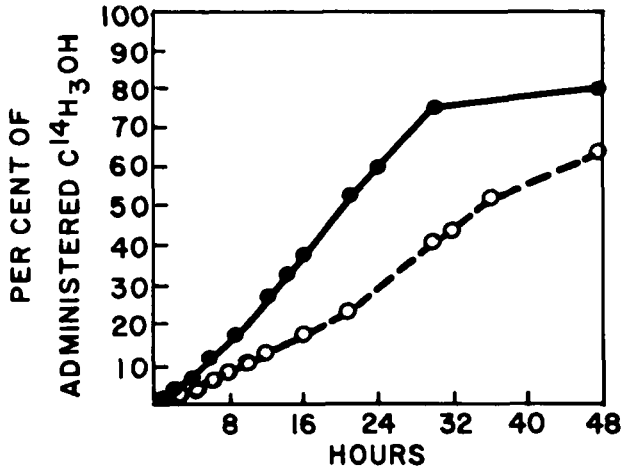

FIGURE 2. Effect of 3-amino-1,2,4-triazole (AT) on the rate of *in vivo* oxidation of ^{14}C-methanol in the rat. From data of Tephly, Parks & Mannering.[8] ●——●, without AT; ○- - -○, with AT. Dose of ^{14}C-methanol: 1 g/kg, I.P., one hour before the administration of ^{14}C-methanol. Rate of ^{14}C-methanol oxidation was based on the rate of pulmonary $^{14}CO_2$ collection. Each point represents the average of four rats.

the first 20 hours methanol oxidation is reduced by about 50%. AT has no effect on the rate of ethanol oxidation in the rat.[8]

FIGURE 3 illustrates an experiment that may be unique in that an enzyme is progressively "titrated" from the *in situ* liver using an inhibitor, and the *in vivo* metabolism of a substrate metabolized by that enzyme is measured concomitantly. In this study rats were injected with various amounts of AT and a saturating dose of ^{14}C-methanol, then were placed in metabolism chambers and their pulmonary $^{14}CO_2$ was collected as a measure of the rate of methanol oxidation. After four hours the rats were killed and the catalase activities of their livers were determined. It was observed that the rate of methanol oxidation was not altered with declining hepatic catalase activities until the catalase activity reached a level of about 400 Kat_f units/g of liver, after which it declined precipitously. This is interpreted to mean that at levels of catalase activity higher than 400 Kat_f units/g of liver, the steady state among peroxide-generating systems, hydrogen peroxide, catalase-hydrogen peroxide complex, and methanol functions maximally for whatever amount of H_2O_2 is being produced and, assuming that AT does not change the rate of peroxide generation, new steady states are established with each declining level of catalase activity below 400 Kat_f units.

In FIGURE 4 the effects of AT on methanol oxidation are compared in the rat, guinea pig, mouse, and rhesus monkey. Methanol oxidation is effected by AT similarly in all three rodents, but AT has no effect on methanol oxidation in the monkey. This observation suggested the possibility that hepatic catalase is not inhibited by AT in the monkey as it is in the rat. This proved not to be the case.[10] In accordance with expectations, AT had no effect on the rate of ethanol oxidation in the monkey.[10]

The effect of AT on methanol oxidation by the isolated, perfused liver of the rat is similar to that seen in the intact animal.[12]

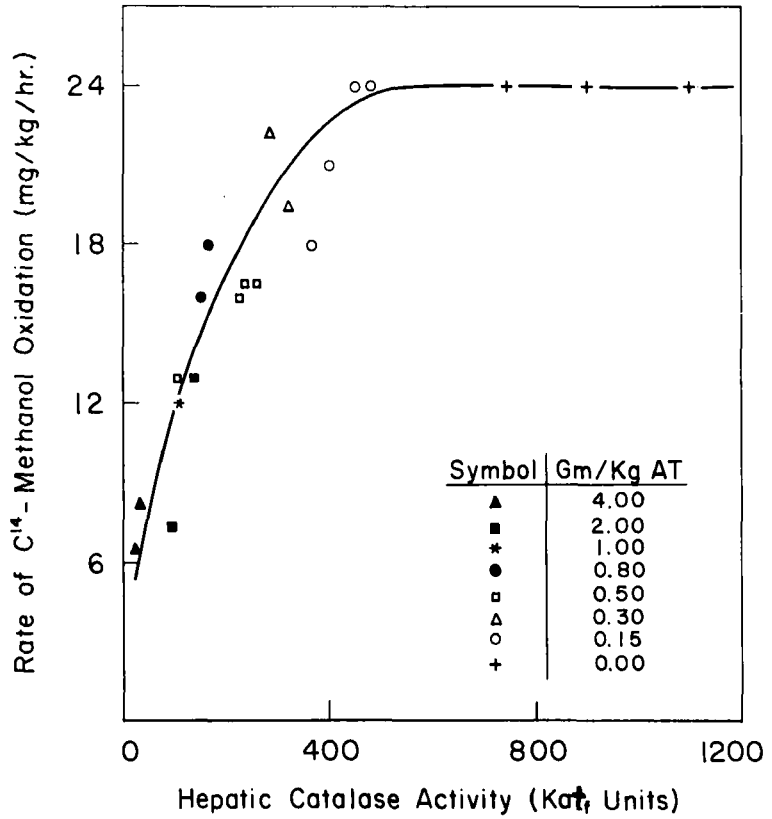

FIGURE 3. Effect of various doses of 3-amino-1,2,4-triazole (AT) on the rate of *in vivo* oxidation of ^{14}C-methanol and on the hepatic catalase activity in the rat. From data of Van Harken.[9] Dose of ^{14}C-methanol: 1 g/kg, I.P. AT was given (I.P.) one hour before the administration of ^{14}C-methanol. Rates of ^{14}C-methanol oxidation ($^{14}CO_2$ formation) were determined at hourly intervals for four hours after which the rats were killed and their hepatic catalase activities determined.

Effects of Ethanol and 1-Butanol on the In Vivo *Oxidation of ^{14}C-Methanol*

Ethanol and methanol are about equally reactive with the isolated catalase peroxidative system,[4] whereas with the purified horse ADH system, the K_m of ethanol, 2mM,[13] is about ten- to 50-fold lower (depending upon the pH at which the reaction is conducted) than the K_m of methanol for monkey ADH.[2] If horse, monkey, and rat ADH possess similar reactivities with methanol and ethanol, then in the studies to be reported, an equimolar amount of administered ethanol should inhibit *in vivo* methanol oxidation by about 50% if the peroxidative system is the primary oxidative pathway involved, and by more than 90% if the ADH system predominates. The reactivity of 1-butanol (K_m = 0.22 mM) is greater than that of ethanol (K_m = 2 mM) for the ADH system,[13] but much less reactive with the peroxidative system than ethanol or methanol.[4] Thus, if methanol is oxidized peroxidatively, 1-butanol should have little effect on its rate of oxidation,

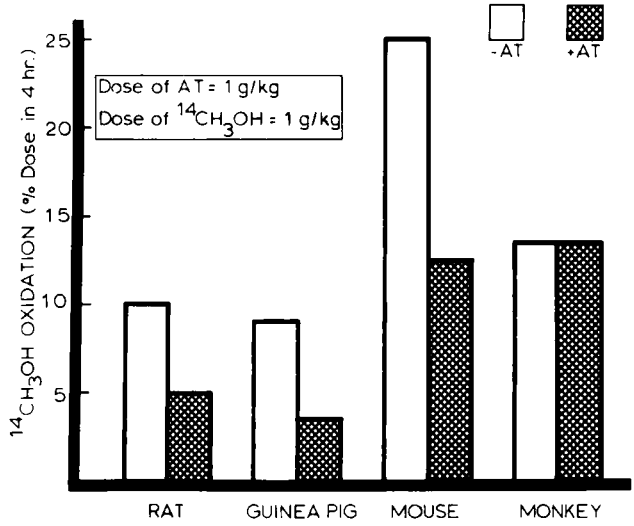

FIGURE 4. Effect of 3-amino-1,2,4-triazole (AT) on the *in vivo* rate of ^{14}C-methanol oxidation in the rat, guinea pig, mouse, and monkey. From data of Tephly, Parks & Mannering,[8] Makar, Tephly and Mannering,[10] and Makar and Mannering.[11] AT was given (I.P.) one hour before the administration of ^{14}C-methanol. Rates of ^{14}C-methanol oxidation ($^{14}CO_2$ formation) were determined at hourly intervals for four hours.

but a profound depression of methanol oxidation should occur if the oxidation of methanol is mediated through the ADH system.

In FIGURE 5 it is seen that in the rat ethanol inhibits methanol oxidation by about 50%, but in the monkey inhibition is about 80%. 1-Butanol has only a slight effect on methanol oxidation in the rat, but a large effect in the monkey. The results obtained with 1-butanol cannot be compared directly with the results obtained with ethanol because of the smaller molar equivalent of 1-butanol employed with respect to methanol. This was necessary because of the toxicity of 1-butanol. However, it should be noted that the difference in the degrees of inhibition caused by 1-butanol in the rat and monkey is marked.

The studies employing competitive substrates support the view that, in the rat, methanol is oxidized largely by the peroxidative system and ethanol by the ADH system, whereas in the monkey, both alcohols are oxidized by the ADH system.

Effects of Methanol and 1-Butanol on the In Vivo *Oxidation of 1-^{14}C-Ethanol*

Employing the same reasoning given in the preceding section, methanol should inhibit ethanol oxidation by about 50% if ethanol is oxidized by the peroxidative system but should have negligible inhibitory effect if ethanol is oxidized primarily via the peroxidative system. 1-Butanol would be expected to have a profound inhibitory effect on ethanol metabolism if ethanol is oxidized by the ADH system, but it should have only a small inhibitory effect if ethanol is oxidized peroxidatively. In FIGURE 6 it is seen that methanol had no effect on the oxidation of ethanol when the molar ratio of methanol to ethanol was 8:1. Results were quite similar in the monkey when the molar ratio of methanol to ethanol was 4:1. The inhibitory effect of 1-butanol on ethanol oxidation was marked in both

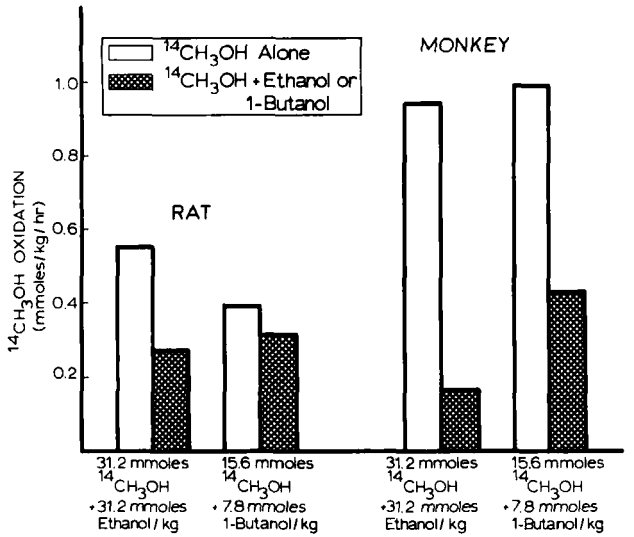

FIGURE 5. Effects of ethanol and 1-butanol on the *in vivo* oxidation of ^{14}C-methanol by the rat and monkey. From data of Tephly, Parks & Mannering[8] and Makar, Tephly and Mannering.[10] Rate of ^{14}C-methanol oxidation was based on rate of pulmonary $^{14}CO_2$ collection.

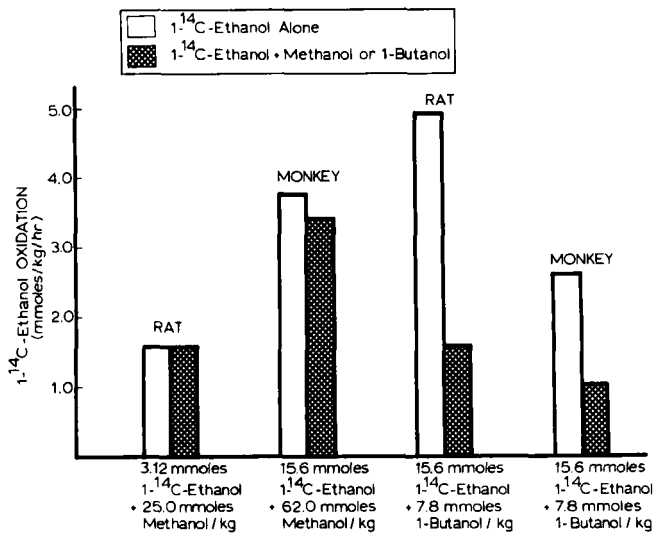

FIGURE 6. Effects of methanol and 1-butanol on the *in vivo* oxidation of 1-^{14}C-ethanol by the rat and monkey. From data of Tephly, Parks & Mannering[8] and Makar, Tephly & Mannering.[10] Rate of 1-^{14}C-ethanol oxidation was based on rate of pulmonary $^{14}CO_2$ collection. The low rate of 1-^{14}C-ethanol oxidation seen in the methanol study is due to the low dose of 1-^{14}C-ethanol employed (3.12 mmoles).

the rat and monkey. The results support the view that ethanol is oxidized primarily by the ADH system in both the rat and the monkey.

The studies employing the three alcohols were repeated using the isolated, perfused liver of the rat.[12] Results were very similar to those obtained with the intact animal.

Stimulation of the In Vivo *Rate of ^{14}C-Methanol Oxidation with Ethylene Glycol*

The observation of von Wartburg[14] that ethylene glycol reacts with the ADH system suggested that it might be used as a substrate inhibitor of ethanol oxidation without interfering with methanol oxidation. From what had previously been learned about the difference in the way methanol is metabolized in the rat and monkey, it seemed probable that ethylene glycol would inhibit methanol metabolism in the monkey, but not in the rat. The effect of ethylene glycol on ethanol oxidation in the monkey proved to be negligible or at least equivocal, but contrary to expectations, ethylene glycol greatly stimulated the rate of methanol oxidation in the rat.[9] The probable reason for the stimulatory effect of ethylene glycol became apparent after reviewing what was known about ethylene glycol metabolism (FIGURE 7). Glycolic acid, a metabolite of ethylene glycol, and molecular oxygen react through the action of the flavin enzyme, glycolic acid oxidase, to form glyoxylic acid and hydrogen peroxide.[16,17] Because it is the catalase · H_2O_2 complex rather than catalase itself that reacts with methanol,[4] and the amount of catalase in the rat appears to be more than sufficient for maximum utilization of the H_2O_2 produced under normal conditions, any increase in the production of H_2O_2, such as that promoted by ethylene glycol, would be expected to increase methanol oxidation. In the case of ethanol oxidation, the picture would be more complex because the peroxidative oxidation of ethanol would be increased while oxidation via the ADH system would be decreased due to the action of ethylene glycol as a substrate inhibitor.

In FIGURE 8 it is seen that, when administered in a dose equimolar to that of methanol, the oxidation of methanol is approximately doubled. The metabolites of ethylene glycol, glycoaldehyde and glycolic acid, are also seen to exert a stimulatory effect on methanol oxidation (FIGURES 9 and 10).

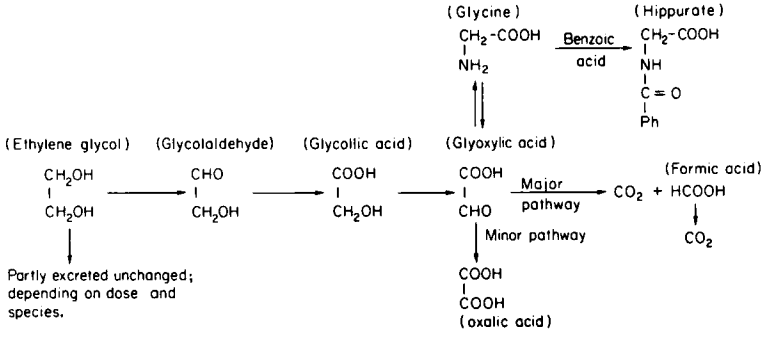

FIGURE 7. Ethylene glycol metabolism. Scheme proposed by Gessner, Parke & Williams.[15]

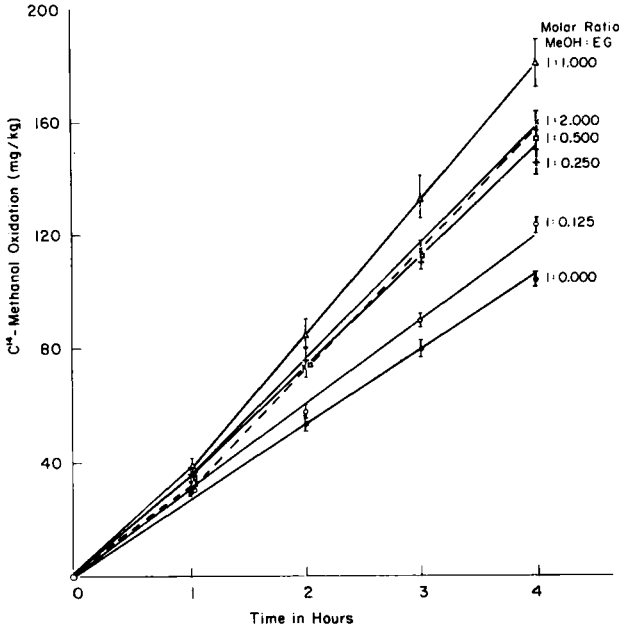

FIGURE 8. Effect of ethylene glycol on the *in vivo* rate of ^{14}C-methanol oxidation in the rat. From data of Van Harken.[9] 1 g of ^{14}C-methanol/kg was injected simultaneously (I.P.) with the various doses of ethylene glycol. Rate of ^{14}C-methanol oxidation was based on rate of pulmonary $^{14}CO_2$ collection. Each point represents the mean value of four rats.

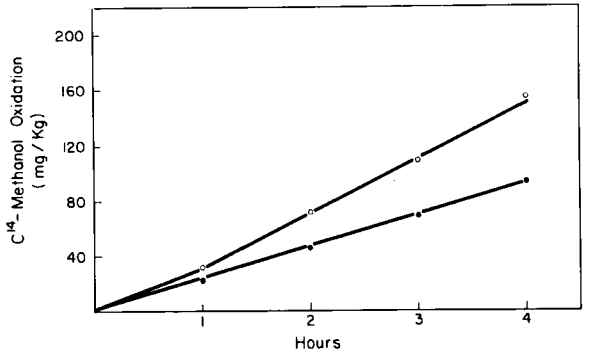

FIGURE 9. Effect of glycoaldehyde on the *in vivo* rate of ^{14}C-methanol oxidation in the rat. Data from Van Harken.[9] 1 g of ^{14}C-methanol and 0.25 g of glycoaldehyde/kg were injected simultaneously (I.P.). Rate of ^{14}C-methanol oxidation was based on rate of pulmonary $^{14}CO_2$ collection. ●——●, ^{14}C-methanol alone; ○——○, ^{14}C-methanol plus glycoaldehyde. Each point represents the mean value of four rats.

Ethylene glycol, glycoaldehyde and glycolic acid also stimulate methanol oxidation in the isolated, perfused liver.[9]

Effect of Pyrazole on the In Vivo *Oxidation of 1-^{14}C-Ethanol*

Pyrazole is a potent inhibitor of ADH both *in vivo*[18] and *in vitro*.[19] Since it has no effect on the peroxidative action of catalase *in vitro* and does not inhibit catalase *in vivo*,[20] this compound was useful in providing additional evidence for the role or lack of role of the peroxidative mechanisms in the oxidation of methanol in the rat and monkey.

The dramatic inhibition of 1-^{14}C-ethanol oxidation in the rat (FIGURE 11) illustrates the importance of ADH in the oxidation of ethanol in this species. The effect of pyrazole on methanol oxidation is shown in FIGURE 12. The oxidation of ^{14}C-methanol is seen to be inhibited by about 40%. It would appear that while the peroxidative mechanism is important in the oxidation of methanol in the rat, the ADH system also plays a significant part in the metabolism of this alcohol. This would explain why AT inhibited methanol oxidation by only 50% (FIGURE 2). In the monkey, pyrazole caused about as great an inhibition of methanol as it did of ethanol in the rat (FIGURE 13), which again supports the view that methanol oxidation proceeds almost entirely by way of the ADH system in the monkey.

Role of the Intracellular Distribution of Hepatic Catalase in the Peroxidative Oxidation of Methanol

The catalase activity of monkey liver was found to be about 4,000 Kat_f units/g of tissue, which is about four times that found in rat liver.[10] With a liver size relative to total body weight of about half that of the rat, the monkey possesses about twice as much hepatic catalase activity as the rat on a per-kilogram of body weight basis. Even thought the main pathway for methanol oxidation in the monkey appears to proceed via the ADH system, one might have expected to have seen some effect of AT on the rate of methanol oxidation in this species. AT reduced the oxidation of methanol in the rat 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 was asked why the hepatic catalase in

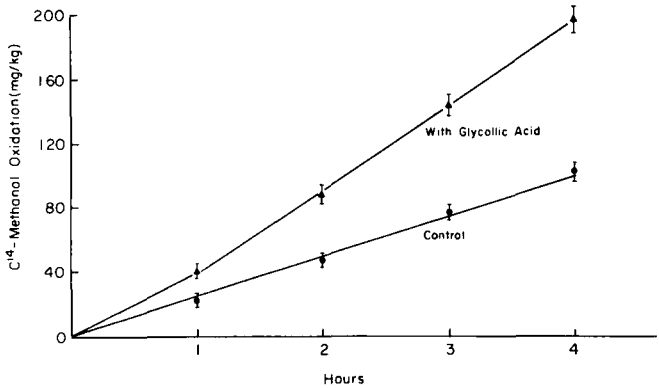

FIGURE 10. Effect of glycolic acid on the *in vivo* rate of ^{14}C-methanol oxidation in the rat. Data from Van Harken.[9] 1 g of ^{14}C-methanol and 0.2 g of glycolic acid/kg were injected simultaneously (I.P.). Rate of ^{14}C-methanol oxidation was based on rate of pulmonary $^{14}CO_2$ collection. Each point represents the mean value of four rats.

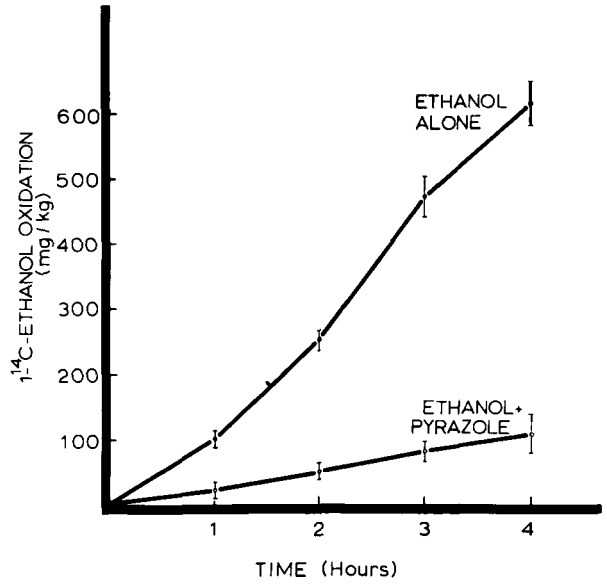

FIGURE 11. Effect of pyrazole on the *in vivo* rate of 1-^{14}C-ethanol oxidation in the rat. Data from Watkins, Goodman & Tephly.[20] 1 g. of 1-^{14}C-ethanol was injected/kg (I.P.). Pyrazole (200 mg/kg) was injected (I.P.) 15 minutes prior to 1-^{14}C-ethanol. Rate of 1-^{14}C-ethanol oxidation was based on pulmonary $^{14}CO_2$ collection. Each point represents the mean value of four rats ± S.E.

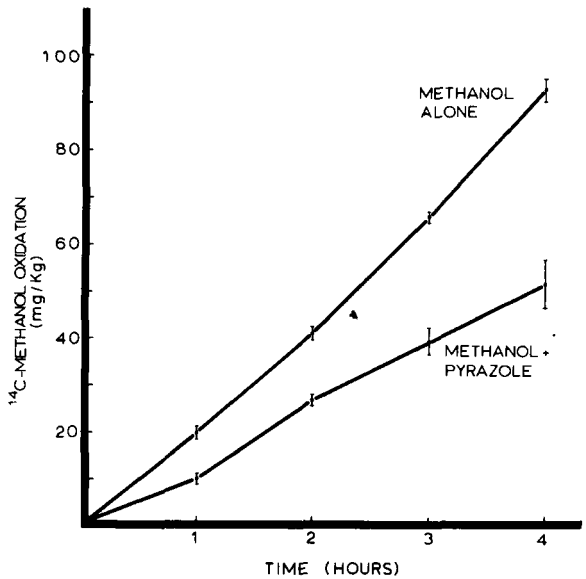

FIGURE 12. Effect of pyrazole on the *in vivo* rate of ^{14}C-methanol oxidation in the rat. Data from Watkins, Goodman & Tephly.[20] 1 g of 1-^{14}C-ethanol was injected/kg (I.P.). Pyrazole (200 mg/kg) was injected (I.P.) 15 minutes prior to ^{14}C-methanol. Rate of ^{14}C-methanol oxidation was based on pulmonary $^{14}CO_2$ collection. Each point represents the mean value of four rats ± S.E.

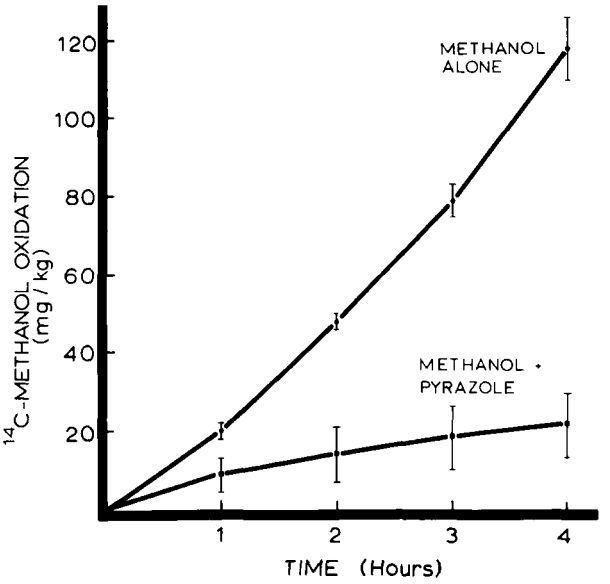

FIGURE 13. Effect of pyrazole on the *in vivo* rate of ^{14}C-methanol oxidation in the monkey. Data from Watkins, Goodman & Tephly.[20] 1 g of ^{14}C-methanol was injected/kg (I.P.). Pyrazole (200 mg/kg) was injected (I.P.) 15 minutes prior to ^{14}C-methanol. Rate of ^{14}C-methanol oxidation was based on rate of pulmonary $^{14}CO_2$ collection. Each point represents the mean of three animals ± S.E.

the monkey is not used for the peroxidative oxidation of methanol. Three possibilities were considered: (a) the distribution of catalase in the hepatic cell of the monkey may be such that it does not have intimate access to the peroxide-generating systems; (b) the hepatic catalases of the rat and monkey may differ such that the peroxidative activity of monkey catalase is less with respect to its catalatic activity than is the case for rat liver catalase; and (c) there may be lesser amounts of the hepatic peroxide-generating systems in the monkey than in the rat.

Role of the Distribution of Catalase Between Soluble and Particulate Fractions of the Hepatic Cell on the In Vivo *Oxidation of ^{14}C-Methanol by the Mouse, Rat, Guinea Pig and Monkey*

The idea that the distribution of catalase within the cell might have some bearing on the problem stemmed from the finding of de Duve and associates[21,22] that the peroxide-generating enzymes uricase and D-amino acid oxidase are found together in the peroxisomes. In seemed quite possible that catalase might require an intimate morphological association with peroxide-generating enzymes for it to function peroxidatively. Any relationship between the catalase activity of the hepatic cell and the *in vivo* oxidation of methanol would then relate to the catalase present in the cell particles, not to the total catalase present in the cell.

The distribution of catalase between soluble and particulate fractions from the livers of the four species is shown in FIGURE 14. The distributions seen in the rodents are very similar to those reported by Feinstein and associates.[25]

In FIGURE 15 comparisons are made between the *in vivo* rates of ^{14}C-methanol oxidation in four species and the catalatic and peroxidatic activities of soluble and

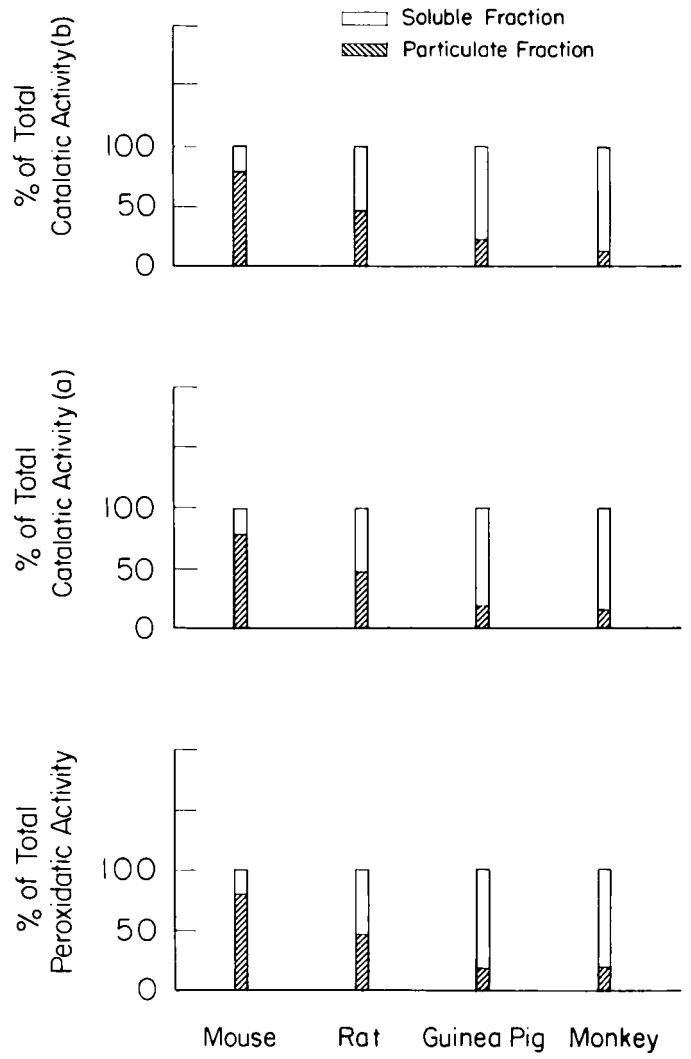

FIGURE 14. Distribution of catalatic and peroxidatic activity between the particulate and soluble fractions of liver homogenates from different species. Data from Makar and Mannering.[11] Particulate and soluble fractions were obtained by centrifuging homogenates at 20,000 × g for 20 min. The particulate catalase was solubilized with Triton X-100. Catalatic activity (a) is expressed in Adams units (23)/g of liver; catalatic units (b) is expressed in Kat_f units (24)/g of liver. Peroxidatic activity is expressed in micromoles of methanol oxidized (HCHO formation)/g of liver tissue/hr.

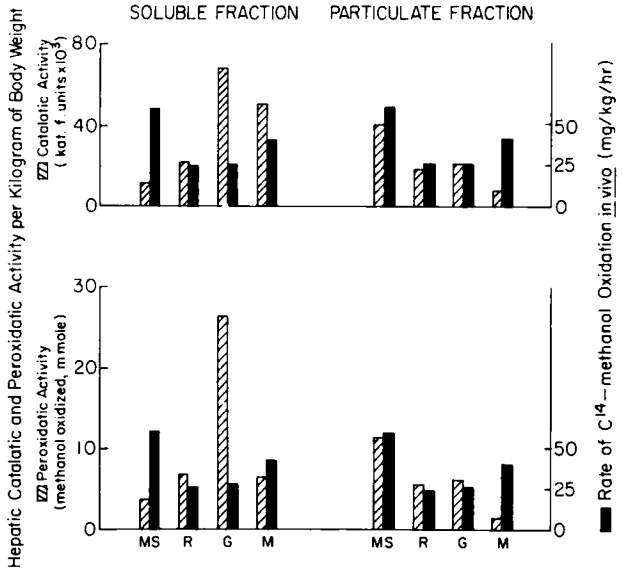

FIGURE 15. Relationship between the *in vivo* rate of ^{14}C-methanol oxidation and the catalatic and peroxidatic activities of liver fractions. Data from Makar and Mannering.[11] Animals were injected (I.P.) with ^{14}C-methanol (1 g/kg) and the rate of ^{14}C-methanol oxidation was calculated from the collection of $^{14}CO_2$ over a four hour period. Immediately after completion of the *in vivo* experiments, livers were removed and homogenized. The units of catalatic and peroxidatic activity are given in the legend to FIGURE 14. Soluble and particulate fractions were prepared as described in the legend to FIGURE 14. Extrapolations of *in vitro* values of hepatic catalatic and peroxidatic activities to values based on the weights of the whole animal were made by using the known weight of liver/kg of body weight in each species: 70 g for the mouse (Ms), 40 g for the rat (R), and guinea pig (G), and 20 g for the monkey (M).

particulate fractions from the same animals. The catalatic and peroxidatic activities of the *in situ* livers were estimated from both *in vitro* studies and the established weights of the livers in each of the animals. It can be seen that there are no consistent relationships between the rates of *in vivo* methanol oxidation and the catalatic and peroxidatic activities of the soluble fractions, but that in the mouse, rat, and guinea pig the catalatic and peroxidatic activities of the particulate fractions closely parallel rates of *in vivo* methanol oxidation. The monkey is seen to bear little resemblance to the rodents when its *in vivo* rate of methanol oxidation is compared with catalatic and peroxidatic activities; much more methanol is oxidized *in vivo* than can be accounted for by the catalase activity of the particulate fraction. This observation adds weight to the view that methanol must be oxidized in the monkey by some enzyme system other than one involving hepatic catalase.

The question invariably arises as to what effect homogenization may have had on the partition of cellular components between soluble and particulate fractions of the cell. The remarkable degree of correlation between the peroxidative activity seen *in vivo* as determined by measurement of the rate of ^{14}C-methanol oxidation and the amount of catalatic and peroxidatic activities found in the particulate fractions from the livers of the mouse, rat, and guinea pig, suggests that the partition

of catalase activity seen *in vitro* was not greatly different from that which existed *in vivo*.

Relationship Between Catalatic and Peroxidatic Activities of Hepatic Catalase in the Mouse, Rat, Guinea Pig and Monkey

FIGURE 16 shows the ratios of peroxidatic to catalatic activity in liver fractions from four species. With respect to relative catalatic and peroxidatic activities, there appears to be little qualitative difference in the catalase found in the soluble and particulate fractions from the liver of any given species. However, it is seen that monkey catalase has a much lower ratio of peroxidatic to catalatic activity than that seen in any of the rodents. This qualitative difference in the catalases from monkeys and rodents further explains why the peroxidative mechanism involving catalase is of lesser importance in the metabolism of methanol in the monkey than it is in rodents.

Role of Peroxisomal Oxidases in the Oxidation of Methanol in the Rat and Monkey

Hepatic oxidases were studied in homogenates and peroxisomes from the liver of rats and monkeys for their ability to generate hydrogen peroxide for the me-

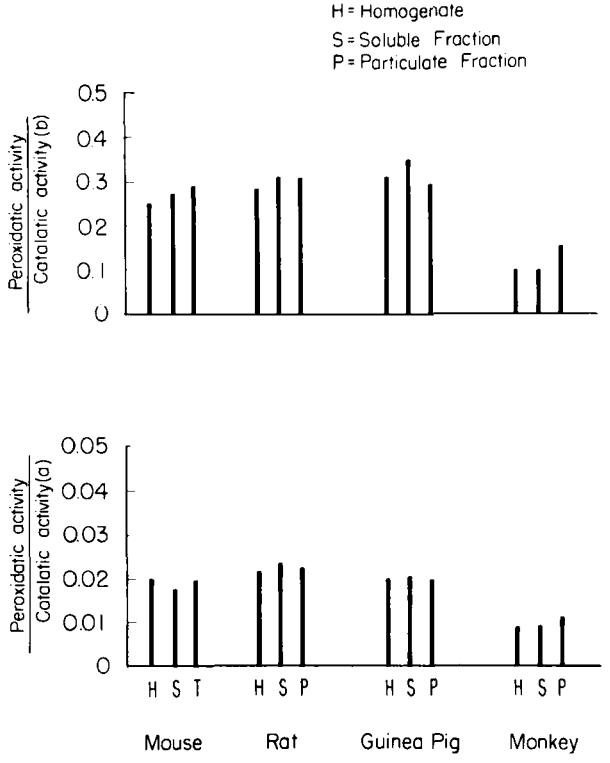

FIGURE 16. Ratio of peroxidatic to catalatic activity in liver fractions from different species. From data of Makar and Mannering.[11] The units of catalatic and peroxidatic activity as well as the method of preparing the fractions are given in the legend to FIGURE 14.

tabolism of methanol.[26] For each of the oxidases studied, much less activity was found in monkey than in rat liver homogenates (TABLE 1). In both rat and monkey liver, urate oxidase and glycolate oxidase were the most active peroxide-generating enzymes, but the activities of urate and glycolate oxidase of monkey liver were only 15% and 38%, respectively, of the activities found in rat liver. Very little xanthine, D-amino acid, or L-α-hydroxy acid oxidase were found in the livers of either species, but again activities were lower in the monkey livers. Addition of crystalline catalase did not affect rates of methanol oxidation.

Similar studies employing hepatic peroxisomes rather than homogenates are also summarized in TABLE 1. Oxidase activities are seen to be much lower in monkey peroxisomes than in rat peroxisomes.

The finding of low oxidase activities in the homogenates and peroxisomes of monkey liver adds to the explanation for the lack of peroxidative oxidation of methanol in this species.

TABLE 1*

OXIDASE-DEPENDENT METHANOL OXIDATION BY LIVER PREPARATIONS

Oxidase	Homogenates†		Peroxisomes‡	
	Rat	Monkey	Rat	Monkey
Urate	95	12	11.8	2.0
Glycolate	52	20	7.2	3.5
Xanthine	5	0	—	—
D-Amino Acid	3	1.5	1.0	0.5
L-α Hydroxy Acid	0	0	0	0

* From data of Goodman and Tephly.[26]

† Micromoles of methanol oxidized/g of liver/hr.

‡ Millimicromoles of methanol oxidized/mg of protein/min.

Summary

Using several experimental approaches, the role of the peroxidative system involving hepatic catalase in the *in vivo* oxidation of methanol was established in the rat. The alcohol dehydrogenase (ADH) system does not appear to be completely inactive in the oxidation of methanol in the rat. In the monkey, the peroxidative system is not important in the oxidation of methanol. Ethanol is oxidized by the ADH system in both species. The failure of the monkey to employ the peroxidative system in the oxidation of methanol is due to the combination of several factors: 1) Hepatic catalase appears to function peroxidatively in the oxidation of methanol only when it is closely associated with the peroxide-generating enzymes found in the particulate fraction of the cell. This close association exists in the peroxisomes. Although monkey liver contains abundant hepatic catalase, only a small proportion is found in the particulate fraction of the cell. 2) The peroxidative activity of monkey hepatic catalase, with respect to its catalatic activity, is only about half that of rat hepatic catalase. 3) There are lesser amounts of the hepatic peroxide-generating enzymes in the monkey than in the rat.

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Discussion of the Paper

DR. J. F. HOGG (*Queens College, Flushing, N.Y.*): In view of the very low proportion of particulate catalase in the primate liver, is there a lower frequency of microbodies in primate liver as compared to rodent liver?

DR. HRUBAN (*University of Chicago, Chicago, Ill.*): No. I did not count them but it seems to me that it cannot be a great difference.

DR. NOVIKOFF: I think I would confirm that.