The Effects of Ethanol on the Activity of Rat Liver Tryptophan Pyrrolase

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The activity of haem-dependent tryptophan pyrrolase (L-tryptophan-O_2 oxidoreductase, EC 1.13.1.12) is enhanced in livers of rats treated with the porphyrogen 2-allyl-2-isopropylacetamide (Feigelson & Greengard, 1961), and patients with porphyria exhibit increased urinary excretion of tryptophan metabolites (Price, 1961). Ethanol produces

Fig. 1. Effects of ethanol on rat liver tryptophan pyrrolase activity in vivo
Ethanol (5ml/kg) was injected at zero time. Each point represents the mean value for four rats except that the zero-time points are means for 20 animals. The enzyme activity was measured as described in the text. ○, Total enzyme activity (that measured in the presence of 2μM-haematin); ●, holoenzyme activity (that in the absence of added cofactor).
porphyria (Gajdos, 1968) and enhances the activity of 5-aminolaevulinate synthetase (Shanley et al., 1968), which is the rate-limiting enzyme in the biosynthetic pathway of porphyrins and haem (Granick, 1966). The present report provides evidence suggesting that acute ethanol poisoning enhances the activity of rat liver tryptophan pyrrolase by a cofactor-type mechanism. The effects of chronic ethanol poisoning and of subsequent withdrawal are also described.

Male (225–300g) Wistar rats, maintained on M.R.C. cube diet 41B and water, were killed at between 12:00 and 15:00h and their livers were examined for tryptophan pyrrolase activity by the method of Feigelson & Greengard (1961b) either in the absence (holoenzyme) or in the presence (total enzyme) of added haematin. Ethanol, in intraperitoneal doses of 2–5ml/kg body wt., significantly increased the pyrrolase activity. At 5min after the injection of a 5ml/kg dose (Fig. 1) both holoenzyme and total enzyme activities were almost doubled; the holoenzyme activity returned to the basal value at 15min, and the total activity was moderately inhibited at 15–20min before returning to the basal value at 30min. At 3h almost all the endogenous apoenzyme became saturated with its haem activator. The holoenzyme activity then rose to a fivefold maximum at 5h and returned to normal after 12h. The initial increase in the enzyme activity (at 5min) may be due to an altered \([\text{NAD}^+] / [\text{NADH}]\) ratio, since the effect can be reproduced \textit{in vitro} by either NADH or ethanol plus NAD\(^+\) but not by ethanol or NAD\(^+\). The results of experiments involving actinomycin D, combined injections with cortisol or tryptophan and holo-/apo-enzyme ratio determinations suggest that the subsequent induction of pyrrolase activity by ethanol is cofactor in nature.

Chronic ethanol poisoning was produced by giving the rats 5\% (v/v) ethanol in
drinking water *ad libitum* and a daily intraperitoneal injection of 3 ml of ethanol/kg body wt. in the form of a 25% (v/v) solution in 0.9% (w/v) NaCl at between 11:00 and 12:00 h. On the day of killing, each group of four rats was not injected but was allowed free access to the 5% ethanol. For withdrawal experiments the ethanol injections were discontinued and the 5% ethanol was replaced with drinking water. Weight and fluid intake were recorded daily through the entire experimental period. The results are shown in Fig. 2. Both holoenzyme and total enzyme activities were significantly decreased ($P < 0.001$) from day 4 of ethanol treatment and remained depressed until day 13. The apoenzyme activity (total minus holoenzyme activity) was more sensitive to inhibition.

Groups of rats were withdrawn after 10 days of chronic ethanol treatment. The holoenzyme activity was the first to recover on withdrawal; the total enzyme activity reached normal values after 4 days of withdrawal, then both activities rose to 2.5 times the basal values ($P < 0.02$) a day later before falling to normal 10 days after ethanol withdrawal. It is suggested that the inhibition of the pyrrolase activity by chronic ethanol treatment is the result of an allosteric effect of NADH *in vivo* (Cho-Chung & Pitot, 1967), and that on withdrawal induction occurs by increased enzyme synthesis since the holo-/apo-enzyme ratio on day 5 of withdrawal is 0.87 compared with the basal value of 0.85. Further work with rats given ethanol orally is in progress and preliminary results agree with the present findings. Further experiments are being planned to investigate the relationship (Curzon, 1969) between tryptophan pyrrolase activity and brain 5-hydroxytryptamine concentration in rats chronically treated with ethanol, and also the excretion of products of both pathways of tryptophan metabolism in normal and alcoholic human subjects during ethanol treatment and withdrawal will be examined.

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**Investigation of the 5-Hydroxylation of Thiabendazole in Rat Liver Microsomal Preparations**

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Thiabendazole [2-(4′thiazolyl)benzimidazole], a broad-spectrum anthelmintic affecting gastrointestinal parasites of domestic animals, has been shown to be rapidly absorbed, metabolized and excreted in sheep, cattle, goats and swine (Tocco *et al.*, 1964). After oral administration of thiabendazole, most of the drug was excreted in the urine and faeces within 48 h as either free 5-hydroxythiabendazole or as the glucuronide or sulphate conjugate.

A method for the determination of the enzymic 5-hydroxylation of thiabendazole has been developed from the chemical assay procedure described by Tocco *et al.* (1964). By using this method thiabendazole 5-hydroxylation was found to require an NADPH-generating system and O$_2$ and was inhibited by CO, and occurred in the microsomal fraction of both rat liver and kidney but not in the heart, lung, gastrointestinal tract or serum.