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Ethanol binding to hepatic microsomes—Its increase by ethanol consumption*

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RECENT studies have established that, in addition to its direct hepatotoxicity, ethanol shares several properties with drugs such as phenobarbital.[1–3] Chronic administration of ethanol increases the following in the liver: (1) smooth endoplasmic reticulum; (2) microsomal protein (in male rats); (3) activities of microsomal drug-metabolizing enzymes; and (4) cholesterol biosynthesis.[4,5] Moreover, a hepatic microsomal system which oxidizes ethanol has been described.[6] For drugs to be oxidized by microsomes, it is generally thought that they must first bind to hemoprotein.[7,8] To determine whether ethanol shares with other drugs this property of binding to microsomal hemoprotein, we investigated the effect on ethanol of the spectral characteristics of hepatic microsomes.

Female Sprague–Dawley rats, weighing 150–200 g, were fed an adequate diet in liquid form[9] for 24 days. Pair-fed littermaters were given a similar diet in which ethanol, isocalorically substituted for carbohydrate, provided 36 per cent of total calories. With this regimen, blood ethanol concentrations, measured according to Bonnichsen,[10] in specimens obtained during periods of random access to ethanol, reached an average of 70 mg/100 ml and rarely exceeded 100 mg/100 ml. The rats were decapitated, livers were homogenized in isotonic KCl, and hepatic microsomes free from hemoglobin were prepared and suspended in 0·1 M phosphate buffer at a concentration of 3 mg of microsomal

TABLE 1. EFFECT OF CHRONIC ETHANOL PRETREATMENT ON THE BINDING OF CO, ETHYL ISOCYANIDE AND ETHANOL TO HEPATIC MICROSOMES

Rats*	CO difference spectra† ($\times 10^3$)	Ethyl isocyanide difference spectra 430 peak‡ ($\times 10^3$)	Ethyl isocyanide difference spectra 455 peak§ ($\times 10^3$)	Ethanol difference spectra‖ ($\times 10^2$)
Control	71·4 ± 3·7¶	49·8 ± 4·6	41·7 ± 2·7	0·238 ± 0·024
Ethanol-treated	129·8 ± 7·2	92·7 ± 6·5	71·2 ± 4·6	0·719 ± 0·065
	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

* Ethanol-treated rats were fed ethanol for 24 days. Control rats were fed a similar diet except that carbohydrate isocalorically replaced ethanol.
† Δ O.D. 450–500 nm/mg microsomal protein.
‡ Δ O.D. 430–500 nm/mg microsomal protein.
§ Δ O.D. 455–500 nm/mg microsomal protein.
‖ Ethanol concentration, 100 mM; Δ O.D. 415–500 nm/mg of microsomal protein.
¶ Mean ± standard error.

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protein per ml. Difference spectra were recorded in an Aminco-Chance split beam, dual wavelength recording spectrophotometer in the split beam mode. Cytochrome P-450 concentration was determined from the CO difference spectrum of dithionite-reduced microsomes.[11] The ethyl isocyanide difference spectrum of dithionite-reduced microsomes was determined as described previously.[12] Protein was determined by the method of Lowry *et al.*[13]

Chronic ethanol administration increased mean P-450 concentration 82 per cent ($P < 0.001$; Table 1). The spectrum of the ethyl isocyanide complex with the reduced hemoprotein displayed peaks at 430 and 455 nm.[14] The magnitude of both peaks was increased by chronic ethanol administration, and the ratio of the 455 nm peak to that at 430 nm was comparable in control and treated animals (Table 1). The hemoprotein induced by chronic feeding of ethanol had spectral characteristics similar to those induced by phenobarbital in that the ratio of the ethyl isocyanide peaks was unchanged from untreated animals,[14] and the maximum absorption of the CO spectra occurred at 450 nm.[12]

The difference spectra resulting from the addition of 100m M ethanol to the microsomal suspensions from control or ethanol-treated rats had a trough at about 390 nm and a peak at 415 nm (Fig. 1). In

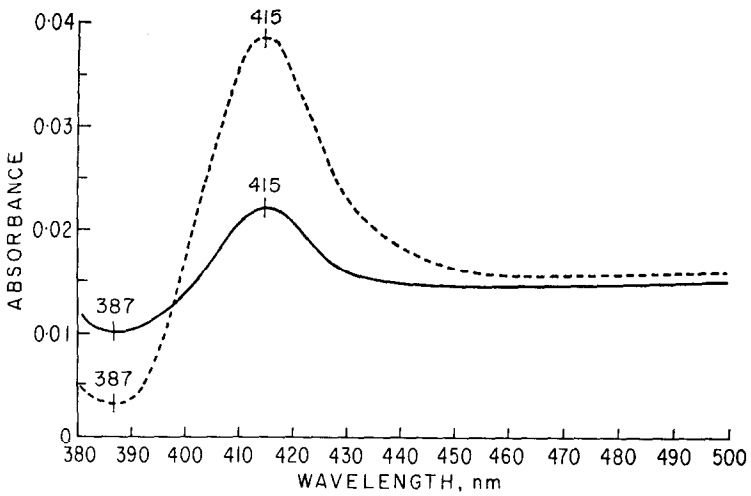

FIG. 1. Binding of ethanol to microsomes from untreated and ethanol-pretreated rats. Microsomal suspensions equivalent to 100 mg liver (wet weight) per ml of 0·1 M phosphate buffer were placed in sample and reference cuvettes. Ethanol (100 mM, final concentration) was added to the sample cuvette and the difference spectrum was recorded. Straight and dashed lines represent spectra from microsomes obtained from untreated and ethanol-treated rats respectively.

animals pretreated with ethanol, the mean magnitude of this peak (415–450 nm) per milligram of microsomal protein was tripled compared to controls ($P < 0.001$; Table 1). To ascertain whether the magnitude of the spectral change varied with ethanol concentration in a manner similar to that observed with binding of many drugs to microsomal hemoprotein, we used microsomes from two ethanol-treated rats, since the increased height of the 415 nm peak in such animals facilitated measurements. A double reciprocal plot of spectral change versus concentration of ethanol (25–125 mM) resulted in a straight line, with a spectral dissociation constant (K_s) of about 100 mM.

In studies using microsomes from untreated rats, Imai and Sato[15] attributed the ethanol-induced spectral change to reversible conformational changes in the hemoprotein, such as occur with the binding of many drugs. This seems a more likely explanation than a solvent effect or denaturation of the protein by ethanol, since; (1) chronic consumption of moderate amounts of ethanol, which results in low blood ethanol levels, increased the magnitude of the spectral change, indicating an effect *in vivo* of ethanol; and (2) chronic ethanol administration increases the activities of hepatic microsomal drug-metabolizing enzymes and P-450 concentration,[2,3] whereas a decrease would be anticipated on the basis of protein denaturation. It seems, therefore, more likely that the spectral change represents binding of ethanol to microsomal hemoprotein. This is supported by the location of the peak in the visible spectrum where hemoproteins absorb and by the linearity of the double reciprocal plots of spectral change versus ethanol concentration.

The spectral characteristics of ethanol binding are similar to, although not necessarily identical with, those observed upon the addition of hexobarbital to hepatic microsomes from animals treated with polycyclic hydrocarbons, which have been described as a modified type 2 binding.[16,17] However, the discrepancy between the increase of P-450 concentration (82%) produced by chronic ethanol feeding and the much larger increase in the ethanol-induced spectral change (300 %) in these animals indicates that the increased magnitude of ethanol binding in the induced animals does not strictly parallel cytochrome P-450 concentration. Ullrich[18] has suggested, on the basis of studies on rats treated with phenobarbital, that induced cytochrome P-450 may be more capable of forming the enzyme–substrate complex.

In conclusion, chronic ethanol administration *in vivo* induces microsomal hemoprotein having characteristics similar to the hemoprotein induced by phenobarbital. However, the spectral changes caused by the addition *in vitro* of ethanol to hepatic microsomes are similar to the changes seen when hexobarbital is added to microsomes from animals previously treated with polycyclic hydrocarbons.

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