# Ethanol Metabolism in Rats after Microsomal Metabolizing Enzyme Induction[1] (34374)

CURTIS D. KLAASSEN
(Introduced by J. Doull)

*Department of Pharmacology, Clinical Pharmacology and Toxicology Center, University of Kansas Medical Center, Kansas City, Kansas 66103*

The hepatic enzyme responsible for ethanol oxidation is thought to be alcohol dehydrogenase (ADH), which is localized in the supernatant fraction of the homogenized liver cell. Recently, a second liver enzyme localized in the microsomal fraction was reported by Lieber and DeCarli (1) to have the *in vitro* capacity to metabolize ethanol. This pathway has been termed the microsomal ethanol-oxidizing system (MEOS). The quantitative role of the MEOS *in vivo* remains unknown.

Feeding ethanol to rats results in an increase in activity of hepatic MEOS (1). Iber *et al.* (2) recently reported that in alcoholics ethanol disappears from the blood approximately twice as fast as it does in nonalcoholics. They suggested that the enhanced rate of disappearance may be due to induction of an enzyme for oxidizing ethanol other than ADH.

Many chemicals and drugs increase microsomal drug-metabolizing enzymes (3, 4). Pretreatment of rats with phenobarbital (PB) and 3, 4-benzpyrene has been reported by Roach *et al.* (5) to result in a stimulation of the MEOS 100 and 70%, respectively. The purpose of the present investigation was to determine the effect of a number of agents, known to stimulate microsomal drug metabolizing enzymes, on the disappearance of ethanol from the blood of rats.

*Methods. Animals and treatments.* Simonsen Sprague-Dawley male rats (150–200 g) were used throughout. The following agents known to stimulate microsomal drug-metabolizing enzymes (3, 4) were employed: phenobarbital sodium (75 mg/kg), gamma-chlordane (50 mg/kg), chlorcyclizine hydrochloride (25 mg/kg), and 3-methylcholanthrene (20 mg/kg). The agents were administered intraperitoneally, once daily for 3 days, in aqueous solution except chlordane and 3-methylcholanthrene (3-MC) which were made up in corn oil. The aqueous solutions delivered the proper dosage in a final volume of 0.01 ml/g and the corn oil solutions in a volume of 0.005 ml/g.

*Ethanol intoxication and blood sampling.* Food was removed approximately 10 hr before ethanol administration. Ethanol (3 g/kg) was diluted to 37.5% and administered by gavage (10 ml/kg) 24 hr after the last pretreatment. Blood samples were obtained from the end of the rat's tail at hourly intervals for 8 hr. The blood (100 $\mu$l) was added to a tube containing 0.4 ml of 2% perchloric acid. The tubes were capped, shaken, and then refrigerated until the analyses were performed.

*Blood ethanol analysis.* Ethanol was measured by an enzymatic alcohol dehydrogenase procedure (6). Fifty $\mu$l of the blood–perchloric acid supernatant was added to 1.5 ml of 0.075 *M* sodium pyrophosphate, pH 9.2, containing 0.075 *M* semicarbazide, 0.022 *M* glycine, 0.387 mg/ml of $\beta$-diphosphopyridine nucleotide (NAD), and 1.0 mg/ml of yeast alcohol dehydrogenase (Sigma). After approximately 1 hr of incubation, the concentration of NADH was determined on a Gilford 240 spectrophotometer at 340 m$\mu$.

*Zoxazolamine paralysis time.* Rats were injected daily for 3 days with the various microsomal drug metabolizing enzyme inducers. Twenty-four hr after the last treatment, the

[1] This work was supported by funds from the Public Health Service Grant GM 15956.

duration of zoxazolamine (100 mg/kg) paralysis was measured. Paralysis time was defined as the time between loss and return of the righting reflex. The zoxazolamine solution was prepared for intraperitoneal injection (0.005 ml/g) by adding 14.4 ml of 1 *N* HCl to 1.2 g of zoxazolamine and diluting with 45 ml of 0.9% NaCl.

*Statistics.* The data were compared by an analysis of variance; when the analysis indicated that a significant difference existed, the means were compared by the Student's *t* test (7).

*Results.* To determine if the various treatments stimulated microsomal drug metabolizing enzymes, zoxazolamine paralysis time was measured. Table I illustrates that all of the treatments significantly decreased zoxazolamine paralysis time. Phenobarbital, chlorcyclizine, and chlordane decreased the paralysis time by one-half while 3-MC decreased it approximately 20-fold.

The concentration of ethanol in the blood at hourly intervals after administration is recorded in Table II. These data indicate that peak concentrations of ethanol are detected in the blood 2 hr after administration and disappear at a linear rate from 4 to 8 hr. Phenobarbital treatment did not alter the concentration of ethanol in the blood at any time interval examined. Chlorcyclizine had a tendency to increase the blood ethanol concentrations at all time intervals; the differences were significant at the 3- and 4-hr collection period. Chlordane also had a tendency to increase the blood ethanol concentration, but the difference was significant only at the 3-hr interval. The 3-MC did not alter the concentration of ethanol in the blood at any of the time intervals.

TABLE I. Zoxazolamine[a] Paralysis Time of Rats Pretreated with Various Microsomal Enzyme Inducers.

| Pretreatment[b] | Paralysis time (min) |
|---|---|
| Control | 232 ± 16[c] |
| Phenobarbital | 101 ± 20[d] |
| Chlorcyclizine | 110 ± 7[d] |
| Chlordane | 106 ± 27[d] |
| 3-Methylcholanthrene | 10 ± 3[d] |

[a] Zoxazolamine administered intraperitoneally (100 mg/kg).

[b] Rats were treated 3 days with the various agents.

[c] Each value represents the mean ± SE of 4 or 5 rats.

[d] Significance, $p < 0.05$.

Data for the rate of elimination of ethyl alcohol for the linear portion of the disappearance curve (4–8 hr) is also demonstrated by Table II. Control rats eliminated ethanol from the blood at a rate of 44 mg/100 ml/hr. When rats were treated with the various microsomal enzyme inducers, no significant alteration in the rate of ethanol disappearance was detectable.

*Discussion.* Many drugs and nutritional substances were examined to determine their effects on alcohol metabolism, and only a few had an effect. Theoretically, agents could alter one of many steps to enhance the removal of ethanol from the body, for example, by increasing (a) the amount of the ADH enzyme; (b) the amount of NAD, the hydrogen acceptor of ADH; (c) urinary excretion; and/or (d) respiratory elimination. However, of the agents studied to date that increased ethanol metabolism, either increased NAD concentrations or increased respiration appears to be the mechanism. This is exemplified by pyruvic acid which increases ethanol metabolism (8) by enhancing the reoxidation of NADH to NAD, and by dinitrophenol which increases respiration (9).

With the recent demonstration that the microsomal fraction of the liver has the *in vitro* capacity to metabolize ethanol, additional mechanisms for enhanced ethanol metabolism are provided. One might suspect that agents which enhance microsomal drug-metabolizing oxidative pathways and decrease the biological half-life of many drugs, might also enhance the elimination of ethanol from the blood. In fact, pretreatment with two agents that are known to stimulate microsomal drug metabolism (PB, and 3, 4-benzpyrene) has been shown to enhance the *in vitro* microsomal metabolism of ethanol (5). However, from the present

TABLE II. Effect of Microsomal Enzyme Inducers on the Concentration of Ethanol in the Blood at Various Times after Administration (3 g/kg).

| Pretreatment[a] dose (mg/kg) | No. of rats | After ethanol (hr) | | | | | | | Δ Blood ethanol (mg/100 ml)/hr |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | |
| Control | 14 | 259 ± 13[b] | 273 ± 16 | 255 ± 16 | 255 ± 17 | 180 ± 18 | 135 ± 17 | 95 ± 17 | 55 ± 18 | 44 ± 3 |
| Phenobarbital (75) | 11 | 270 ± 19 | 285 ± 17 | 268 ± 12 | 228 ± 13 | 192 ± 16 | 147 ± 16 | 103 ± 14 | 60 ± 14 | 44 ± 3 |
| Chlorcyclizine (25) | 8 | 273 ± 21 | 315 ± 22 | 300 ± 16[c] | 273 ± 17[c] | 225 ± 17 | 177 ± 19 | 131 ± 22 | 85 ± 25 | 47 ± 5 |
| Chlordane (50) | 9 | 287 ± 22 | 300 ± 16 | 295 ± 14[c] | 270 ± 16 | 217 ± 18 | 170 ± 21 | 120 ± 19 | 76 ± 21 | 47 ± 1 |
| 3-Methylcholanthrene (20) | 9 | 238 ± 16 | 250 ± 15 | 250 ± 8 | 237 ± 12 | 182 ± 12 | 135 ± 8 | 90 ± 7 | 52 ± 11 | 43 ± 3 |

[a] Rats were treated for 3 days with the various agents.
[b] Each value represents mean ± SE (mg/100 ml).
[c] Significantly different ($p < 0.05$) from control values.

study it would appear that these microsomal drug-metabolizing inducers have little or no effect on the overall disappearance of ethanol from the blood of rats. The zoxazolamine paralysis data present evidence that the various agents did increase microsomal drug-metabolising enzyme activity. Even though the zoxazolamine paralysis was decreased to one half and in one instance to one twentieth of control values, no increase in the rate of ethanol metabolism was apparent.

Control rats eliminate ethanol from the blood at a rate of 44 mg/100 ml/hr. This is a relatively rapid rate compared to the 16 mg/100 ml/hr reported for man and dog (10). However, it is comparable to the rates observed in smaller animals such as the 37 mg/100 ml/hr observed for chickens (10) and 55–70 observed for bats (11). This relatively high rate observed in rats supports the conclusion (12) that smaller animals with higher metabolic rates eliminate ethanol from the blood at a greater rate than do larger animals. In the present study, stimulation of the microsomal drug-metabolizing enzymes in the rat did not enhance the disappearance of ethanol from the blood. However, it is possible that increasing the microsomal enzyme activity of larger species, which have a slower rate of ethanol metabolism, could have a more pronounced effect on the disappearance of ethanol from the blood than it does in smaller species.

*Summary.* The ability of a number of microsomal drug-metabolizing enzyme inducers to alter ethanol metabolism was examined. Phenobarbital, chlordane, chlorcyclizine, and 3-methylcholanthrene significantly increased drug metabolism as measured by a decrease in zoxazolamine paralysis time. Control rats metabolized ethanol at a rate of 44 mg/100 ml/hr and the rate was not altered when rats were treated with the various microsomal enzyme inducers. Therefore, it appears that agents which increase the miccrosomal metabolism of many drugs have little quantitative importance in determining the rate at which ethanol is metabolized by the rat.

The author wishes to acknowledge the very able technical assistance of **Mr. W. H.** Whittley.

1. Lieber, C. and DeCarli, L., Science **162**, 917 (1968).

2. Iber, F., Carulli, N., and Kater, R., Federation Proc. **28**, 626 (1969).

3. Conney, A., Pharmacol. Rev. **19**, 317, (1967).

4. Mannering, G., *in* "Selected Pharmacological Testing Methods" (A. Burger, ed.), p. 51. Dekker, New York (1968).

5. Roach, M., Reese, W., and Creaven, P., Federation Proc. **28**, 546 (1969).

6. Bonnichsen, R. and Theorell, H., Scand. J. Clin. Invest. **3**, 58 (1951).

7. Steele, R. and Torrie, J., "Principles and Procedures of Statistics" McGraw-Hill, New York (1960).

8. Westerfeld, W., Stotz, E., and Berg, E., J. Biol. Chem. **144**, 657 (1942).

9. Newman, H. and Tainter, M., J. Pharmacol. Exptl. Therap. **57**, 67, 1936.

10. Newman, H. and Lehman, A., Arch. Intern. Pharmacodyn. **55**, 440 (1937).

11. Greenwald, E., Martz, R., Harris, P., Brown, D., Forney, R., and Hughes, F., Toxicol. Appl. Pharmacol. **13**, 358, (1969).

12. Jacobsen, E., Pharmacol. Rev. **4**, 107 (1952).

Received May 22, 1969. P.S.E.B.M., 1969, Vol. 132.