system. Dipyridamole is a relatively potent inhibitor of adenosine uptake (Huang & Daly 1974).

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In vitro hepatic oxidative metabolism of antipyrine, phenytoin and phenylbutazone in uraemic rabbits

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Earlier (Van Peer et al 1978) we calculated from serum concentrations that in uraemic rabbits the intrinsic clearance of free drug, i.e. the inherent metabolizing ability of the liver (Rowland et al 1973; Wilkinson & Shand 1975), is not altered for antipyrine (phenazone), increased for phenytoin and decreased for phenylbutazone. Conflicting results on the biotransformation rate of drugs in renal failure have been reported from in vivo and in vitro experiments (Reidenberg 1977). We decided therefore to study in vitro the hepatic oxidative metabolism of antipyrine, phenytoin and phenylbutazone in acute uraemic rabbits. We also measured amidopyrine *N*-demethylase and aniline hydroloxylase, commonly used markers of the hepatic oxidative metabolism.

Female albino rabbits were used; five of the animals received 0·9% NaCl (saline) intravenously and five of the animals the nefrotoxic agent uranyl nitrate (2 mg kg^{-1}) (Van Peer et al 1978). Five days after the injection, when serum urea was markedly (700%) increased in the uranyl nitrate-treated rabbits, control and treated rabbits were killed after an overnight fast. The livers (which showed only slight cytolysis in the treated animals) were homogenized in 4 volumes of 1·15% KCl–0·01 M sodium phosphate buffer pH 7·4, and 9000 *g* fractio s and microsomes were prepared.

Microsomal protein (Lowry et al 1951), cytochrome P-450 (Schoene et al 1972), amidopyrine *N*-demethylase (Mazel 1971) and aniline hydroxylase (Mazel 1971) were measured as described in the literature.

For antipyrine hydroxylase, 2 ml incubation mixture contained antipyrine 20, glucose-6-phosphate 16, NADP 2, $MgCl_2$ 12 μmol, 0·1 M sodium phosphate buffer pH 7·4, 0·8 ml; 9000 *g* fraction or microsomal fraction 1 ml, and glucose-6-phosphate dehydrogenase 1.4 I.U. After incubation at 37°C for 60 min, the reaction was stopped by addition of 0·5 ml 20% trichloroacetic acid. The 4-hydroxyantipyrine formed was measured spectrophotometrically as described by Tabarelli-Poplawski & Uehleke (1977).

For phenytoin hydroxylase, 1 ml incubation mixture contained [^{3}H]phenytoin (specific activity 4 nCi $nmol^{-1}$)12, NADP 1, glucose-6-phosphate 8, $MgCl_2$ 6 nmol, 0·1 M sodium phosphate buffer pH 7·4 0·8 ml, 9000 *g* fraction or microsomal fraction 0·2 ml and glucose-6-phosphate dehydrogenase 0·7 I.U. After incubation of the mixture at 37° C for 20 min the unmetabolized phenytoin was extracted with 1-chlorobutane as described by Gerber et al (1971) and the radioactivity measured. The amount of phenytoin transformed during the incubation was calculated from the difference in extracted radioactivity between a sample kept at 0° C and the sample incubated at 37° C.

For phenylbutazone hydroxylase, 1 ml incubation mixture contained phenylbutazone 162, NADP 1, glucose-6-phosphate$_2$ 8, MgCl 6 μmol, 0·1 M sodium phosphate buffer pH 7·4 0·7 ml, and 9000 *g* fraction or microsomal fraction 0·3 ml and glucose-6-phosphate dehydrogenase 0·7 I.U. Incubation time was 45 min at 37° C. After the incubation, phenylbutazone was measured spectrophotometrically by the permanganate oxidation method of Jähnchen & Levy (1972) which also measures γ-hydroxyphenbutazone. The amount of phenylbutazone transformed during the incubation was calculated from the difference in extinction of a sample kept at 0° C and a sample after incubation at 37° C. In the same 9000 *g* fractions of control and uraemic rabbits the activity of phenylbutazone hydroxylase was measured before and after dialysis of the 9000 *g* fraction for 16 h at 4° C.

Table 1 shows that in the 9000 *g* fraction and in the microsomes the content of microsomal protein and

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Table 1. In vitro hepatic oxidative metabolism in the 9000 *g* fraction and the microsomers of the liver of 5 control rabbits and 5 uraemic rabbits. Means ± standard error of the mean are given. ** $P < 0.01$ (Mann-Whitney U-test). Serum urea is 58 ± 3 mg% in control rabbits and 411 ± 43 mg% in uraemic rabbits ($P < 0.01$). Protein content is expressed as mg g^{-1} liver, cytochrome P-450 as nmol g^{-1} liver and nmol mg^{-1} microsomal protein, activities of the enzymes as nmol substrate transformed g^{-1} liver min^{-1} and nmol substrate transformed mg^{-1} microsomal protein min^{-1}. Phenylbutazone hydroxylase was measured on the same 9000 *g* fractions before and after dialysis at 4° C for 16 h.

	9000 g fraction		Microsomes	
	Control	Uraemic	Control	Uraemic
Protein	—	—	24±2	22±1
Cytochrome P-450	31±2	27±2	1·13±0·05	0·96±0·04
Amidopyrine *N*-demethylase	97±10	79±3	2·89±0·11	2·41±0·09
Aniline hydroxylase	17±2	21±2	0·34±0·01	0·36±0·01
Antipyrine hydroxylase	62±7	53±3	1·47±0·09	1·42±0·06
Phenytoin hydroxylase	2·7±0·3	2·5±0·1	0·094±0·002	0·090±0·007
Phenylbutazone hydroxylase			0·62±0·03	0·59±0·02
before dialysis	23±1	12±1**		
after dialysis	19±3	20±2		

cytochrome P-450, and the activity of amidopyrine *N*-demethylase, aniline hydroxylase, antipyrine hydroxylase and phenytoin hydroxylase in uraemic rabbits, are not significantly different from those in control rabbits. In the 9000 *g* fraction of the uraemic rabbits the activity of phenylbutazone hydroxylase was decreased ($P < 0.01$, Mann-Whitney U-test). The Lineweaver-Burk plot for phenylbutazone hydroxylase in the 9000 *g* fraction indicated a non-competitive inhibition: the average V_{max} was decreased from 55 ± 12 nmol g^{-1} liver min^{-1} in control rabbits to 25 ± 2 nmol g^{-1} liver min^{-1} in uraemic rabbits ($P < 0.05$, Mann-Whitney U-test), while the average K_m was not significantly different in both groups (265 ± 19 μM in control and 315 ± 35 μM in uraemic rabbits). After dialysis of the 9000 *g* fraction the difference in activity between control and uraemic rabbits disappeared.

There is a good agreement between these in vitro results and our previously published in vivo findings, i.e. that in uraemic rabbits the intrinsic clearance of antipyrine is unchanged and the intrinsic clearance of free phenylbutazone is decreased (Van Peer et al 1978). For phenytoin, the unchanged in vitro metabolism is in contrast with the increased intrinsic serum clearance of free phenytoin previously reported (Van Peer et al 1978). However, when calculation of the intrinsic clearance of free phenytoin is done using blood concentrations instead of serum concentrations, no change between control and uraemic rabbits is found (unpublished results).

Our in vitro data are in agreement with other data of literature, showing an unchanged or decreased hepatic oxidative metabolism in uraemic animals (Van Peer & Belpaire 1977; Leber et al 1978; Terner et al 1978).

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