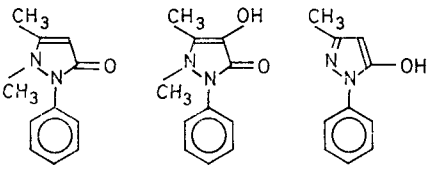
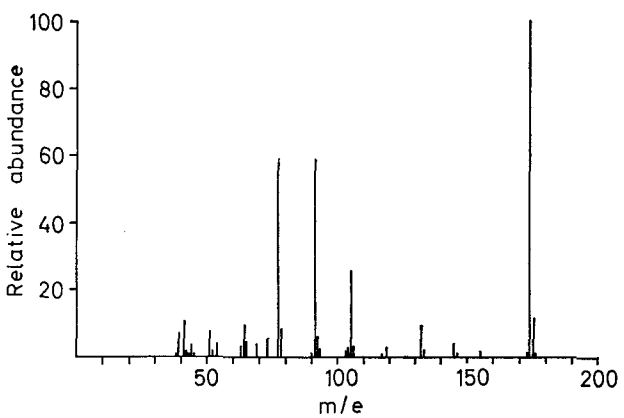
Norphenazone, a new metabolite of phenazone in human urine

Phenazone (2,3-dimethyl-1-phenyl-5-pyrazolone (I) still has a use in metabolic studies in man. According to Brodie & Axelrod (1950) it is metabolized to a 4-hydroxylated derivative (II) which is excreted as a glucuronide. We have recently found norphenazone (3-methyl-1-phenyl-5-pyrazolone) (III), previously reported to occur in the urine of phenazone-treated rats (Schüppel, 1966), also to be present in the urine of man.



I II III

The 24 h urine from 6 fasted (9 h) male volunteers who had each ingested 600 mg of phenazone, was pooled and stored at −20°. The samples were hydrolysed by a crude β-glucuronidase enzyme preparation (12 h; pH 4·5; 20°) in acetate buffer. They were then extracted at pH 2, 7 and 14, using methylene chloride (2× vol of urine). The extracts were analysed using a Pye 104 chromatograph combined with an MS 12 mass spectrometer and a DS 30 data system (Associated Electrical Industries Limited). Chromatography conditions used were: glass column, 7 ft × $\frac{1}{8}$ in; packing: 3% OV1 on 100/120 mesh Gas Chrom Q; oven temperature 180°; carrier gas He at 30 ml min^{-1}. A compound whose mass spectrum is shown in Fig. 1 was thought to be a demethylated phenazone. 3-Methyl-1-phenyl-5-pyrazolone was synthesized according to Koike, Iida & others (1954), and the retention time and mass



spectrum of this material were found to be identical with those of the suspected metabolite. The latter compound co-ran with authentic norphenazone (R_F 0·46) on thin layers of silica gel using benzene–ethyl acetate–ethanol (4:5:1). The compound cannot be extracted from hydrolysed urine at pH 14, an observation which suggests that norphenazone exists as shown (III). It cannot be extracted from unhydrolysed urine, hence it must exist in a conjugated form as a glucuronide or sulphate.

The results were correlated with known weights of norphenazone, phenazone and 4-hydroxyphenazone added to blank urine and analysed in an identical manner.

For the detection of the 4-hydroxyphenazone, the extracts were converted to trimethylsilyl ethers before injection on the column, since we were unable to obtain satisfactory results with the free compound. The retention times of the compounds relative to phenazone ($t_R = 1{\cdot}0$) using the same g.l.c. conditions as above, except N_2 at 20 ml min^{-1} was the carrier gas, were: norphenazone 0·32, trimethylsilyl 4-hydroxyphenazone 0·9. The retention time of phenazone under these conditions was 9·6 min. Approximately 6% of the total dose of phenazone was excreted as norphenazone in the 24 h following ingestion of the drug. [In subjects taking phenazone after a course of phenobarbitone, the amount of norphenazone produced was not affected even though there was a considerable increase in the amount of the 4-hydroxyphenazone excreted.]

We thank the Medical Research Council for the provision of the mass spectrometer and data system; and the United Liverpool Hospitals Medical Research Committee for the gas chromatograph combined to the mass spectrometer. We are indebted to Mrs. P. A. Robinson for skilled technical assistance.

Pharmacogenetics Division.
Department of Medicine,
University of Liverpool,
Liverpool, L69 3BX, U.K.
September 5, 1972.

J. D. BATY
D. A. PRICE EVANS

REFERENCES

BRODIE, B. B. & AXELROD, J. (1950). *J. Pharmac. exp. Ther.*, **98**, 97–104.

KOIKE, E., IIDA, H., OKAUA, M., & KASHIOKA, A. (1954). *J. chem. Soc. Japan*, **57**, 56–58. (See *Chem. Abs.* (1955), 49, p. 11629.)

SCHÜPPEL, R. (1966). *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **255**, 71–72.

Effects of intracerebral injections of 6-hydroxydopamine on sleep and waking in the rat

Monoamines in the central nervous system are considered to play an important role in the onset and maintenance of the different forms of sleep (Jouvet, 1969). Noradrenergic neurons are implicated not only in the induction of paradoxical sleep (PS) (Roussel, 1967) but have also received attention of being possible mediators of waking (Jones, Bobillier & Jouvet, 1969; Jones, 1969, 1972). Suppression of PS occurred (Jouvet, 1967) after destruction of the noradrenaline-containing neurons in the locus coeruleus (Dahlström & Fuxe, 1964). The ascending axons of these neurons probably branch and give off collaterals to the cerebellar cortex, reticular formation, the colliculi and the thalamus on their way to the cortical areas of the forebrain (Loizou, 1969; Ungerstedt, 1971a; Olson & Fuxe, 1971).

Recent studies indicate, that sedative drugs like barbiturates and benzodiazepine derivatives decrease the turnover of cortical noradrenaline (Taylor & Laverty, 1969; Corrodi, Fuxe & others, 1971; Lidbrink, Corrodi & others, 1972a), suggesting a way by which these drugs may partly exert hypnotic as well as PS suppressant effects. We have now followed sleep and waking in the rat after intracerebral injections of 6-hydroxydopamine (6-OH-DA) (Ungerstedt, 1968; 1971b) in the mesencephalon causing small and selective lesions of the so called dorsal noradrenaline pathway which contains the axons from the locus coeruleus (Ungerstedt, 1971a; see also Fuxe, Hökfelt & Ungerstedt, 1970).