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Effect of phenobarbital administration on ethanol oxidizing enzymes and on rates of ethanol degradation

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THE PRINCIPAL enzyme responsible for the oxidation of ethanol is alcohol dehydrogenase present in the supernatant fraction of liver cell homogenates.[1] Recently, a microsomal enzyme system was shown likewise to be capable of the oxidation of ethanol.[2,3] Administration of ethanol to rats was found to induce the microsomal ethanol oxidizing enzyme system;[2] when alcoholic patients were shown to have faster rates of ethanol disappearance from the blood than control subjects, it was suggested that microsomal enzyme induction was responsible for the increased rates.[4] The purpose of the present study was to determine whether or not the administration of phenobarbital, a well known inducer of microsomal enzymes, would increase both the activity of ethanol oxidizing systems and the rates of ethanol disappearance from the blood.

Twenty-four male albino Wistar rats, weighing initially between 210–230 g, were studied. Purina chow and water were fed to the rats *ad lib.* Phenobarbital sodium (100 mg/kg) was given daily by intraperitoneal injection as a 25 per cent solution in normal saline. The durations of the treatment were 3 days (four animal pairs) and 5 days (eight animal pairs). The animals were sacrificed by a blow on the head 24 hr after the last intraperitoneal injection after a 14-hr fast. The livers were immediately removed, washed in ice-cold physiologic saline solution, weighed, minced with scissors and then homogenized in a Potter–Elvehjem homogenizer for 3 min with a volume of buffer equivalent to two times the liver weight. The composition of the buffer was as follows: tris–HCl (0·05 M), KCl (0·08 M), $MgCl_2$ (0·01 M), sucrose (0·25 M) pH 7·8. The homogenate was centrifuged at 9000 *g* for 10 min in a Sorvall refrigerated centrifuge. The resulting nuclear fraction was discarded and the supernatant centrifuged at 106,000 *g* for 60 min in a B-50 International refrigerated ultracentrifuge (Rotor 211A). The supernatant fraction obtained at this point was separated for the analysis of alcohol dehydrogenase activity, and the microsomal pellet washed with 8 ml of the above buffer and then recentrifuged at 106,000 *g* for 1 hr. The washed microsomal pellet was finally resuspended in 8 ml of 0·1 M NaH_2PO_4-K_2HPO_4 buffer, pH 7·4.

Alcohol dehydrogenase activity was determined by the method of Bonnichsen and Brink,[5] the microsomal NADPH-dependent ethanol oxidizing activity as described by Lieber and DeCarli,[3] aniline hydroxylase according to Imai and Sato,[6] and cytochrome P-450 according to Omura and Sato.[7] Protein concentration was determined by the Folin-Ciocalteau method of Lowry *et al.* with bovine serum albumin used as a standard.[8] The rates of ethanol disappearance from the blood were determined in all the animals over a 6-hr time interval prior to sacrifice. Ethanol (4 g/kg) was given as a 20% solution in water by stomach tube. Eighty μl of blood was obtained from the retro-orbital plexus of each animal with a heparinized capillary tube hourly for 6 hr. After centrifugation of 2000 *g* for 10 min, the separated plasma sample was analyzed for ethanol concentration by gas–liquid chromatography.[9] Ethanol concentrations in blood when plotted against time followed a linear function. The rate of ethanol disappearance from the blood was obtained from the slope of the regression line calculated by the method of least squares[10] and expressed in milligrams of ethanol cleared per 100 ml of blood per hour.

Phenobarbital administration resulted in significant increases in liver weight, microsomal protein concentration, aniline hydroxylase activity and cytochrome P-450 (Table 1). The ethanol oxidizing enzymes, alcohol dehydrogenase and NADPH microsomal ethanol oxidizing activity were not increased when expressed per milligram of supernatant and microsomal protein respectively (Table 2). However, the calculated total hepatic activities of the ethanol oxidizing enzymes were increased, alcohol dehydrogenase at 5 days ($P < 0{\cdot}02$) and NADPH-dependent ethanol oxidizing activity at 3 and 5 days ($P < 0{\cdot}001$) after phenobarbital administration. Total alcohol dehydrogenase increased 27 per cent and microsomal ethanol oxidizing system 40 per cent over control values after 5 days of phenobarbital administration. The increases in total liver alcohol dehydrogenase activity can be explained by the effect of phenobarbital in enhancing liver weight, while those in microsomal ethanol oxidizing activity reflect both an increase in liver weight and microsomal protein concentration. Despite the increases in the total activity of the ethanol oxidizing enzymes, the rates of ethanol

TABLE 1. EFFECT OF PHENOBARBITAL ADMINISTRATION ON LIVER WEIGHT MICROSOMAL PROTEIN CONCENTRATION ANILINE HYDROXYLASE AND CYTOCHROME P-450*

	Period of treatment			
	3 Days		5 Days	
Determination	Control (No. = 4)	Phenobarbital (No. = 4)	Control (No. = 8)	Phenobarbital (No. = 8)
Liver weight (%)†	3·5 ± 0·2	4·5 ± 0·5‡	3·3 ± 0·1	4·4 ± 0·2§
Microsomal protein (mg/g)	19·8 ± 1·7	25·0 ± 2·3‖	24·1 ± 1·4	32·0 ± 2·4§
Aniline hydroxylase (mμmoles/mg/hr)	11·7 ± 0·9	18·2 ± 1·7§	12·1 ± 2·2	23·1 ± 5·6§
Cytochrome P-450 (mμmoles/mg)	1·0 ± 0·04	1·8 ± 0·01§	1·2 ± 0·01	2·3 ± 0·40§

* The values are expressed as means ± S.D.
† Gram of wet weight × 100/body weight. Significance (vs. control).
‡ $P < 0·01$.
§ $P < 0·001$.
‖ $P < 0·02$.

TABLE 2. EFFECT OF PHENOBARBITAL ADMINISTRATION ON ETHANOL OXIDIZING ENZYMES AND RATES OF ETHANOL DISAPPEARANCE FROM THE BLOOD*

	Period of treatment			
	3 Days		5 Days	
Determination	Control (No. = 4)	Phenobarbital (No. = 4)	Control (No. = 8)	Phenobarbital (No. = 8)
	(mμmoles/mg protein†/min)			
Alcohol dehydrogenase	9·9 ± 1·9	9·3 ± 1·7	11·8 ± 1·9	10·2 ± 2·4
Microsomal ethanol oxidizing activity	12·6 ± 1·7	10·8 ± 1·8	11·0 ± 2·3	9·4 ± 1·1
	(μmoles/liver/min)			
Alcohol dehydrogenase	9·3 ± 1·4	9·4 ± 1·4	8·2 ± 1·9	10·4 ± 1·4‡
Microsomal ethanol oxidizing activity	0·26 ± 0·02	0·33 ± 0·02§	0·20 ± 0·03	0·28 ± 0·04§
	(mg/100 ml/hr)			
Rates of ethanol disappearance from the blood	28·8 ± 4·0	30·1 ± 5·1	29·1 ± 5·3	32·6 ± 5·6

* Values are expressed as means ± S.D.
† Milligram of supernatant protein for alcohol dehydrogenase and milligram of microsomal protein for microsomal ethanol oxidizing activity. Significance (vs. control).
‡ $P < 0·05$.
§ $P < 0·001$.

disappearance from the blood were not significantly altered by phenobarbital administration. Fischer and Oelssner[11,12] reported increases in ethanol elimination of up to 20 per cent in mice and rabbits after hexobarbital and phenobarbital treatment; however, more recent studies by Klaassen[13] showed no changes in the rates of ethanol elimination in rats after phenobarbital despite decreases in zoxazolamine paralysis times. In conclusion, it appears that factors other than the absolute changes in ethanol oxidizing enzymes regulate the rates of ethanol disappearance from the blood.

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