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.

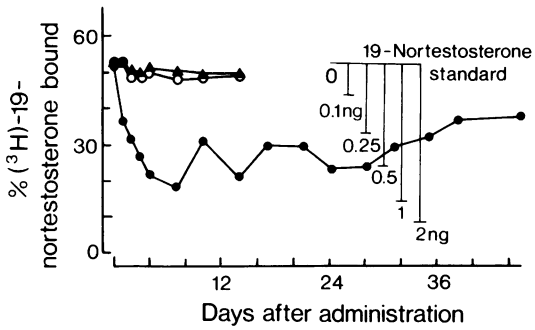

Figure 1 The depression of binding of [^{3}H]-19-nortestosterone in horse urine after administration of 19-nortestosterone laurate (200 mg, i.m.). Radioimmunoassay with (●) 1 ml chloroform/methanol extract (○) 0.01 ml urine (▼) 0.01 ml urine after solvent extraction.

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.

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Hypothalamic polypeptides and analogues on corticotrophin production by rat adenohypophysial tissue *in vitro*

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Corticotrophin releasing hormone (CRH), the hypothalamic releasing factor which controls the secretion of corticotrophin (ACTH) by the adenohypophysis, appears to be a polypeptide similar to but not identical with vasopressin. We have attempted to obtain more information about its precise nature by studying the corticotrophin releasing activity of hypothalamic polypeptides and their analogues. Their ability to stimulate corticotrophin synthesis and release by pituitary tissue *in vitro* was assessed. Segments of rat adenohypophysial tissue were incubated in medium containing the putative releasing hormone and the ACTH contents of the tissue and the incubation medium were then determined using a modification (Alaghband-Zadeh, Daly, Bitensky & Chayen, 1974) of the sensitive and precise cytochemical assay method (Chayen, Loveridge & Daly, 1972). Pressinoic acid, its amide, oxytocin, 8-alanine vasopressin, and the tail fragment (proline-arginine-glycinamide) of arginine vasopressin were ineffective and the desglycinamide derivatives of lysine- and arginine vasopressin were only slightly effective in stimulating ACTH synthesis and release. Arginine- and lysine vasopressin caused dose related increases in ACTH production but both were less active than oxypressin and arginine vasotocin. Arginine vasotocin was the most active compound studied and, in small doses which alone did not influence ACTH production, also markedly potentiated the action of hypothalamic extract.

The corticotrophin releasing activity of the polypeptides was also tested in pentobarbitone/chlorpromazine treated rats (de Wied, 1967) and, although the method is considerably less sensitive than the *in vitro* technique, the results were similar. Comparison of the corticotrophin releasing potencies of such compounds with hypothalamic extract, using different assay systems, may lead to a clearer understanding of the nature of the corticotrophin releasing hormone(s).

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