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

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SUMMARY

The peroxidative system involving catalase plays an important role in the oxidation of methanol in the rat, but is of little importance for this purpose in the monkey. Since there is abundant hepatic catalase in the monkey, the question arose why it does not function measurably in the peroxidative oxidation of methanol in this species. Two possibilities were investigated: (a) catalase may be distributed in the hepatic cell in such a way that it is not as accessible to peroxide-generating systems as it is in the rat, and (b) hepatic catalase from the monkey may be less active peroxidatively than that found in the rat. Evidence was presented to show that both these factors combine to explain, at least in part, the failure of the peroxidative system to function appreciably in the oxidation of methanol in the monkey. The mouse and the guinea pig resemble the rat in that they also utilize the peroxidative system for the oxidation of methanol. The rate of methanol oxidation *in vivo* was found to bear a direct relationship to the amount of particulate catalase in the livers of the rat, mouse, and guinea pig.

INTRODUCTION

In the preceding study (1) it was concluded that whereas the peroxidative system involving hepatic catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6) plays an important role in the oxidation of methanol in the rat, it is of little importance for this purpose in the monkey. This conclusion was based partly on the observation that 3-amino-1,2,4-triazole greatly decreased the rate of methanol oxidation in the rat *in vivo* (2) but had no measurable effect on methanol oxidation in the monkey. Because AT[2] almost completely inhibited hepatic catalase activity in both species, and because the monkey harbors an abundance of hepatic catalase, the question was raised as to why AT was without at least some recognizable effect on methanol oxidation in the monkey, even though alcohol dehydrogenase seemed to be mainly responsible for methanol oxidation in this species. The amount of peroxidative activity that can occur in the rat appears to depend not so much upon the quantity of hepatic catalase present as upon the rate of hydrogen peroxide generation (3, 4). If the peroxide-generating systems are more deficient in the monkey than they are in the rat, this would account for the failure of the peroxidative system to exert a role in methanol oxidation in the monkey. An evaluation of the rate of hydrogen peroxide generation *in vivo* is not readily amenable to experimental design. However, two other possibilities can be studied readily and they could account for the low level of the peroxidative activity in the monkey: (a) while there is abundant catalase in the hepatic cell of the monkey, its distribution may be such that much of it does not have intimate access to the

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

peroxide-generating systems, or (b) the hepatic catalases of the rat and monkey differ so that the peroxidatic activity (methanol-oxidizing activity) of monkey catalase is less with respect to its catalatic activity (H_2O_2-oxidizing activity) than is the case for hepatic catalase from the rat. The experimental evidence to be presented shows that both these factors combine to explain why the peroxidative system can account for only a relatively small percentage of the total oxidation of methanol in the monkey.

The idea that the distribution of catalase within the cell might have some bearing on the problem stemmed from the observations of de Duve and associates (5, 6), who showed that the peroxide-generating enzymes uricase and D-amino acid oxidase are found together with catalase in the subcellular particles known as microbodies. It seemed quite possible that catalase might require an intimate morphological association with peroxide-generating enzymes for it to function peroxidatively. Any relationship between peroxidatic activity and catalatic activity would then relate to the catalase present in the cell particles, not to the total catalase present in the cell. Feinstein and co-workers (7) were the first to show that the intracellular distribution of hepatic catalase varies greatly in different species. For example, 73% of the hepatic catalase in the mouse is found in the particulate fraction, but in the guinea pig only 18% of the catalase is particulate. The mouse and the guinea pig were included in the current study for comparison with the rat and monkey because they represent extremes with respect to their intracellular distributions of catalase.

MATERIALS AND METHODS

Chemicals. Methanol-^{14}C was purchased from New England Nuclear Corporation; its specific activity was determined as described previously (2). 3-Amino-1,2,4-triazole was generously supplied by the American Cyanamid Company; it was purified as described previously (8). Triton X-100 was obtained from Rohm and Haas Company. Glucose oxidase (β-D-glucose:O_2 oxidoreductase, EC 1.1.3.4) was a purified preparation purchased from Nutritional Biochemicals Corporation.

Animals. The following animals were employed (males): rhesus monkeys (1.6–2.7 kg), Sprague-Dawley rats (250–350 g), English shorthair guinea pigs (300–400 g), and Webster Swiss mice (19–25 g).

Fractionation of liver homogenates. The animal was decapitated; the liver was removed quickly, blotted on filter paper, and weighed; and a 10% (w/v) homogenate was prepared in ice-cold 0.25 M sucrose solution. Excessive homogenization is known to affect the subcellular distribution of catalase activity in liver homogenates (9); therefore, homogenization was restricted to 16 hand strokes in a glass homogenizer. The homogenate was centrifuged at 20,000 *g* for 20 min. The supernatant was designated the *soluble fraction.* The pellet was washed once by resuspending it in 0.25 M sucrose solution and centrifuging it at 20,000 *g* for 10 min. The washed pellet, designated the *particulate fraction,* was resuspended in sufficient amounts of 0.25 M sucrose solution or 0.25 M sucrose solution containing 0.5% Triton X-100 to restore its initial volume. Triton X-100 was used to solubilize the particulate catalase, thereby enabling assessment of the total catalase activity of the particulate fraction (9). Homogenization and fractionation procedures were conducted at 0–5°.

Measurement of hepatic catalase activity. Two methods were used to measure the catalatic activities of the soluble and particulate liver fractions. Feinstein's procedure (10) utilizes sodium perborate as a substrate at 37°. Adams' method (11) employs H_2O_2 as a substrate at low temperature, and the procedure was performed as described originally except that the reaction was conducted at 4° rather than at 0°. When Feinstein's method was used, catalase activity was expressed in Kat. f. units as defined by von Euler and Josephson (12). When Adams' method was used, catalase activity was expressed in Adams units derived from a predetermined standard curve (11).

Measurement of methanol metabolism

by liver preparations. Measurement of the peroxidative activity of the liver preparations was based on the original observation of Strittmatter (13), later confirmed by Tephly and co-workers (14), that without suitable supplementation with coenzymes rat liver homogenates do not oxidize methanol beyond the formaldehyde stage. One milliliter of appropriately diluted liver preparation was mixed with 8 ml of a solution containing the following materials: semicarbazide, 150 μmoles; nicotinamide, 80 μmoles; magnesium chloride, 40 μmoles; phosphate buffer (pH 7.4), 24 μmoles; glucose, 20 mg; and purified glucose oxidase preparation, 0.1 mg. The mixture was incubated at 37° in stoppered 25-ml Erlenmeyer flasks containing air in a Dubnoff metabolic shaker (120 oscillations/min). After an equilibration period of 10 min, the reaction was started by adding 1.0 ml of a solution containing 100 μmoles of methanol. Two-milliliter aliquots of the reaction mixture were removed at 0 and 20 min (during which the time the reaction had been determined to proceed at a constant rate) and placed in 50-ml pear-shaped distilling flasks containing 4 ml of a 30% trichloracetic acid solution. The mixture was distilled and the distillate (4 ml) was assayed for its formaldehyde content by the method of MacFadyen (15). All values were corrected for a predetermined 10% distillation loss.

Studies in vivo. The metabolism *in vivo* of methanol-^{14}C in rats, guinea pigs, mice, and monkeys was studied as described previously (1, 2). Rats, guinea pigs, and monkeys were placed singly in the metabolism chambers, but mice were studied in groups of five. Immediately upon completion of the experiments *in vivo*, livers were removed from the animals for determination of their catalatic and peroxidatic activities.

RESULTS

Effect of AT on the oxidation of methanol-^{14}C by the mouse and guinea pig. In Figs. 1 and 2 it can be seen that AT inhibits the oxidation of methanol-^{14}C in the intact mouse and guinea pig by about 50%,

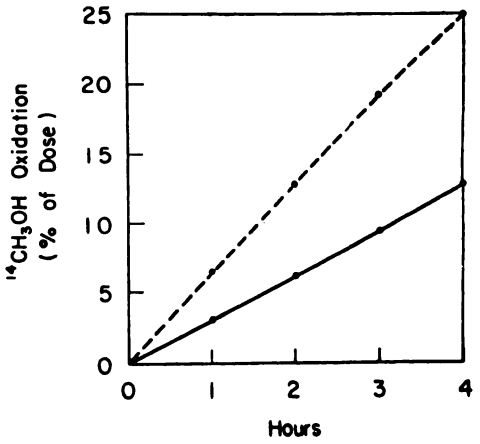

FIG. 1. *Effect of 3-amino-1,2,4-triazole on methanol-^{14}C oxidation in the mouse in vivo*

●---●, Methanol-^{14}C (1g/kg); ○——○, methanol-^{14}C (1 g/kg) 1 hr after the administration of AT (1 g/kg). Rates of $^{14}CO_2$ production are significantly different from control rates at each time interval ($p < .01$). Each point represents data obtained from three groups of five mice. All injections were made intraperitoneally.

which is about the same degree of inhibition produced by AT in the rat (2). This is interpreted to mean that in all three rodents catalase plays an important role in the peroxidative oxidation of methanol.

Intracellular distribution of catalase in the monkey, rat, guinea pig, and mouse. In Fig. 3 comparisons are made of the dis-

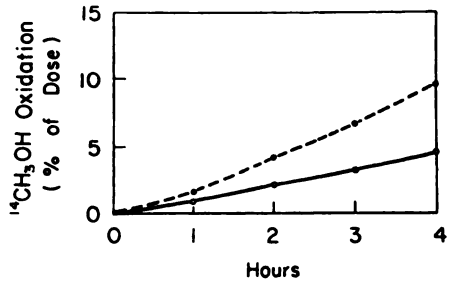

FIG. 2. *Effect of 3-amino-1,2,4-triazole on methanol-^{14}C oxidation in the guinea pig in vivo*

●---●, Methanol-^{14}C (1 g/kg); ○——○, methanol-^{14}C (1 g/kg) 1 hr after the administration of AT (1 g/kg). Rates of $^{14}CO_2$ production are significantly different from control rates at each time interval ($p < .01$). Each point represents data obtained from three animals. All injections were made intraperitoneally.

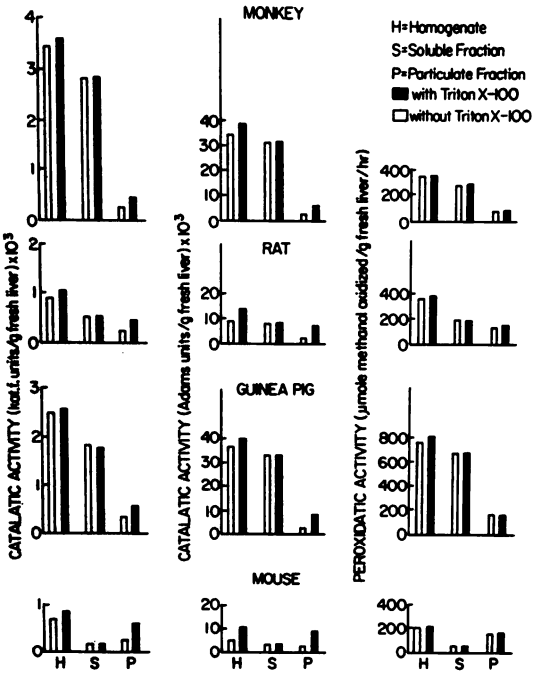

FIG. 3. *Catalatic and peroxidatic activity of liver homogenates and their soluble and particulate fractions from the monkey, rat, guinea pig, and mouse*

Each bar represents data obtained from three liver samples.

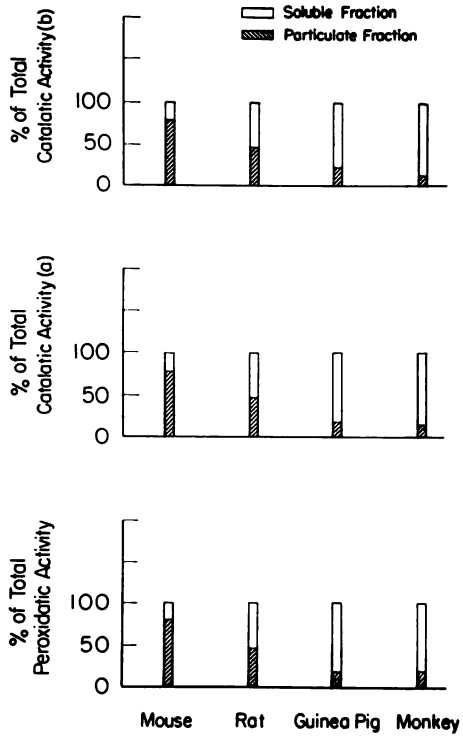

FIG. 4. *Distribution of catalatic and peroxidatic activity between the particulate and soluble fractions of liver homogenates from different species*

Catalatic activity (a) is expressed in Adams units per gram of liver. Catalatic activity (b) is expressed in Kat. f. units per gram of liver. Peroxidatic activity is expressed in micromoles of methanol oxidized per gram of liver tissue per hour.

tribution of catalase between soluble and particulate fractions of the livers from four animal species. The figure also illustrates the effectiveness of Triton X-100 in liberating catalase from the particles. In all subsequent studies (Figs. 4–6) Triton X-100 was used when particulate catalase activity was measured. As would be predicted, Triton X-100 did not increase catalase activity in the soluble fraction. It is also to be noted that Triton X-100 had little if any effect on the peroxidative activity of the particulate fraction, which suggests that during the assays of catalatic and peroxidatic activities, methanol more readily penetrates the particles than does hydrogen peroxide.

The total catalase activities of the liver homogenates are seen to vary greatly from species to species. The variability is seen largely in the amount of catalase found in the soluble fraction. The total amount of particulate catalase varies little in the four species.

The distribution of catalase between soluble and particulate fractions from the four species is shown in Fig. 4. The distributions seen in the mouse, rat, and guinea pig are very similar to those reported by Feinstein and associates (7). With about 80% of its catalase located in the soluble fraction, the monkey resembles the guinea pig in its intracellular distribution of catalase.

Relationship between the oxidation of methanol in vivo and the catalatic and peroxidatic activities of liver fractions. In Fig. 5 comparisons are made between the measured rates of methanol-^{14}C oxidation *in vivo* in four animal species and the

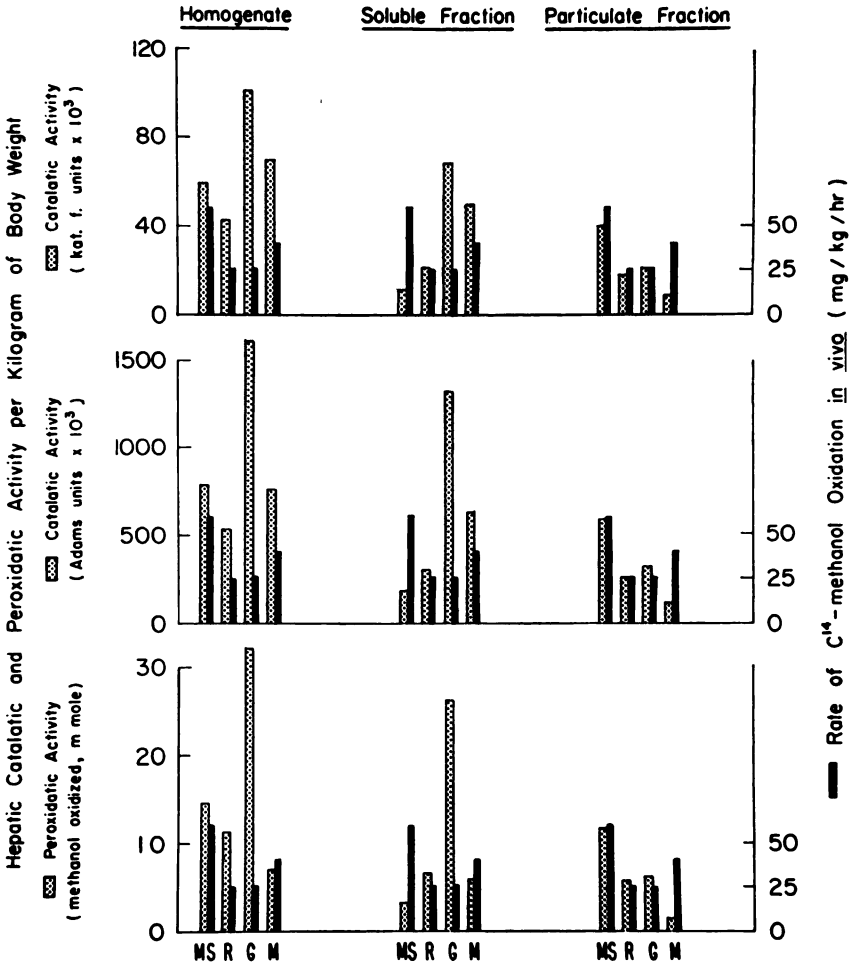

FIG. 5. *Relationship between the oxidation of methanol in vivo and catalatic and peroxidatic activities of liver fractions*

Extrapolations of values of hepatic catalatic and peroxidatic activities *in vitro* to values based on the weights of the whole animal were made by using the known weight of liver per kilogram of body weight in each species: 70 g for the mouse (*Ms*), 40 g for the rat (*R*), 40 g for the guinea pig (*G*), and 20 g for the monkey (*M*).

catalatic and peroxidatic activities of whole homogenates and of soluble and particulate fractions from the same animals. The catalatic and peroxidatic activities of the intact livers were estimated from the studies *in vitro* and the known weights of the livers in each of the animals studied. It can be seen that there are no consistent relationships between the rates of methanol-^{14}C oxidation *in vivo* and the catalatic and peroxidatic activities of either the whole homogenates or the soluble fractions, but that in the mouse, rat, and guinea pig the catalatic and peroxidatic activities of the particulate fractions parallel the rates of methanol oxidation observed to occur in the intact animals. Thus, the mouse, with total particulate catalatic and peroxidatic activities about twice those found in the whole livers of the rat and guinea pig, oxidizes methanol-^{14}C *in vivo* at about twice the rates seen in the other two rodents. Comparing Fig. 3 with Fig. 5, it can be seen that the mouse possesses

greater total particulate catalatic and peroxidatic activities than the rat and guinea pig, not because of higher activities per gram of liver, but because the mouse has a larger liver per unit of body weight.

The monkey is seen to bear little resemblance to the rodents when its rate of methanol-^{14}C oxidation is related to catalatic and peroxidatic activities; much more methanol is oxidized *in vivo* than can be accounted for by the catalase activity of the particulate fraction. Taken with the previous finding that AT has no effect on the oxidation of methanol in the intact monkey (1), this observation strengthens the view that methanol must be oxidized in this species by some enzyme system other than one involving hepatic catalase.

Relationship between catalatic and peroxidatic activities of hepatic catalase in different species. In Fig. 6, data taken from previous figures have been applied to show the ratio of peroxidatic to catalatic activities in liver fractions from four species. With respect to relative catalatic and peroxidatic activities, there would appear 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 apparent that when compared to the enzyme from rodents, which shows relatively similar ratios of the two activities, the hepatic catalase from the monkey has a much lower ratio of peroxidatic to catalatic activity. This qualitative difference between hepatic catalase from the monkey and hepatic catalase from rodents further explains why the peroxidative mechanism involving catalase may be of lesser importance in the metabolism of methanol in the monkey than in rodents.

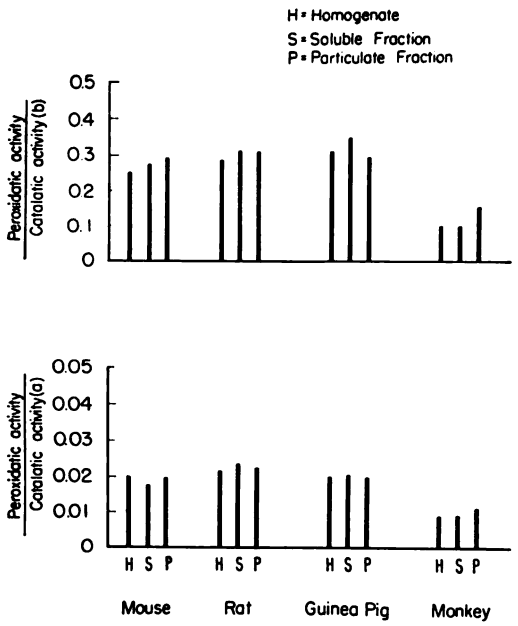

FIG. 6. *Ratio of peroxidatic to catalatic activity in liver fractions from different species*

Catalatic activity (a) is expressed in Adams units per gram of liver tissue; catalatic activity (b) is expressed in Kat. f. units per gram of liver tissue, and peroxidatic activity is expressed in micromoles of methanol oxidized per gram of liver tissue per hour.

DISCUSSION

Several factors combine to explain why 3-amino-1,2,4-triazole depresses methanol oxidation in rodents, but not in the monkey. The rate of methanol oxidation *in vivo* in rodents is directly related to the amount of particulate catalase in the liver. The monkey has a higher concentration of catalase in the liver than any of the rodents in this study, but the intracellular distribution of this catalase is such that the amount in the particulate fraction per gram of liver is about the same or slightly less than that found in the rodents. However, the weight of the liver in the monkey, relative to the body weight, is less than half that of the mouse. Thus, on a per kilogram of body weight basis, the amount of particulate hepatic catalase in the monkey is one-half that of any of the rodents, or less. The potential for peroxidative oxidation in the monkey is further reduced by the relatively low peroxidatic activity of monkey catalase as compared to that of catalases found in rodents.

If there is a direct correlation between particulate hepatic catalase activity and the amount of methanol oxidation that can occur peroxidatively *in vivo*, as the evidence strongly suggests, it can be calculated that the monkey possesses a functional peroxidative mechanism that is only

20% of that found in the rat. AT reduces the rate of methanol oxidation in the intact rat from the normal rate of 24 mg/kg/hr to 12 mg/kg/hr (2). By analogy, methanol oxidation should be reduced by AT in the monkey by 0.2×12, or 2.4 mg/kg/hr. This would represent a reduction of only 6% of the 37 mg/kg/hr of methanol known to be oxidized by the monkey *in vivo* (1). A reduction of this magnitude would not be revealed readily by the methods of this study. This calculation is made with the assumption that the rates of hydrogen peroxide generation are about equal in the rat and the monkey. The studies of Goodman and Tephly (16, 17) suggest that the monkey generates less H_2O_2 than the rat. This would mean that even less than 6% of the rate of methanol metabolism in the monkey could be accounted for by peroxidative activity.

From the data *in vitro* it can be calculated that only about one-fifth of the particulate catalase is functioning maximally in the oxidation of methanol *in vivo* in the rat, mouse, and guinea pig. This could mean that not all the catalase found in the particulate liver fraction is morphologically located so that it can couple with the peroxide-generating mechanisms. It could also mean that the rate of H_2O_2 generation is rate-limiting in the over-all peroxidative reaction. When measurements of peroxidative activity are made *in vitro*, the liver fractions are highly diluted and excess H_2O_2 is provided, conditions that favor maximum conversion of catalase to catalase–H_2O_2, the complex required for the oxidation of methanol (18). However, this abundance of H_2O_2 does not exist *in vivo*, and the amount of catalase–H_2O_2 present at any given moment will relate to the equilibria existing between H_2O_2, catalase, catalase–H_2O_2, and methanol.

AT exerts its inhibitory effect not on catalase per se, but on the catalase–H_2O_2 complex (19–21). Because a peroxidative reaction is required for AT to produce its inhibitory effect, and because AT is as effective an inhibitor of catalase in the monkey as it is in the rat, it might be argued that peroxidation proceeds well in both species and therefore, if catalase is involved in methanol oxidation, it should play as large a role in the monkey as it does in the rat. This argument would ignore the qualitative features of the two peroxidative reactions involving AT and methanol. The reaction of AT with catalase–H_2O_2 is irreversible or virtually so. Thus only a small amount of H_2O_2 is needed to inactivate all or most of the catalase contained in the liver, whereas 1 molecule of H_2O_2 is required each time 1 molecule of methanol is converted to formaldehyde. Neither monkeys nor rodents produce enough H_2O_2 to permit full utilization of their hepatic catalase for peroxidative functions, but enough H_2O_2 is generated to allow AT to inhibit their hepatic catalases almost completely.

The question invariably arises when studies are made of the partition of cellular components between soluble and particulate fractions of a cell as to what effect homogenization may have had. There is no doubt that prolonged homogenization releases catalase from the particulate to the soluble fraction (9). In the current studies it is not known how much catalase was released from the particles through homogenization, but the remarkable degree of correlation between peroxidative activity *in vivo*, as measured by methanol metabolism, 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*.

ACKNOWLEDGMENTS

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