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 12:00h. 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Groups of rats were withdrawn after 10 days of chronic ethanol treatment.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.


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 48h 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.
Inducers of drug-metabolizing enzymes such as phenobarbitone, administered to rats for 14 days in the drinking water (1 mg/ml, ad lib.), promoted a threefold increase in the specific activity of the thiabendazole 5-hydroxylase, with comparable increases in cytochrome P-450 and other drug-metabolizing enzyme activities. Pretreatment of male rats with 3-amino-1,2,4-triazole, an inhibitor of haem synthesis (Baron & Tephly, 1969), decreased thiabendazole 5-hydroxylase activity as well as the hepatic cytochrome P-450 concentration and aniline hydroxylase activity. From these observations it is concluded that the 5-hydroxylation of thiabendazole is cytochrome P-450-dependent. 

M and V values were 0.484 pmol of 5-hydroxythiabendazole produced/h per mg of protein. This is comparable with the $K_m$ values obtained for other xenobiotics, e.g. aniline (hydroxylation) ($4.1 \times 10^{-4}$ M; Kato et al., 1970) and aminopyrine (demethylation) $3.6 \times 10^{-4}$ M; Kato et al., 1969).

As thiabendazole is a nitrogen heterocycle and forms stable complexes with a number of metals including iron (Robinson et al., 1965), it might be expected to give a type II binding spectrum with cytochrome P-450. This suggestion is supported by the finding that thiabendazole 5-hydroxylation in vitro is uncompetitively inhibited by the type I substrate compound SKF-525A, whereas it is competitively inhibited by the type II substrates aniline and 1-dodecylimidazole. The type II binding spectrum of thiabendazole with cytochrome P-450 was confirmed in microsomal preparations from phenobarbitone-pretreated rats.

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The Relationship between Locust Gut β-Glucosidase and β-Galactosidase Activities

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The Enzyme Commission classification and nomenclature of glycosidases is based on observations of a typically very high (or absolute) group specificity exhibited by these enzymes to the glycone moiety of the substrate with a much lower order of specificity to the aglycone. Thus β-glucosidase (EC 3.2.1.21) refers to an activity catalysing the hydrolysis of a wide range of β-D-glucopyranosides in which the aglucone may be derived from an alcohol, phenol or sugar. Substrates for this enzyme would therefore include the disaccharides cellobiose and gentiobiose as well as alkyl and aryl β-D-glucosides. Similarly β-galactosidase (EC 3.2.1.23) is considered to exhibit an equally broad specificity to a series of β-D-galactopyranosides, including the disaccharide lactose, which are the 4-epimers of the β-D-glucoside substrate series.

In natural sources of glycosidases the two activities are often found together, sometimes apparently resulting from the presence of a single enzyme. The precise relationship between β-glucosidase and β-galactosidase is often complex and varies markedly with the source of the enzymes (see, e.g., Price & Robinson, 1966). Sometimes different enzymes appear to be responsible for the hydrolysis of aryl β-D-glycosides and the corresponding disaccharides. For these reasons the use of 'convenience' substrates such as nitrophenyl and 4-methylumbelliferyl glycosides requires caution and, in