The quantitation of metyrapone and its reduced derivative in urine

We have previously shown (Sprunt, Browning & Hannah, 1968) that the principal metabolite of metyrapone [(2-methyl-1,2-di(3-pyridyl)propan-1-one] in man is reduced metyrapone [2-methyl-1,2-di(3-pyridyl)propan-1-ol]. We now report a method for quantitating metyrapone and reduced metyrapone in urine, using gas-liquid chromatography.

The samples of urine were obtained from 9 patients undergoing a metyrapone test (Liddle, Estep & others, 1959). 24-h urines were collected on the day before metyrapone administration (Day 0), the day of metyrapone administration (Day 1), and the two days following metyrapone (Days 2 and 3). Metyrapone was administered orally in a dose of 750 mg every 4 h, the total dose being 4·5 g.

The urines were stored at −10° until the analysis could be done. The pH of the 24-h urines was adjusted to 7·0, and then 5 ml aliquots were taken. These were extracted with 2 × 10 ml methylene chloride to obtain free metyrapone and free

Table 1. *The mean values of metyrapone and reduced metyrapone recovered from the urine of 9 patients undergoing the metyrapone test. Metyrapone was administered orally on Day 1 in a dose of 750 mg every 4 h (total dose 4·5 g).*

	Day 1				Day 2			
	Metyrapone (mg)		Reduced metyrapone (mg)		Metyrapone (mg)		Reduced metyrapone (mg)	
	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.
Mean	19·2	185·9	98·7	729·9	2·7	30·6	41·4	754·5
± s.e.	3·8	9·4	7·9	11·4	0·9	5·4	10·2	14·8

reduced metyrapone. Following this extraction, the pH of the urine was adjusted to 5·0 and 1 ml of 0·5M acetate buffer added. The urine was then incubated with β-glucuronidase (prepared from the visceral hump of the limpet, *Patella vulgata*) for 36 h at 37°. The pH of the urine was re-adjusted to 7·0 before again extracting with methylene chloride (2 × 10 ml). This extract contained metyrapone and reduced metyrapone which had been excreted as the glucuronides.

The methylene chloride extracts were taken to dryness before acetylation with 0·1 ml acetic anhydride and 0·1 ml pyridine, overnight at 37°. Metyrapone remains unchanged, but reduced metyrapone acetylates to a more stable derivative. After removal of the pyridine, the extracts were taken up in chloroform, and SU 9055 [3-(1,2,3,4-tetrahydro-1-oxo-2-naphthyl)pyridine] was added as internal standard.

A Pye Series 104 chromatograph was used. A 7 foot $\frac{1}{4}$ inch o.d. silicanized glass column was packed with 100/120 mesh Gas Chrom Q coated with 3% XE-60 (Applied Sciences). The gas flow rates were nitrogen 40 ml/min, hydrogen 45 ml/min, and air 600 ml/min measured at room temperature. The operating temperature of the column was 215°.

5 μl samples were injected into the chromatograph. In the range 1–5 μg, the ratio of the peak heights of metyrapone and the acetylated reduced metyrapone to those of the standard, SU 9055, were linear. The retention times under the stated conditions were metyrapone 2·3 min, acetylated reduced metyrapone 4·2 min, and SU 9055 5·7 min. The recoveries of metyrapone and reduced metyrapone added to urines were 94·2% ± 9·8 (s.d.) and 93·9% ± 7·3 respectively.

In Table 1 are shown the mean values for metyrapone and reduced metyrapone measured in the urine of the 9 patients undergoing the metyrapone test. Both compounds were excreted mainly as the glucuronides. Reduced metyrapone occurred in appreciably larger amounts than metyrapone, particularly on Day 2 when little change was seen in the level of reduced metyrapone, but the level of metyrapone fell markedly. Small but significant quantities (108 mg $\pm$ 14·9 s.e.) of reduced metyrapone could still be detected on Day 3. The mean quantity of metyrapone plus reduced metyrapone recovered from the urine on Days 1–3 was 1·97 g $\pm$ 0·13 s.e.

Reduced metyrapone is quantitatively an important metabolite of metyrapone. It is not an inactive metabolite, being as potent an inhibitor of ox and human adrenal 11β-hydroxylase as metyrapone (Sprunt & Hannah, 1968).

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Alterations in noradrenaline turnover in the peripheral sympathetic neurons induced by stress

It has long been known that injected noradrenaline can be taken up and concentrated in peripheral sympathetic nerves (Whitby, Axelrod & Weil-Malherbe, 1961; Hamberger, Malmfors & others, 1964). Furthermore, noradrenaline incorporation is greatest in tissues with rich sympathetic innervation (Kopin, 1966). In addition to the normal endogenous noradrenaline content, the uptake of noradrenaline into tissues after intravenous injection is related to the proportion of cardiac output delivered to the tissue. Endogenous transmitter and exogenously given tritiated noradrenaline (^{3}H-NA) can be released by nerve impulses which, together with other evidence, indicate that the latter is present in the same store as the endogenous catecholamine (Iversen, 1967). Thus, the disappearance rate and the impulse flow seems to be correlated. I now report the influence of various stress conditions, which might increase sympathetic tone, on the disappearance rate of administered ^{3}H-NA.

Male albino rats were injected with ^{3}H-NA 1 μg/kg, i.v., 3 h before stress was induced. The animals were stressed for 3 h and then killed by exsanguination under light chloroform anaesthesia. Various peripheral organs were dissected and homogenized with an "Ultra-Turrax" apparatus in ice cold 0·4 N perchloric acid. After centrifugation, filtration and neutralization, the samples were passed through Dowex 50 cation-exchange columns from which the noradrenaline was eluted (Carlsson & Waldeck, 1963). The ^{3}H-NA content was measured by liquid scintillation counting technique (Waldeck, 1968).

Three different kinds of stress were investigated, (1) cold stress, (2) stress induced by electric shock, (3) immobilization stress. For (1) the rats were shaved the day before the experiment under light ether anaesthesia. They were maintained at an environmental temperature of +3–4° for 3 h. The rectal temperature was +32–35° at