KNOLL, J. & MAGYAR, K. (1972). Some puzzling pharmacological effects of monoamine oxidase inhibitors. *Adv. Biochem. Psychopharmac.*, **5**, 393–408.

ROTH, J.A. & GILLIS, C.N. (1975). Multiple forms of amine oxidase in perfused rabbit lung. *J. Pharmac. exp. Ther.*, **194**, 537–544.

SOUTHGATE, J. & COLLINS, G.G.S. (1969). The estimation of monoamine oxidase using ^{14}C-labelled substrates. *Biochem. Pharmac.*, **18**, 2285–2287.

YANG, H.Y.T. & NEFF, N.H. (1973). β-Phenylethylamine: a specific substrate for type B monoamine oxidase of brain. *J. Pharmac. exp. Ther.*, **187**, 365–371.

The metabolic fate of [^{14}C]-phenobarbitone in the rat and the effect of chronic administration and dose size

J. CALDWELL, JANE E. CROFT, R.L. SMITH & W. SNEDDEN†

Department of Biochemical and Experimental Pharmacology, St. Mary's Hospital Medical School, London W2 1PG and †Mass Spectrometry Group, St. Bartholomew's Hospital, London EC1A 7BE

The metabolic fate of phenobarbitone (5-ethyl-5-phenylbarbituric acid) has been studied in the rat as part of an examination of the biochemical basis of the tolerance which occurs to this drug. The effect of dose size and chronic administration on its metabolism has also been examined. [^{14}C]-Phenobarbitone, labelled in the 2-position of the barbituric acid ring (50 mg/kg; 10 μCi/animal), was injected intraperitoneally into female Wistar albino rats (body weight 180–200 g). Urine and faeces were collected for 8 days after administration and $^{14}CO_2$ in the expired air collected for 2 days. The excretion of ^{14}C was monitored by liquid scintillation counting and the metabolites present in the urine examined by thin layer chromatography followed by radiochromatogram scanning, reverse isotope dilution, gas-liquid chromatography and gas chromatography-mass spectrometry (g.c.-m.s.). Over the 8 days of the experiment, some 88% of the administered ^{14}C was recovered, with 79% in the urine, 0.14% as $^{14}CO_2$ in the expired air and 9% in the faeces. The bulk (75%) of the urinary ^{14}C was excreted in the first 3 days, with 43% appearing in the first 24 hour. After the 4th day, some 0.5–1% of the dose was excreted in the urine per day. Analysis of the 0–24 h urine by the techniques described revealed the presence of unchanged phenobarbitone (14% of dose), *p*-hydroxy-phenobarbitone, excreted free (16%) and as its glucuronide conjugate (9%) and an unknown acidic water-soluble metabolite (4%). The identities of these metabolites were confirmed by comparison of their g.c.-m.s. properties with standards, and the g.c.-m.s. of the unknown suggested that it was ethylphenyl-malonylurea, arising from the ring opening of phenobarbitone. The nature of the faecal radioactivity was not determined. Phenobarbitone and its metabolites were excreted at similar rates, and since the excretion half life of total ^{14}C was quite long (ca. 1.25 days) it would be expected that phenobarbitone and its metabolites would be accumulated when the drug was administered on a daily basis.

In other experiments, the excretion of [^{14}C]-phenobarbitone by bile-duct cannulated rats was examined. After the i.p. injection of 50 mg/kg (10 μCi/animal), 35% of the dose was excreted in the bile in 24 h with a further 18% in the urine. The excretion of ^{14}C in the bile proceeded at a fairly constant rate of about 1.5% of dose/h over the 24 h of the experiment. The metabolites present in bile were examined as above, and comprised phenobarbitone (5% of dose) *p*-hydroxyphenobarbitone glucuronide (21%) and the unknown metabolite (9%). No free *p*-hydroxyphenobarbitone was found in bile.

The possible variation in the metabolism of phenobarbitone with dose was also studied. Using the methods described, rats were dosed with 5 mg/kg and 100 mg/kg [^{14}C]-phenobarbitone by i.p. injection (10 μCi/animal), and the excreta analysed for ^{14}C and metabolites. With both these doses, the rate of excretion and nature of the metabolic products was essentially similar to the findings reported with a 50 mg/kg dose.

Analysis of the tissue distribution of ^{14}C 24 h after the i.p. injection of [^{14}C]-phenobarbitone (50 mg/kg; 20 μCi/animal) showed that the bulk of the radioactivity remaining unexcreted was associated with the muscle, fat and bones of the carcass (16%) and in the blood (red cells 3%/ml; plasma 1%/ml). Some 7% of the dose was associated with the vital organs, in the order liver > kidney > lung > heart > gut wall > brain, the brain containing 0.2% of dose.

In further experiments, rats were treated daily with phenobarbitone (50 mg/kg i.p.) (treated group) or the injection vehicle, 90% propane-1,2-diol (2 ml/kg i.p.) (control group) for up to 14 days. Groups of rats were taken after 4 days or 14 days chronic administration, injected with [^{14}C]-phenobarbitone (50 mg/kg; 10 μCi/animal) and their urine and faeces collected for 3 days. Analysis of ^{14}C and metabolites in the urine was performed as described. The excretion of ^{14}C in the urine and faeces of both treated and control

groups was the same as after the single 50 mg/kg dose, and the urine contained the same four metabolites. There were no marked differences between the groups, but there was a trend for the treated animals to excrete more of the acidic unknown metabolite and less free *p*-hydroxyphenobarbitone than after the acute administration.

It has long been known that phenobarbitone is a powerful inducer of the drug metabolizing enzymes of the liver microsomes (Remmer, 1962), and it is therefore interesting to see that repeated phenobarbitone administration does not markedly affect its rate of excretion or pattern of metabolism, although this is the case with many other compounds. In view of this, and of the possible accumulation of phenobarbitone and its metabolites in the body on chronic administration, it provides further support for the view that the tolerance which occurs to this drug does not have its origin in altered drug disposition (see Caldwell & Sever, 1974).

This work was supported by a grant from the Mental Health Trust and Research Fund. We thank the Wellcome Trust and the Joint Research Board, St. Bartholomew's Hospital for funds for mass spectrometry.

References

CALDWELL, J. & SEVER, P.S. (1974). The biochemical pharmacology of abused drugs II. Alcohol and barbiturates. *Clin. Pharmacol. Ther.*, **16**, 737–749.

REMMER, H. (1962). Drug tolerance. In *Ciba Symposium on Enzymes and Drug Action*, ed. Mongar, J.L. & DeReuck, A.V.S., pp. 276–298. London, J. & A. Churchill, Ltd.

Self induction of metabolism by ketamine in the rat

A. LIVINGSTON & A.E. WATERMAN

Department of Pharmacology, University of Bristol, Bristol BS8 1TD

In a previous communication to the Society (Livingston & Waterman, 1976) we indicated that tolerance following repeated administration of ketamine was due to enzyme induction. We have now confirmed that pretreatment with ketamine shortens its duration of action and we have also investigated its effect on plasma levels of ketamine and its metabolites as well as on the rate of metabolism of ketamine by liver homogenate preparations *in vitro*.

The pretreatment schedule consisted of 10 daily injections of ketamine hydrochloride (40 mg/kg i.p.) the last injection being given 24 h prior to further experiment (control rats received saline injections). The duration of action of a single dose of ketamine (75 mg/kg i.p.) was then measured in control and treated rats. In addition, animals were killed at various times after injection in order to obtain blood for the assay of plasma levels of ketamine and its metabolites as previously described (Livingston & Waterman, 1976).

The pretreated rats, when compared to controls, showed a significant decrease in sleeping time (11.73 ± 0.95 compared to 23.11 ± 1.4 min), there was a significant decrease in their plasma levels of ketamine and metabolite I and a significant increase in their plasma metabolite II levels (Figure 1).

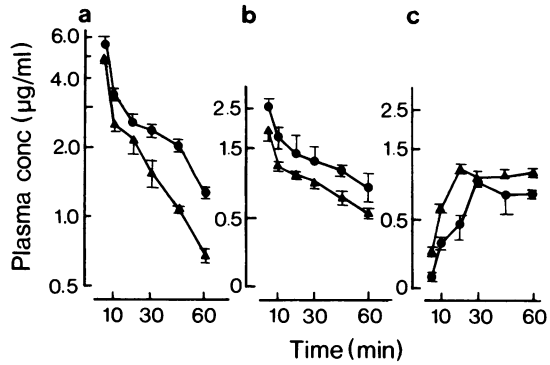

Figure 1 Plasma levels of Ketamine (A) Metabolite I (B) and Metabolite II (C) after i.p. administration of Ketamine (75 kg/mg) to rats, pretreated with either Ketamine (▲) or saline (●). The rats were killed at the times indicated after injection and blood collected for assay of plasma levels of ketamine and its metabolites. Each point represents the mean of 4 animals ± s.e. mean.

The metabolism of ketamine *in vitro* was investigated in homogenates of livers from control and ketamine pretreated rats using the method of Cohen & Trevor (1974). Samples (0.1 ml) were taken from the reaction vessels at zero time and after, 5, 10, 15, 30, 60 and 120 min incubation with ketamine (initial conc. 100 µg/ml) for the assay of ketamine and its metabolites.