more specific binding: n is small and the sites are rapidly saturated. The second negative slope corresponds with the binding to the second class of sites which are of lower affinity but in greater number; these sites are not saturable. These Scatchard plots are in complete disagreement with those obtained previously by Judis whose positive Scatchard plots indicate that n and/or K_a decrease as the protein concentration increases. One could attribute (Bowmer & Lindup 1978) this phenomenon of positive slopes to the contaminants of the albumin commercial preparation, like fatty acids or tryptophan. We have used simultaneously a normal albumin and an albumin free from fatty acids with no change in the binding percentage. The phenomenon of positive slope can also be attributed to a cooperative binding.

This would be obvious when the protein concentration increases. The Scatchard plot of methadone is the same for a 0·4% albumin or 4% albumin: n is only a little smaller for the 4% albumin, this could be due to the masking of some sites by the folding of the protein molecules.

December 10, 1979

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On the urinary disposition of phenformin and 4-hydroxy-phenformin and their rapid simultaneous measurement

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Phenformin (β-phenethylbiguanide) is an orally active hypoglycaemic agent used in the treatment of maturity-onset diabetes. It has been found to be metabolized by oxidation to form a single hydroxylated derivative, 4-hydroxy-phenformin, which is excreted, together with unchanged parent drug, in the urine (Beckmann 1967). We have studied the urinary disposition of phenformin and its metabolite in a single subject given phenformin and we describe the method used. A normal male volunteer was given an oral dose of 50 mg phenformin (Dibotin, Winthrop Laboratories). Urine samples were then collected hourly for the first 8 h following this dose and at 10, 13, 24 and 26 h. After recording the volume of each sample an aliquot was stored at −20 °C before analysis as described below. Both parent drug and metabolite could readily be detected in all the urine samples collected and the rates of excretion for the two substances were plotted on a logarithmic scale against time (Fig. 1). Maximum rate of excretion for phenformin and 4-hydroxy-phenformin coincided in the same urine sample, that obtained from 1 to 2 h after dosing, suggesting the existence of a significant first-pass effect. Thereafter the rates of excretion declined exponentially with time enabling estimates of elimination half-life to be estimated which for phenformin was 3·7 h and 4-hydroxy-phenformin 3·8 h. The total recovery of the drug in this subject was 27·6 mg (56·2%) comprising 18·5 mg unchanged phenformin and 9·1 mg metabolite.

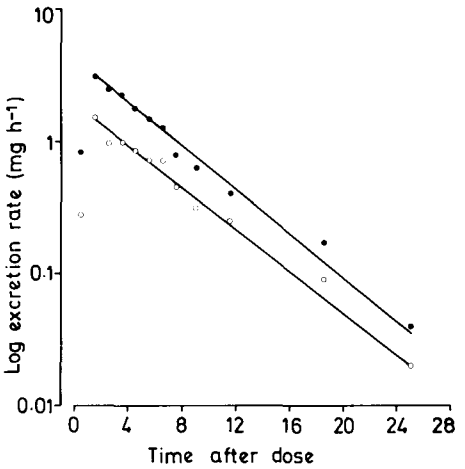

FIG. 1. The rates of excretion of phenformin (●) and 4-hydroxy-phenformin (○) by a single male subject following an oral dose of phenformin (50 mg).

Whereas a number of methods have been described for the estimation of phenformin (Matin et al 1975; Alkalay et al 1976; Hill & Chamberlain 1978), this has not been so for the metabolite. 4-Hydroxyphenformin possesses the strongly basic biguanide group and a weakly acidic phenolic hydroxyl residue, thereby

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giving it an amphoteric nature which severely limits its extraction into non-polar solvents. It is not possible, therefore, to derivatize it in the same way as the parent drug (Matin et al 1975). We were unsuccessful in our attempts to derivatize the metabolite in the dried residues of urine samples as described by Mottale & Stewart (1975) so an h.p.l.c. method was devised.

In normal urine there is an unidentified substance with similar chromatographic properties to those of 4-hydroxy-phenformin and separation of drug and metabolite from this is essential. The ability of Amberlite XAD-2 resin to adsorb polar aromatic compounds such as phenformin and metabolite suggested to us that this could be a means of purification.

Small columns (5 × 0·5 cm) were packed with Amberlite XAD-2 resin (BDH) which were prepared for use by washing successively with 3 ml each of acetone, methanol and water. To each column was applied 1 ml of a solution of phenacetin (100 μg ml^{-1}) which acted as an internal standard directly followed by a urine sample (1–5 ml) or calibration standard within the range 40–200 μg phenformin and 4–20 μg 4-hydroxy-phenformin. After the columns had been washed with water (3 ml), elution was carried out with methanol (3 ml) and the eluates collected in 50 ml ground-glass stoppered tubes. These extracts were then dried by rotary evaporation under vacuum at 50 °C and chromatographed using an h.p.l.c. column packed with a bonded reversed phase material (Waters Radial Compression Column Pak A) through which a mobile phase of 30% (v/v) acetonitrile in 0·05 M KH_2PO_4 was pumped (Pye Unicam LC-XPS) at ambient temperature at 3 ml min^{-1}. Detection was achieved by u.v. absorption at 230 nm (Pye Unicam LC-UV). The dried residues obtained from the eluates from the XAD-2 columns were redissolved in the h.p.l.c. solvent (100 μl), 5 μl of which was injected on to the column. Retention volumes: 4-hydroxy-phenformin, 5·1 ml; internal standard (phenacetin), 12·6 ml; and phenformin, 18·9 ml.

From the calculated peak height ratios, calibration curves were obtained for both phenformin and 4-hydroxy-phenformin. The curves were linear over the range of concentrations used and enabled quantitation of as little as 1·0 μg ml^{-1} phenformin and 0·5 μg ml^{-1} 4-hydroxy-phenformin. Recovery from urine was assessed by analysis of six control urine samples to which phenformin and 4-hydroxy-phenformin had been added at concentrations of 200 and 20 μg ml^{-1} respectively. For both compounds the recovery was high, giving mean (with s.d.) values of 97·0 (2·5%) for phenformin and 95·5 (2·0%) for 4-hydroxy-phenformin respectively.

The authors gratefully acknowledge the financial support of the Wellcome Trust. Phenformin and 4-hydroxy-phenformin standards were a kind gift from Dr L. J. King, Department of Biochemistry, The University of Surrey.

May 7, 1980

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