young and old rats respectively were plasma clearance, 0·27 and 0·29 litre h^{-1}; plasma half-life, 5·4 and 5·6 h; apparent volume of distribution, 2·1 and 2·4 litre kg^{-1}. In contrast the concentrations of radioactivity in the brain were consistently and significantly two or three times greater in the old rats (Fig. 1). There was no consistent difference of this magnitude between young and old animals with the other organs examined; the mean old: young tissue radioactivity concentration ratio in the spleen, kidney, heart, liver and lung was 1·1.

The highest concentrations of radioactivity were found in the kidney and liver, followed by the spleen, heart and lung, with the lowest values in the plasma and brain (Fig. 1). High concentrations of radioactivity (similar to those found in the liver and kidney) were also measured in the wall of the small intestine (data not presented) although this could have been a direct result of injecting the drug intraperitoneally. Peak concentrations of radioactivity were achieved in plasma and brain by 2 h,but in some other tissues, and particularly in the old rats, peaks tended to occur later (Fig. 1). Over the period of the experiment the erythrocyte/plasma radioactivity concentration ratio was 0·6–0·7.

In the brain or liver of the young rats killed at 3 h at least 85% of the radioactivity could be identified chromatographically as unchanged nitrazepam. The remaining radioactivity on the chromatograms appeared to be "background" and did not correspond clearly to any particular chromatographic locus.

Our results in young rats essentially agree with those of previous workers using [^{14}C]nitrazepam in rats (Tanayama & others, 1974; Yanagi & others, 1975) in that the brain: plasma radioactivity concentration ratio was about 0·7 and that the liver concentrations of radioactivity were markedly higher than in the plasma and brain. The higher concentration of [^{14}C]nitrazepam-derived radioactivity in the brain of old rats was surprising and it is possible that an analogous distribution change in man could account, wholly or in part, for the increased sensitivity to nitrazepam seen in elderly subjects and patients.

We thank F. Hoffman-La-Roche and Co., Basle, Switzerland for a gift of 5[^{14}C]nitrazepam.

December 9, 1977

REFERENCES

CASTLEDEN, C., GEORGE, C., MARCER, D. & HALLETT, C. (1977), *Br. med. J.*, **1**, 10–12.
DIXON, R. L., ADAMSON, R., BEN, M. & RALL, D. (1965). *Proc. Soc. exp. Biol. Med.*, **118**, 756–759.
EVANS, J. & JARVIS, E. (1972). *Br. med. J.*, **4**, 487.
IISALO, E., KANGAS, L. & RUIKKA, I. (1977). *Br. J. Clin. Pharmac.*, **4**, 646P–647P.
TANAYAMA, S., MOMOSE, S. & KANAI, Y. (1974). *Xenobiotica*, **4**, 229–337.
YANAGI, Y., HAGA, F., ENDO, M. & KITAGAWA, S. (1975). *Ibid.*, **5**, 245–257.

Ethanol and the disposition of amylobarbitone: effect of dose and significance as a mechanism for increased toxicity

W. J. TILSTONE*, P. C. REAVEY**, *Forensic Science Unit, Department of Pharmaceutical Chemistry, University of Strathclyde, Glasgow, U.K. and **Department of Biochemistry, Royal Infirmary, Glasgow, U.K.*

Although there is a general acceptance that the clinical toxicity of barbiturate hypnotics is increased by concomitant ingestion of ethanol (Patel, Roy & Wilson, 1972) the results of animal experiments conflict about whether the interaction is one of synergism (Wiberg, Coldwell & Trenholm, 1969), addition (Smith & Hexheimer, 1969) or indeed of antagonism (Curry & Scales, 1973). Considering only those studies which indicate that ethanol increases the toxicity of barbiturates, there remains disagreement about the mechanism. In particular, it is not certain to what extent impairment of drug metabolism by ethanol, with a consequent prolongation of half-life of the barbiturate, is the cause of the increased toxicity (Wiberg & others, 1969; Schuppel, 1972).

* Correspondence.

Schuppel has reviewed the results of his group's experiments on the *in vitro* effects of ethanol on microsomal mixed function oxidase activity and on the *in vivo* elimination of a number of drugs in the rat when ethanol is co-administered, and concludes that inhibition of barbiturate metabolism due to ethanol is the cause of the increased toxicity of barbiturates which they have observed (Schuppel, 1972).

We have examined the effects of hypnotic and non-hypnotic doses of ethanol on the effects, distribution and elimination of amylobarbitone in adult male Wistar rats. The drugs were given as intraperitoneal injections of aqueous solutions. The plasma disappearance of amylobarbitone was measured by collecting blood from groups of 4 rats decapitated at various intervals between 20 and 300 min after injection of 100 mg kg^{-1} amylobarbitone sodium, or of 100 mg kg^{-1} amylobar-

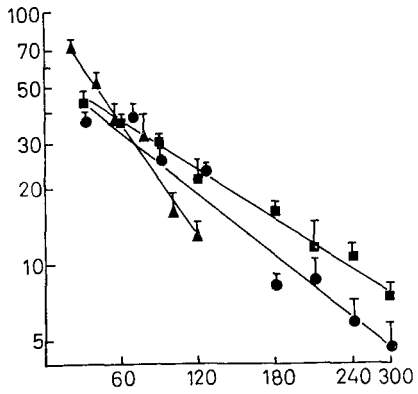

FIG. 1. Plasma amylobarbitone in the rat following intraperitoneal injection of 100 mg kg^{-1} (▲—▲); 100 mg kg^{-1} together with ethanol, 0·8 g kg^{-1} (●—●; and 100 mg kg^{-1} together with ethanol, 3·2 g kg^{-1} (■—■). Results are mean and standard deviation. Ordinate: Plasma amylobarbitone (μg ml^{-1}). Abscissa: Time (min).

bitone sodium together with 0·8 or 3·2 g kg^{-1} ethanol. Dose-response curves were constructed for doses of 20–160 mg kg^{-1} amylobarbitone sodium alone or together with 0·64–5·1 g kg^{-1} ethanol. A dose-response curve was also constructed for ethanol by itself at 0·5 to 7·0 g kg^{-1}. The response studied was loss of righting reflex. Plasma amylobarbitone concentrations were measured by a gas chromatographic method based on that of Berry (1969), with a measured accuracy of 99·0% and precision of 3·5%. The overall efficiency of elimination of amylobarbitone was assessed by measuring its clearance from plasma (dose/area under plasma concentration-time curve from 0 to infinity). The slope of the log-linear part of the plasma concentration-time curve was computed by linear least squares analysis to give the first order rate constant for amylobarbitone elimination. Examination of the data showed a one compartment model adequate to describe the time course of the plasma amylobarbitone concentration, and the volume of distribution was thus calculated from the intercept of the linear regression. Since the elimination rate constant is the fraction of the volume of distribution cleared of amylobarbitone in unit time, its

Table 1. *The influence of sub-hypnotic (0·8 g kg^{-1}) and hypnotic (3·2 g kg^{-1}) doses of ethanol on amylobarbitone (100 mg kg^{-1}) disposition in Wistar rats.*

Ethanol (g kg^{-1}, i.p.)	Volume distribution ml kg^{-1}	Elimination rate constant h^{-1}	Clearance from plasma ml min^{-1}	Half-life h
0 (n = 24)	939	1·06	16·6	0·65
0·8 (n = 32)	1800**	0·57**	17·1	1·22**
3·2 (n = 24)	1803**	0·39**	11·7**	1·78**

** Different from amylobarbitone alone, $P < 0.05$, by analysis of variance.

magnitude, and thus the half-life of amylobarbitone, depends on both clearance and volume of distribution (Gibaldi & Perrier, 1975).

In all experiments, administration of ethanol increased the duration of loss of righting reflex but did not affect the minimum effective dose of amylobarbitone (mean 25·6 mg kg^{-1}). The minimum effective dose of ethanol was found to be 2·55 g kg^{-1}. Administration of 0·8 g kg^{-1} ethanol together with amylobarbitone did not affect the clearance of amylobarbitone from plasma. That is, there is no evidence of impaired amylobarbitone metabolism at this dose of ethanol. The volume of distribution of amylobarbitone was markedly increased by the ethanol and therefore the fractional elimination rate fell, leading to a significantly prolonged half-life. In contrast, administration of 3.2 g kg^{-1} of ethanol reduced amylobarbitone clearance in addition to increasing its volume of distribution. The prolongation of amylobarbitone half-life by this hypnotic dose of ethanol is thus due partly to redistribution and partly to impaired amylobarbitone metabolism. The results are summarized in Table 1 and Fig. 1. The original data are available from the authors on request.

The results show that the fractional elimination of amylobarbitone is reduced by concomitant administration of ethanol, the effect of hypnotic doses thereby being prolonged, but only at high doses of ethanol do the results support the *in vitro* observations of altered barbiturate metabolism. The interaction occurs with doses of ethanol which are well below the measured minimum hypnotic dose of ethanol given by itself.

Supported by the Scottish Hospitals Endowments Research Trust.

December 14, 1977

REFERENCES

BERRY, D. J. (1969). *J. Chromat.*, **42**, 39–44.
CURRY, S. H. & SCALES, A. H. (1973). *J. Pharm. Pharmac.*, **25**, 142P.
GIBALDI, M. & PERRIER, D. (1975). *Pharmacokinetics*, New York: Dekker.
PATEL, A. R., ROY, M. & WILSON, G. M. (1972). *Lancet*, **2**, 1099–1102.
SCHUPPEL, R. (1972). *Proc. Eur. Soc. Study Drug Tox.*, **13**, 52–62.
SMITH, S. E. & HEXHEIMER, A. (1969). *J. Pharm. Pharmac.*, **21**, 869–870.
WIBERG, G. S., COLDWELL, B. B. & TRENHOLM, H. L. (1969). *Ibid.*, **21**, 232–236.