Catalase Activity and Ethanol Metabolism in the Rat.* (22609)

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Until recently the sole function of catalase in living cells was thought to be that of preventing accumulation of toxic concentrations of hydrogen peroxide. Keilin and Hartree (1.2), however, indicated that catalase may catalyze a peroxidatic oxidation of ethanol to aldehyde. If this reaction does occur *in vivo*, ethanol oxidation conceivably may be carried out by both catalase and alcohol dehydrogenase, and not by the latter alone. Jacobsen (3) has even postulated that catalase is responsible in man for oxidation of 20% of a given dose of ethanol. Based on a review of the literature, and on his own work, Bartlett (4) concluded that alcohol dehydrogenase alone is responsible for the first step in the oxidation of ethanol. Westerfeld(5) agreed in principle with Bartlett when the former expressed the view that the catalase mechanism is not of major importance in alcohol metabolism. One of us(6) has recently attempted to determine the importance of catalase in alcohol metabolism in dogs by correlating rate of ethanol metabolism and hepatic catalase activity in the same animal. The coefficient of correlation between the two determinations was statistically significant, 0.602. Liver weight alone, however, when correlated with rate of metabolism yielded a coefficient of 0.797. In view of these findings, it appears that liver weight may be the determining factor in the calculations, and the relatively high value of the coefficient is merely due to the preponderant effect of liver weight. In view of these considerations, and because a new research technic has become available, a different approach was made to the problem of ascertaining the importance of catalase activity in ethanol metabolism.

The new approach was made possible when Heim *et al.*(7) injected rats intraperitoneally with 3-amino-1, 2, 4-triazole (designated hereafter as AT) and found that hepatic catalase activity was markedly depressed for at least 12 hours. The general plan of the present investigation involved determination of the rate of ethanol metabolism in rats, over a 4 hour period after AT had been injected, followed by determination of the ethanol oxidizing capacity of the liver, believed to be a measure of alcohol dehydrogenase activity, and hepatic catalase activity. The findings in control animals were compared with those in AT-injected experimental animals.

Methods. Twenty per cent ethanol, in saline, in dosages of 3 g/kg, was administered intraperitoneally in adult male Sprague-Dawley rats, 60 minutes after 1 g kg of AT,‡ as a 14% aqueous solution, had been administered intraperitoneally. Control animals were given alcohol in an identical manner but were not injected with AT. Four hours after administration of ethanol, the rat was sacrificed by a blow on the head. A small specimen of liver was removed, weighed, and homogenized. Alcohol dehydrogenase and catalase activities were determined by methods previously described(6). The carcass was comminuted and treated as described by Aull *et al.*(8). To ascertain the degree and persistence of the reduction of hepatic catalase activity, as a result of the action of AT, the catalase activity was determined in groups of 3-4 rats each at hourly intervals over a 5-hour period. The assay method employed(6) was the same as that used with the small specimen in the larger experimental series.

Results. The rate of ethanol metabolism, in mg/kg/hr, and the hepatic alcohol dehydrogenase in mg/g liver/hr, and catalase activities, in m.e./mg liver and m.e./mg N, are

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TABLE I. Rate of Ethanol Metabolism and Enzyme Activities.

	Metabolic rate, mg/kg/hr	Oxid. cap., mg/g/hr	Catalase m.e./mg liver	Catalase m.e./mg N
Controls (10 rats):				
Range	180 – 239	4.5 – 9.5	.992 – 1.448	24.9 – 34.7
Mean ± S.D.	205 ± 18	7.2 ± 1.3	1.170 ± .147	29.1 ± 3.8
Exp. (10 rats):				
Range	143 – 230	6.5 – 12.0	.268 – .372	6.9 – 10.0
Mean ± S.D.	193 ± 27	8.9 ± 1.6	.327 ± .035	8.2 ± 1.0

recorded in Table I. In the rats injected with AT, determination of the liver catalase activity, at hourly intervals for 4 hours, in 3 animals each, showed a reduction of 88.1-81.7% below the average for the control series. In light of the greater depression during the first 4 hours, the catalase activities recorded in Table I probably represent maximum levels of activity reached during the 5 hour period following AT injection. The differences between mean values of both the metabolic rate and the ethanol oxidizing capacity of the liver, in the control and experimental series, were statistically tested. In each case the P value, of less than 0.1, indicates that there is probably no significant difference between control and experimental series.

Discussion. Results of the determinations of hepatic catalase activity over a period of 5 hours, indicate that AT depresses catalase activity promptly and that the depression persists to a marked degree for at least 5 hours. This marked reduction in catalase activity of the liver, averaging at least 71.8%, should be sufficient to affect the rate of ethanol metabolism if catalase plays an important role in alcohol metabolism. The p values, indicating that both the rate of ethanol metabolism and the ethanol oxidizing capacity of the liver are statistically identical in control and experimental series, lead us to conclude that hepatic catalase does not play a significant role in alcohol metabolism. Apparently, AT does not have an appreciable effect upon alcohol oxidizing capacity of the liver. This determination, originally designated as ethanol oxidizing capacity of liver(6) is now believed to be a measure of the alcohol dehydrogenase activity since addition of purified catalase, or of purified catalase plus perborate, to the liver homogenate failed to give any increase in the ethanol oxidizing capacity of liver homogenate. All of the observations, therefore, indicate that liver catalase does not play a significant role in ethanol metabolism.

Summary. Reduction in hepatic catalase activity, of at least 71.8%, over a period of 5 hours, as a result of injection of 3-amino-1, 2, 4-triazole, failed to produce a statistically significant alteration in rate of ethanol metabolism in rats. The ethanol oxidizing capacity of the liver, *e.g.*, alcohol dehydrogenase activity, was statistically the same in control and experimental series. It is concluded that hepatic catalase, in rats, does not play a significant role in ethanol metabolism.

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