## Studies on the metabolic fate of 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) in man

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Many drugs are thought to exert their pharmacological effects by altering brain noradrenaline turnover. It would therefore be useful to have a non-invasive technique for the measurement of brain noradrenaline turnover in man. Measurement of noradrenaline levels in urine or plasma has not proved to be a good index of central noradrenaline turnover, as little, if any, neurotransmitter leaves the brain without undergoing metabolism. A solution would be to assay a metabolite of noradrenaline formed only in the brain. Following the reports of Schanberg and co-workers (Schanberg, Schildkraut, Breese & Kopin, 1968a; Schanberg, Breese, Schildkraut, Gordon & Kopin, 1968b) considerable work has led to the belief that in most species, including man, one of the principal metabolites of noradrenaline in the brain is MHPG and further, that the sulphate conjugate of this metabolite has its origin almost exclusively in the brain.

We have therefore developed sensitive, specific stable isotope dilution assays for MHPG and for its sulphate and its glucuronide conjugates, utilizing computerized gas chromatography-mass spectrometry. MHPG and its sulphate conjugate, labelled specifically with deuterium atoms, were synthesized for use as internal standards. MHPG was isolated from 5 ml aliquots of urine by extraction with ethyl acetate while the two conjugates were measured after extraction on Amberlite XAD-2 resin, followed by chromatographic separation on Sephadex LH-20. Both MHPG and its sulphate could be converted directly to the MHPG *tris*-trifluoracetate derivative by treatment with trifluoracetic anhydride. The glucuronide was not cleaved by this reagent and hence did not interfere with determination of the sulphate.

Urinary excretion of the three metabolites in 10 normal volunteers over 24 h was as follows expressed as mg free MHPG (mean $\pm$ s.d.): MHPG $0.08 \pm 0.03$ mg, MHPG sulphate $1.05 \pm 0.38$ mg, MHPG glucuronide $1.30 \pm 0.40$ mg. Thus most MHPG in urine is conjugated, with almost equal quantities of the two conjugates present. Data from 4 patients with adrenal catecholamine-releasing tumours (phaeochromocytoma) revealed elevated urinary levels of all three metabolites. The ratio of glucuronide to sulphate, however, was 0.94–2.16 and thus similar to control values. In contrast, with lumbar CSF from 42 individuals an average of 84% of the MHPG was unconjugated. Preliminary results with pooled samples of CSF indicate that the remainder is present as the glucuronide. This finding is in conflict with previous reports in which conjugated MHPG in CSF was described as the sulphate (Schanberg *et al.*, 1968b).

It is concluded that MHPG can be conjugated with either sulphuric or glucuronic acids in the periphery whereas in the central nervous system conjugation is a minor pathway and appears to occur only with glucuronic acid.

**References**

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## Studies of the interaction of carbenoxolone sodium and warfarin sodium *in vivo*

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The potentiation of the anti-coagulant activity of warfarin by phenylbutazone is well known (Aggler, O'Reilly, Leong & Kowitz, 1967; O'Reilly & Levy, 1970). Investigations into the mechanism of potentiation both *in vivo* and *in vitro* suggest displacement of warfarin from plasma binding sites as the cause (O'Reilly & Levy, 1970; Jun, Luzzi & Hsu, 1972). We have previously demonstrated a difference in the binding sites on human serum albumin between phenylbutazone, warfarin and carbenoxolone using a technique *in vitro* (Gottfried, Parke, Sacra & Thornton, 1975). We suggested that an interaction, due to displacement from plasma protein binding sites, between carbenoxolone and warfarin, phenylbutazone and other protein-bound drugs was unlikely, having