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).

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Self induction of metabolism by ketamine in the rat

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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).

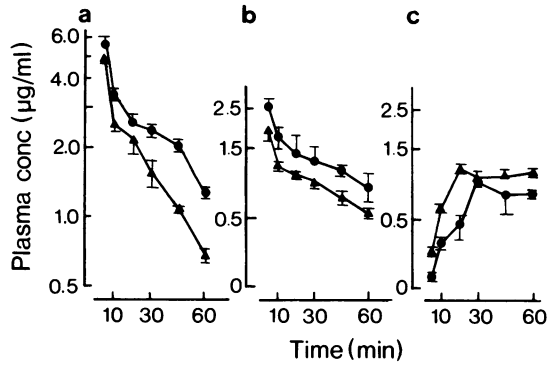

Figure 1 Plasma levels of Ketamine (A) Metabolite I (B) and Metabolite II (C) after i.p. administration of Ketamine (75 kg/mg) to rats, pretreated with either Ketamine (▲) or saline (●). The rats were killed at the times indicated after injection and blood collected for assay of plasma levels of ketamine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

The only detectable product of the metabolism of ketamine *in vitro* was metabolite I (the *n*-demethylated product) and pretreatment increased the rate of *n*-demethylation of the drug by an average of 122%. We have so far been unable to demonstrate the further metabolic conversion of metabolite I *in vitro*.

A.E.W. is a Wellcome Research Training Scholar.

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On the detectability of anabolic steroids in horse urine

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The antibody raised against 19-nortestosterone 3-(*O*-carboxymethyl)oxime-bovine serum albumen in Soay ewes (Jondorf, 1977) has been characterized. It can be used for following the excretion of various anabolic steroid preparations administered to horses in veterinary doses.

Cross-bred and thoroughbred geldings maintained under standard conditions of feeding and exercise in our stables were injected intramuscularly with one of the following: 19-nortestosterone laurate (200 mg; 0.46 mg/kg), 19-nortestosterone phenylpropionate (400 mg; 0.75 mg/kg), 19-nortestosterone cyclohexylpropionate (100 mg; 0.25 mg/kg), 19-nortestosterone (150 mg; 0.40 mg/kg).

Urine samples collected from experimental animals before administration of anabolic steroid, and sequentially after administration, were kept deep-frozen (–20°C) until required for analysis. Urines were then thawed, adjusted to pH 9 and were extracted with equal volumes of chloroform/methanol (9 : 1 v/v) in rotary separators for 20 minutes. Extracts (1 ml) were analysed by radioimmunoassay (Jondorf, 1977) after removal of solvent, with [^{3}H]-19-nortestosterone as marker, and with unlabelled 19-nortestosterone as standards (100–2000 pg). For comparison, unextracted urine samples (0.01 ml) and residues after solvent extraction (0.01 ml) were also analysed in some instances.

The sensitivity of the radioimmunoassay method is such that a biological sample should contain more than 100 pg of 19-nortestosterone equivalent when analysed.

As shown in Figure 1, the urinary fate of 19-nortestosterone laurate can be followed for more than 45 days, at which time the depression of binding is still in the range equivalent to 100–250 pg 19-nortestosterone. It is known from studies on the metabolism of 19-nortestosterone in the rabbit (Both-Miedema *et al.*, 1972) and in the horse (Houghton, 1977) that the drug is metabolized to isomers of 5-oestrane-3,17-diols, and that these isomers cross-react with the antibody used in these assays (Jondorf, 1977). The detectability of anabolic steroid after administration of a veterinary preparation is not therefore dependent solely on the excretion of unchanged, de-esterified parent steroid.

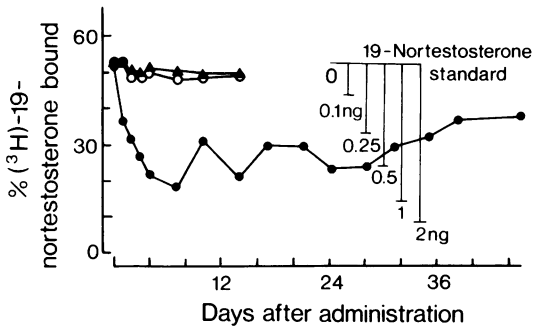

Figure 1 The depression of binding of [^{3}H]-19-nortestosterone in horse urine after administration of 19-nortestosterone laurate (200 mg, i.m.). Radioimmunoassay with (●) 1 ml chloroform/methanol extract (○) 0.01 ml urine (▼) 0.01 ml urine after solvent extraction.

In the corresponding experiments with the administration of other esters, the urinary detectability for the phenylpropionate and the cyclohexylpropionate were >15 days and >10 days respectively. 19-Nortestosterone itself was excreted fairly rapidly in contrast (detectable for ca. 2 days).

Among anabolic steroids cross-reacting with the antibody, and administered under the same conditions, trienbolone acetate (75 mg; 0.17 mg/kg) and 1-dehydrotestosterone undecylenate (250 mg; 0.66 mg/kg) could be detected >5 days and >21 days respectively.