The Role of Hepatic Microbody and Soluble Oxidases in the Peroxidation of Methanol in the Rat and Monkey

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SUMMARY

Hepatic oxidases were studied in rat and monkey liver homogenates and subcellular fractions for their capacity to provide hydrogen peroxide for the catalase-dependent peroxidative oxidation of methanol. Urate oxidase and glycolate oxidase were the most active hepatic hydrogen peroxide-generating enzymes in the rat; much less activity was found in preparations obtained from monkey liver. Supplementation of solubilized hepatic microbodies or cell sap from either species with an exogenous hydrogen peroxide-generating system stimulated methanol oxidation above rates obtained when uric acid or glycolic acid was used to enhance methanol oxidation. Addition of crystalline catalase did not affect the rate of methanol oxidation. These studies indicate that hydrogen peroxide generation is rate-limiting in the peroxidation of methanol in both rat and monkey liver, and the low activity of oxidases in monkey liver may explain the lack of peroxidative oxidation of methanol in this species.

INTRODUCTION

It has been generally accepted that there are two enzymic systems available for the oxidation of methanol in the animal, a pyridine nucleotide-dependent alcohol dehydrogenase and a catalase peroxidative pathway which requires a source of hydrogen peroxide generated from intracellular oxidases. Previous studies have shown that the catalase peroxidative system plays a major role in methanol oxidation in the rat (1) but only a minor role in the monkey (2), although monkey liver contains a higher level of catalase activity than rat liver (3).

Catalase and oxidases capable of generating hydrogen peroxide exist in both the soluble and particulate fractions of the cell (4), and it has been shown that, within the particulate fraction, catalase and several oxidases reside in a distinct particle which can be isolated relatively free of mitochondria, lysosomes, and microsomes (5). These particles have been termed peroxosomes by de Duve and Baudhuin (6), and they correspond to what Rouiller and Bernhard (7) have described as microbodies. Recently, Mannering and Makar (8) observed a correlation between the methanol oxidation *in vivo* in the rat, mouse, and guinea pig and the catalase activity present in the particulate fraction of the hepatic cell. No such correlation was found for the monkey, but the catalase catalatic and peroxidative activity in the particulate fraction was found to be lower in the monkey than in the other species studied. Furthermore, the ratio of peroxidative to catalatic activity in the particulate fraction of monkey liver was observed to be lower than in the rat, mouse, and guinea pig. It was suggested that these findings account at least in part for the low peroxidative oxidation of methanol in the monkey.

In this report hydrogen peroxide-generating oxidases of rat and monkey liver were examined *in vitro* and their role in imposing a rate limitation upon the peroxidative

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oxidation of methanol was demonstrated for both the rat and the monkey. A preliminary account of this work has been published (9).

MATERIALS AND METHODS

Xanthine, hypoxanthine, D-alanine, crystalline beef liver catalase, D-leucine, and uric acid were purchased from Sigma Chemical Company. Triton X-100 was purchased from Rohm and Haas Company, and α-hydroxy-*n*-butyric acid was purchased from K & K Laboratories, Inc. Glycolic acid was purchased from Fisher Scientific Company, and glucose oxidase was purchased from Worthington Biochemical Corporation. All other reagents were employed in the highest purity available.

Preparation of Subcellular Fractions

Subcellular fractions of liver were prepared from male Holtzman rats (250 g) and from male or female rhesus monkeys (6 kg) according to the method of de Duve *et al.* (10). Rats were decapitated, the livers were rapidly removed, and 25% (w/v) homogenates were prepared with a glass hand-homogenizer. A solution containing 0.25 M sucrose with 0.001 M disodium EDTA was used as the suspension medium and for all subsequent washings and dilutions. Monkey liver homogenates were prepared in the same manner except that the animals were anesthetized with ether prior to laparotomy. A pellet containing mitochondria, lysosomes, and microbodies was obtained by centrifugation of the 10,000 g_{min} supernatant at 12,500 *g* for 20 min. This is referred to as the crude mitochondrial fraction. A pink fluffy layer which capped this pellet was removed and saved.

The crude mitochondrial fraction was resolved into mitochondria, lysosomes, and microbodies by density equilibrium centrifugation using a sucrose–glycogen gradient [GH 10 (11)]. After centrifugation, the microbodies were concentrated in the relatively clear portion at the top of the tube, the mitochondria were concentrated in the turbid yellow layer near the bottom of the tube, and the brown pellet at the bottom of the tube contained lysosomes and a small amount of sedimented glycogen.

Enzyme Assays

Urate oxidase. The rate of oxidation of uric acid was measured by following the decrease in optical density at 292.5 mμ (12).

Xanthine oxidase. Xanthine oxidase activity was measured by determining the formation of urate from xanthine at 292.5 mμ (12).

Catalase catalatic activity. The method of Feinstein (13) was employed for the determination of catalase catalatic activity.

Catalase peroxidative activity. Enzyme preparation (0.5 ml) was incubated with a solution containing glucose oxidase (1 mg) and glucose (0.05 mg) in 1.5 ml of 0.05 M Tris-HCl buffer, pH 8.3. After a preliminary incubation period of 6 min, the reaction was started by the addition of 250 μmoles of methanol (0.5 ml). All incubations were carried out with shaking in an atmosphere of air at 37° in a Dubnoff metabolic shaker. After an incubation period of 6 min, 0.3 ml of 70% perchloric acid was added and formaldehyde was determined by the method of Nash (14). Under these conditions formaldehyde formation was linear with time and dependent on catalase concentration.

Acid phosphatase. Acid phosphatase activity was determined by the method of Gianetto and de Duve (15).

Glucose 6-phosphatase. Glucose 6-phosphatase activity was determined by the method of Harper (16).

Cytochrome oxidase. Cytochrome oxidase activity was assayed by the method of Cooperstein and Lazarow (17).

Glycolate oxidase. Glycolate oxidase activity was measured by determining the rate of formation of glyoxylic acid by the 2,4-dinitrophenylhydrazine assay (18).

Protein. Protein was determined by the biuret method (19).

Determination of Methanol Oxidation

The 2.5-ml reaction mixture contained 0.5 ml of enzyme preparation; methanol, 250 μmoles; and Tris, 100 μmoles (pH

9.0 when uric acid was supplied as substrate, pH 8.3 at all other times). Concentrations of substrate for oxidases were 0.002 M for uric acid and glycolic acid, 0.0005 M for xanthine and hypoxanthine, and 0.03 M for D-alanine, D-leucine, and α-hydroxybutyric acid. All substrates were dissolved in 0.05 M Tris buffer (pH 9.0 when uric acid was employed, pH 8.3 when other substrates were used). All incubations were conducted at 37° under an atmosphere of air or 100% oxygen in a Dubnoff metabolic shaker. When preparations of rat liver were used, they were incubated for 6 min prior to the addition of methanol and oxidase substrates. When monkey liver enzyme preparations were used, no initial incubation period was used. An incubation period of 6 min was used for homogenate, nuclear and membrane fraction, crude mitochondrial fraction, fluffy layer fraction, microsomal fraction, and cell sap fraction. When lysosomal, mitochondrial, or microbody fractions were studied, reactions were carried out for 12 min. During these incubation periods, formaldehyde formation was linear with time. All substrate concentrations were at least 10 times the Michaelis constant for the given enzyme, and methanol did not inhibit urate oxidase, catalase, glycolate oxidase, or xanthine oxidase.

Reactions were stopped by the addition of 0.3 ml of 70% perchloric acid. After centrifugation to sediment the denatured protein, an aliquot of the supernatant was assayed for formaldehyde by the method of Nash (14) or by the chromotropic acid method of MacFadyen (20) after distillation (21). Recovery of formaldehyde from these reaction mixtures varied between 90% and 100%.

RESULTS

Stimulation of Methanol Oxidation by Uric Acid

Urate oxidase mediates the oxidation of uric acid to allantoin, carbon dioxide, and hydrogen peroxide and has been shown to be associated with hepatic microbodies isolated from several species (5). Figure 1 shows that when hepatic homogenates and certain subcellular fractions from rat or monkey were supplemented with uric acid a profound stimulation of methanol oxidation occurred above that obtained in the absence of uric acid. A 20-fold increase was found in methanol oxidation in rat liver homogenates, and a 2-fold increase

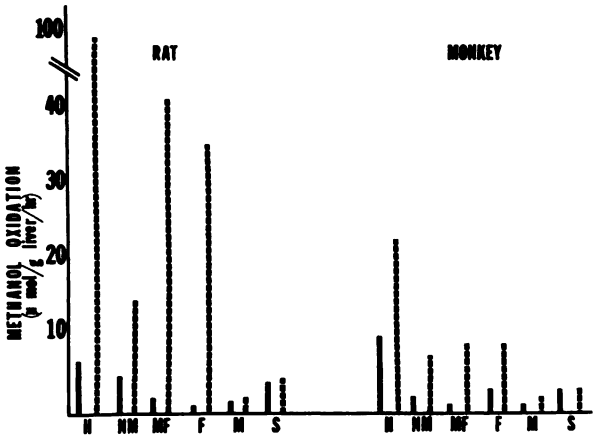

FIG. 1. *Stimulation of methanol oxidation by uric acid in rat and monkey liver homogenates and subcellular fractions*

Solid lines represent results obtained in the absence of uric acid, and dashed lines represent results obtained upon the addition of uric acid (0.002 M); *H* = homogenate; *NM* = nuclear and membrane fraction; *MF* = crude mitochondrial fraction; *F* = fluffy layer fraction; *M* = microsomal fraction; *S* = cell sap fraction. Reactions were carried out at 37°, pH 9.0, with 0.1 M methanol. Rat preparations were incubated for 6 min prior to addition of substrates.

in monkey liver homogenates over the respective endogenous rate. Upon fractionation, the stimulation was confined to those fractions that contain microbodies (the crude mitochondrial fraction and the fluffy layer fraction). This stimulation can be ascribed to the increase in hydrogen peroxide formation induced by the addition of uric acid. In assays in which uric acid oxidation was followed spectrophotometrically along with methanol oxidation, it was found that approximately 1 mole of formaldehyde was formed for every mole of uric acid oxidized. When crystalline beef liver catalase (1600 units) was added to each fraction in addition to uric acid, no further increase in methanol oxidation occurred.

A small amount of urate oxidase activity was found in the nuclei and membrane fraction; this may represent urate oxidase in unbroken cells or particles adsorbed on cell membrane fragments. The fluffy layer fraction was included in these studies in order to assess the total amount and distribution of urate oxidase present within the hepatic cell. This fraction was shown to contain mitochondria, microsomes, lysosomes, and microbodies as determined by assays for cytochrome oxidase, glucose 6-phosphatase, acid phosphatase, and urate oxidase, respectively.

The urate oxidase activity in preparations from monkey liver was uniformly lower than in the corresponding preparations from rat liver. This was expected, since the lack of urate oxidase in the primate is thought to account for uric acid as the end product of purine catabolism in this species. Although low, however, the activity was appreciable.

Stimulation of Methanol Oxidation by Glycolic Acid

Glycolate oxidase is a flavoprotein found in both hepatic microbodies and cell sap and generates hydrogen peroxide in its catalysis of the conversion of glycolic acid to glyoxylic acid (6, 22). Van Harken *et al.* (23, 24) showed that glycolic acid doubled the rate of methanol-^{14}C oxidation to $^{14}CO_2$ in the isolated perfused liver of the rat and in the rat *in vivo*. This stimulation of methanol oxidation in the rat by glycolic acid or by agents such as ethylene glycol, which can be converted to

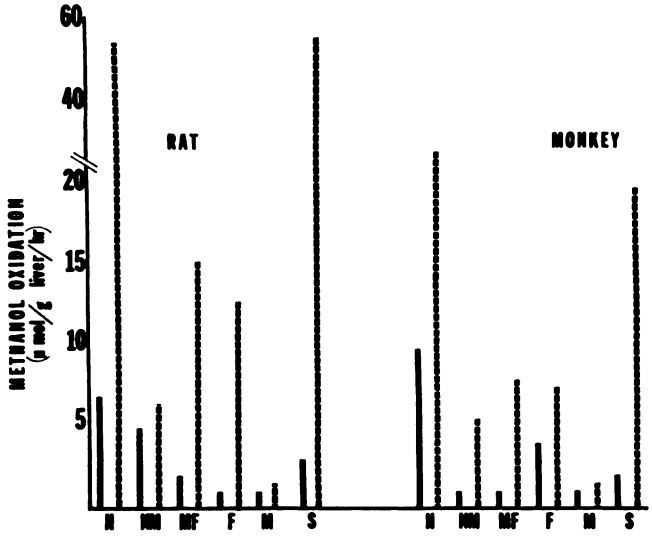

FIG. 2. *Stimulation of methanol oxidation by glycolic acid in rat and monkey liver homogenates and subcellular fractions*

Solid lines represent results obtained in the absence of glycolic acid, and dashed lines represent results obtained in the presence of glycolic acid (0.002 M). Symbols refer to fractions as in Fig. 1. Reactions were carried out at pH 8.3. All other conditions were the same as in Fig. 1.

glycolic acid, has been taken as one of the arguments favoring peroxidative oxidation of methanol as the predominant pathway for methanol metabolism in the rat. Figure 2 shows that methanol oxidation is stimulated in homogenates and in certain subcellular fractions when these preparations are supplemented with glycolic acid. Homogenates of rat and monkey liver yielded 8- and 3-fold increases in methanol oxidation, respectively, over endogenous rates, and the stimulation was seen in fractions containing microbodies and cell sap. When crystalline catalase (1600 units) was added to each fraction, no further stimulation of methanol oxidation was observed.

The Role of Xanthine Oxidase, D-*Amino Acid Oxidase, and* L-α-*Hydroxy Acid Oxidase in the Peroxidative Oxidation of Methanol in Rat and Monkey Liver in Vitro*

In addition to urate oxidase and glycolate oxidase, certain other oxidases capable of generating hydrogen peroxide were examined for their ability to stimulate methanol oxidation in rat and monkey liver homogenates and subcellular fractions. Xanthine oxidase is a soluble enzyme that is known to generate hydrogen peroxide in its conversion of hypoxanthine or xanthine to uric acid. When homogenates of rat liver were supplemented with hypoxanthine only a doubling of the methanol oxidation occurred, and this was found to be confined to the cell sap fraction. The maximum rates obtained were about 10 μmoles/g of liver per hour. This might be compared with rates of 100 μmoles of methanol oxidized per gram of liver per hour when rat liver homogenates were supplemented with uric acid. No increases in methanol oxidation were observed when monkey liver homogenates were supplemented with hypoxanthine. Therefore, xanthine oxidase does not appear to be an important peroxide-generating enzyme in the rat or monkey liver. Studies were performed in which xanthine oxidation to uric acid was measured along with methanol oxidation to formaldehyde. The rate of oxidation of xanthine to uric acid was 7.5 and 5.8 μmoles/g of liver per hour in rat and monkey hepatic cell sap, respectively.

An assessment of the capacity of D-amino acid oxidase to provide hydrogen peroxide for the peroxidative oxidation of methanol was performed in the same manner. When D-alanine or D-leucine was added to rat hepatic homogenates, a 50% increase over the endogenous rate of 5 μmoles/g of liver per hour was obtained, and this slight stimulation was confined to the microbody fraction. D-Alanine or D-leucine produced only a 25% increase in methanol oxidation in monkey hepatic homogenates or microbody fractions. The possibility that the flavin moiety of the D-amino acid oxidase was dissociating from the enzyme was ruled out because the addition of flavin adenine dinucleotide (10^{-4} M) did not change the results.

De Duve and Baudhuin (6) observed that DL-α-hydroxybutyrate served as a substrate for L-α-hydroxy acid oxidase and stimulated hydrogen peroxide generation for the peroxidative oxidation of formate-^{14}C to $^{14}CO_2$. DL-α-Hydrorybutyrate was added to rat or monkey hepatic homogenates and subcellular fractions, and no stimulation of methanol oxidation over endogenous rates was observed. Therefore, hepatic L-α-hydroxy acid oxidase appears to be a minor hepatic peroxide-generating enzyme in both the rat and monkey.

Methanol Oxidation in Hepatic Microbodies from Rat and Monkey Liver

Figure 3 represents experiments performed on fractions resolved by equilibrium density gradient centrifugation of crude mitochondrial fractions derived from rat and monkey liver. Although an imperfect resolution was obtained for crude mitochondrial preparations from rat with reference to the presence of microbodies within the mitochondrial fraction, microbodies were found to be relatively free of mitochondria and lysosomes as determined by the presence of urate oxidase in the mitochondrial fraction and the relative absence of cytochrome oxidase and acid phosphatase in the microbody fraction. An alternative explanation would be that

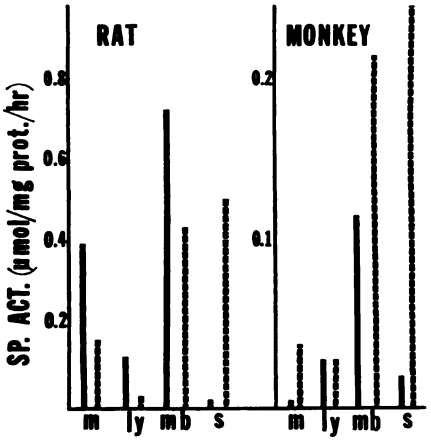

FIG. 3. *Methanol oxidation in particles resolved from the crude mitochondrial fraction and supplemented with uric acid or glycolic acid*

Specific activity refers to micromoles of methanol oxidized to formaldehyde per milligram of protein per hour in the presence of uric acid (solid lines) or in the presence of glycolic acid (dashed lines); *m* = mitochondria; *ly* = lysosomes; *mb* = microbodies; *s* = cell sap. Conditions were as described for Figs. 1 and 2.

there is urate oxidase within mitochondria. The resolution of the crude mitochondrial fraction derived from monkey liver into mitochondria, lysosomes, and microbodies was quite good. Marker enzyme studies showed very little cross-contamination. Upon subtraction of the respective endogenous rates of methanol oxidation it can be seen that the specific activity of urate oxidase in microbodies derived from monkey liver represents about 15% of that seen for rat liver. The specific activity of glycolate oxidase in monkey microbodies is about 50% of that observed for the rat. Included in Fig. 3 are calculations of the specific activity of glycolate oxidase for the cell sap fractions from rat and monkey liver. It can be seen that the activity of glycolate oxidase in the cell sap of both species is higher than that seen for the microbody, and is in agreement with the findings of Baudhuin *et al.* (5) if we assume that the activity that they reported for L-α-hydroxy acid oxidase is that of glycolate oxidase in our studies.

Catalase (catalatic) activity was also determined in fractions seen in Fig. 3. The relative specific activity of catalase followed that for urate oxidase very closely for both rat and monkey preparations. More complete resolution of the crude mitochondrial fraction has been achieved by Baudhuin *et al.* (5) using a sucrose gradient with liver preparations derived from rats treated with Triton WR-1339. They showed that cytochrome oxidase was associated with granules having an average density of less than 1.20 and that urate oxidase and catalase resided in particles with average densities greater than 1.216.

Further studies with hepatic microbodies were performed in order to assess the role of catalase and microbody oxidases in imposing a rate limitation upon the peroxidative oxidation of methanol in these particles. Uric acid, glycolic acid, or their combination was supplied to microbody preparations from rat and monkey liver, and Triton X-100 was used in order to lyse microbody membranes. The addition of Triton X-100 clarified microbody preparations but did not affect the rate of methanol oxidation in either rat or monkey liver microbodies. The addition of crystalline beef liver catalase (1600 units/ml of reaction) to solubilized microbodies did not produce any further stimulation of methanol oxidation above that seen when substrates for microbody oxidases were used to enhance methanol oxidation. Slight but significant stimulation was observed for rat hepatic microbodies when substrates for microbody oxidases were added to microbody preparations under an atmosphere of 100% oxygen. Stimulation by oxygen was not observed with monkey hepatic microbodies. It is curious that an additive effect on methanol oxidation did not occur when uric acid and glycolic acid were added in combination. In the rat liver preparation there was a slight stimulation under 100% oxygen, but this did not produce a rate that would suggest peroxide generated from two sources. It is possible that under the conditions of this assay not enough oxygen could be driven into the reaction mixture to allow for maximal hydrogen peroxide generation

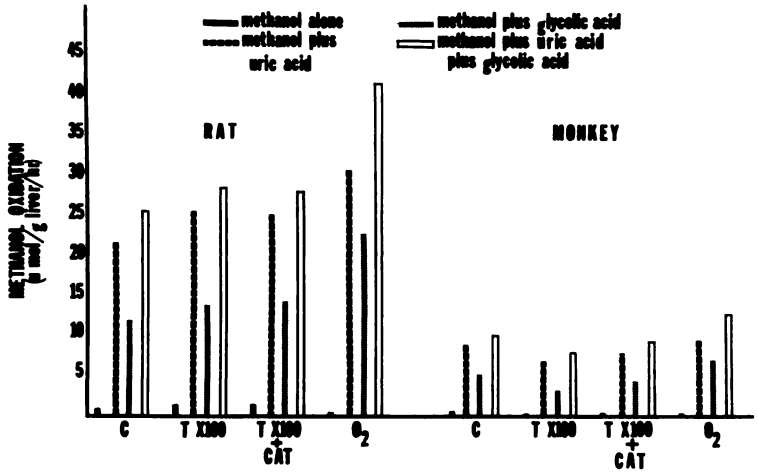

FIG. 4. *The effect of excess catalase or oxygen on the stimulation of methanol oxidation produced by uric acid and/or glycolic acid in hepatic microbodies*

C = control; *T X100* = Triton X-100 (0.2%); *CAT* = crystalline beef liver catalase (1600 units/ml of reaction); O_2 = reactions carried out in 100% oxygen. Conditions were as described for previous figures.

from both urate oxidase and glycolate oxidase. Inhibition of urate oxidase by glycolic acid and the inhibition of glycolate oxidase by uric acid was not observed. These results are shown in Fig. 4.

Figure 5 shows results obtained from rat and monkey liver microbody and cell sap fractions. The peroxidative capacity of catalase in both liver fractions from both species was examined by supplying an exogenous and excess source of hydrogen peroxide in the form of glucose and glucose oxidase. In this manner catalase was made limiting in the peroxidative oxidation of methanol to formaldehyde. It can be seen in Fig. 5 that the amount of catalase present in the cell sap and in the microbody fractions of rat and monkey liver can provide for oxidation rates of methanol in excess of those seen when hydrogen peroxide is maximally generated from endogenous oxidases such as urate oxidase or glycolate oxidase. It would appear from these results that catalase does not impose a rate limitation upon the peroxidative oxidation of methanol in either the cell sap or microbodies from rat or monkey liver. The peroxidative capacity of catalase obtained from monkey microbodies was found to be lower than that of the rat. This agrees with a lower particulate catalase peroxidative activity observed by Makar and Mannering (3). However, whereas they observed a higher peroxidative capacity for the soluble catalase in

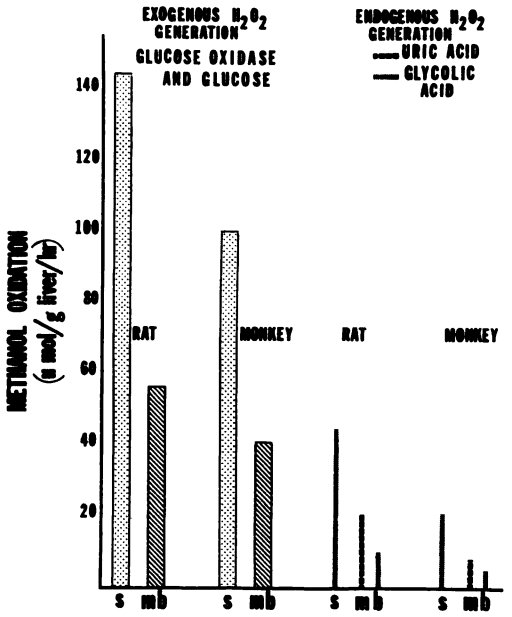

FIG. 5. *Methanol oxidation in hepatic cell sap and microbodies supplemented with an exogenous hydrogen peroxide-generating system or substrates for endogenous oxidases*

s = cell sap; *mb* = microbodies.

the monkey as compared to the rat soluble enzyme, the current studies show that catalase from monkey liver cell sap has a lower peroxidative capacity than that from rat liver cell sap.

DISCUSSION

These studies have been directed toward an understanding of why the monkey oxidizes methanol primarily through an alcohol dehydrogenase pathway (2) rather than through a peroxidative mechanism as is the case with the rat (1). A lack of hepatic catalase in the monkey does not provide an answer, since monkey liver has more catalase catalatic activity than does rat liver. Makar and Mannering (3) have examined the distribution of hepatic catalase and evaluated the peroxidative activity of catalase in a number of species in which methanol appears to be oxidized peroxidatively and in which methanol seems not to be metabolized by this system. In the rat, mouse, and guinea pig, in which methanol is metabolized primarily through a catalase peroxidative system, methanol metabolism *in vivo* correlated well with the catalase activity in the particulate fraction of the liver. In the monkey no correlation between methanol oxidation and particulate catalase was observed. These workers also found that the peroxidative capacity of hepatic particulate catalase activity from monkey was about 20% of that observed for the rat liver particulate catalase. In the current studies another approach was followed. Hepatic oxidases, which are known to generate hydrogen peroxide and represent different subcellular loci, were studied in rat and monkey liver *in vitro* and their capacity to provide hydrogen peroxide for the catalase-dependent peroxidative oxidation of methanol was assessed in homogenates and in subcellular fractions. These experiments indicate that for each oxidase studied much less activity was found in monkey liver than in rat liver (Table 1). In both rat and monkey liver, urate oxidase and glycolate oxidase were the most active peroxide-generating enzymes studied. However, the activities of urate oxidase and glycolate oxidase of monkey liver were only 15% and 38%, respectively, of those found in rat liver. Other peroxide-generating enzymes, such as xanthine oxidase, D-amino acid oxidase, and L-α-hydroxy acid oxidase, were found to have very little capacity for the generation of hydrogen peroxide in both the rat and monkey liver and, where measurable, activity was in each case lower in the monkey (Table 1). Studies by Van Harken *et al.* (23) and Van Harken (24) have indicated that, although methanol is oxidized peroxidatively in the rat, peroxide generation is limiting in this species. It would appear that if any peroxidative oxidation of methanol occurred in the monkey, peroxide generation would also be limiting and impose a much lower oxidative rate than in the rat. That catalase was not a rate-limiting factor in the peroxidative oxidation of methanol in subcellular fractions of liver from either rat or monkey was shown in experiments in which crystalline beef liver catalase was added to solubilized preparations of microbodies or to cell sap fractions. No increases in methanol oxidation could be provoked by catalase addition once maximal activity of microbody or cell sap oxidases was achieved. Furthermore, the peroxidative capacity of microbody or cell sap catalase was shown greatly to exceed the capacity of hydrogen peroxide generation or methanol oxidation provided by addition of excess amounts of substrates for endogenous

TABLE 1

Comparison of oxidase activity in rat and monkey liver

Oxidase	Rat	Monkey
Urate oxidase	95[a]	12
Glycolate oxidase	52	20
Xanthine oxidase	5	0[b]
D-Amino acid oxidase	3	1.5
L-α-Hydroxy acid oxidase	0	0

[a] Each value represents micromoles of methanol oxidized per gram of fresh liver per hour.

[b] Although methanol oxidation was not measurable in the presence of substrates for xanthine oxidase, uric acid formation was observed (5.8 μmoles/g liver/hr).

oxidases. These experiments would explain why methanol oxidation in the monkey does not proceed through a peroxidative mechanism. Ultimately, the control *in vivo* would most likely be the substrate levels for the oxidases generating hydrogen peroxide. That peroxidative metabolism of methanol in the rat, mouse, and guinea pig correlates with particulate catalase (3) may reflect the fact that urate oxidase and catalase reside together in the microbody and that their activities are under similar regulation. A species study of urate oxidase such as that done for catalase (3) has not been performed to date. It is possible that still other oxidases not studied in this report provide more hydrogen peroxide than that produced by urate oxidase or glycolate oxidase. These oxidases were selected because they are known to be very active in hydrogen peroxide production. Monoamine oxidase was not selected because it is a mitochondrial enzyme and in the presence of an organized electron transport chain the likelihood that hydrogen peroxide would be available for catalase of the cell sap or microbody seemed remote.

Evidence provided by Baudhuin *et al.* (5) and experiments reported here indicate that methanol oxidation within the particulate fraction of the hepatic cell is carried out in the microbody. Although resolutions of crude mitochondrial fractions obtained from rat liver were imperfect in these studies, microbodies were quite pure as assessed by the presence of marker enzymes for mitochondria and lysosomes. Monkey liver microbodies were resolved from the mitochondria and lysosomes with good success. Catalase peroxidative capacity in the microbody fractions obtained from rat liver was higher than that observed for the monkey microbody. In disagreement with the results of Makar and Mannering (3), the peroxidative capacity of cell sap catalase derived from monkey liver was lower than that isolated from rat liver. Differences in procedure may account for this discrepancy, since the time required for isolation of subcellular fractions from the liver was much greater for the experiments reported here.

It is interesting that peroxidative reactions can also occur in the cell sap. Although xanthine oxidase activity was observed to be surprisingly low in both species studied, a substantial amount of glycolate oxidase was found in the cell sap. Although kinetic measurements (K_m) of these enzymes indicate that they are very much alike, certain feeding experiments have been performed which show that the glycolate oxidase in cell sap can be diminished without affecting the activity in the microbody fraction. This may mean that the synthesis of the soluble enzyme is under different control than the microbody enzyme or that the soluble enzyme is the same as the microbody enzyme and a transfer mechanism from microbody to cell sap is affected by environmental conditions.

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