The effects of ethanol and of a mixture of ethanol and higher-alcohols on the activity of microsomal aniline hydroxylase in the rat liver

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The chronic administration of ethanol to rats produces a proliferation of the smooth endoplasmic reticulum of the hepatocyte (Rubin, Hutterer & Lieber, 1968; Lieber & de Carli, 1968; Oudea, Collette & Oudea, 1973), and increases the activity of a number of hepatic microsomal enzymes including aniline hydroxylase (Tobon & Mezey, 1971). It has been suggested that the clinically observed resistance of chronic alcoholics to drugs such as sedatives may be due to the ethanol-induced increase in hepatic drug metabolizing enzyme activity (Misra, Lefèvre & others, 1971). However, very few of the drinks consumed by such patients contained ethanol alone (Chapman, 1970). Most also contain relatively small quantitities of higher aliphatic alcohols, amongst which n-propyl, n-butyl and iso-amyl alcohols are the most abundant and ubiquitous (Ginger, 1966). The effects of these alcohols upon the ethanol-induced changes in hepatic microsomal enzyme activity have not been previously studied, despite their obvious relevance to the clinical situation. n-Propyl, n-butyl and iso-amyl alcohol have been added to ethanol in doses only slightly greater than those found in commercial 'spirits' such as whiskey or brandy. The influence of chronic administration of this cocktail on drug metabolizing activity has been compared with ethanol alone.

Eighteen young male Wistar rats were housed individually and given free access to Oxoid laboratory animal breeding diet (Oxoid Ltd.). Six animals received ethanol alone (10% v/v in tap water for 28 days followed by 20% v/v for 42 days); six animals were given ethanol (10% v/v for 28 days and 20% v/v for 42 days) to which had been added n-propanol 804 mg litre^{-1} n-butanol 3·24 and iso-amyl alcohol 2·45 g litre^{-1} and a control group of 6 animals received water. All animals were denied food and allowed free access to tap water for the last 24 h before death.

After 70 days each animal was decapitated and the livers removed and homogenized in ice cold isotonic KCl. The microsomal pellet was obtained by differential centrifugation and the pellet was resuspended in 10·0 ml Na^+/K^+ phosphate buffer at 50 m mol litre^{-1} pH 7·4.

The activity of aniline hydroxylase in the microsomal preparation was measured by the method of Imai, Ito & Sato (1966). Microsomal protein was measured by the method of Lowry, Rosebrough & others (1951). The results were compared using the Mann-Whitney U test.

Chronic administration of ethanol was associated with a 49% increase in microsomal protein concentration ($P < 0{\cdot}05$) and a 49·5% increase in aniline hydroxylase activity ($P < 0{\cdot}02$) confirming previous reports (Tobon & Mezey, 1971). The aniline hydroxylase activity per gram of microsomal protein remained constant (Table 1) indicating an increase in functioning hepatic mass rather than an increase in enzyme activity. Chronic administration of ethanol with higher alcohols was associated with an 81% increase in microsomal protein concentration ($P < 0{\cdot}001$) and a 41% increase in aniline hydroxylase activity ($P < 0{\cdot}03$). The increase in microsomal protein concentration was greater in rats treated with ethanol plus higher alcohols than in those treated with ethanol alone ($P < 0{\cdot}002$). However, the aniline hydroxylase activity was not significantly different. Thus aniline hydroxylase activity per gram of micro-

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Table 1. *The effects of chronic administration of ethanol and the higher alcohols upon the activity of rat liver microsomal aniline hydroxylase (mean with s.d.).*

	Control	Ethanol alone	Ethanol plus higher alcohols
mg Microsomal protein g^{-1} liver	8·06 (2·43)	12·02* (1·30)	14·56* 1·14
μg *p*-Aminophenol formed g^{-1} liver per 20 min	0·99 (0·13)	1·48† (0·29)	1·40† (0·36)
μg *p*-Aminophenol formed mg^{-1} microsomal protein per 20 min	0·13 (0·04)	0·125 (0·03)	0·08‡ (0·03)

P values: Significance of difference from control using Mann-Whitney U Test: P = * <0·005, † <0·03 ‡ <0·02.

somal enzyme was less after treatment with the alcohol mixture than either after ethanol alone ($P<0·03$) or control ($P<0·02$). This suggests a direct effect of the higher aliphatic three alcohols which differs from that induced by ethanol alone.

There is no evidence that the higher aliphatic alcohols are metabolized by the microsomal enzyme system (Orme-Johnson & Ziegler, 1965), however, acute administration of higher aliphatic alcohols competitively inhibits microsomal aniline hydroxylase activity and is more potent in this respect than ethanol (Cohen & Mannering, 1973). This indicates that direct interaction between these alcohols and the microsomal enzyme system can occur. In the present experiment, direct enzyme inhibition was unlikely as the ethanol mixture was withdrawn 24 h before death.

The additional increase in microsomal protein due to the ethanol and higher alcohol mixture, in comparison to ethanol alone, might be either due to a direct inducing effect of the higher alcohols, or to a diversion of ethanol metabolism from cytoplasmic ethanol metabolism to microsomal metabolism, as these alcohols competitively inhibit alcohol dehydrogenase activity in pure enzyme preparations (Theorell & Bonnichsen, 1951) and in the isolated perfused rat liver (Auty & Branch, 1975). However, this does explain the inability of the additional microsomal protein to hydroxylate aniline; whether this microsomal protein has enzyme activity requires further elucidation, but it suggests the possibility of specific enzyme induction.

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