GENETICALLY DETERMINED OXIDATION CAPACITY AND THE DISPOSITION OF DEBRISOQUINE

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1 The disposition in urine of debrisoquine and its hydroxylated metabolites has been studied in subjects of the 'extensive metabolizer' (EM; $n = 5$) and 'poor metabolizer' (PM; $n = 5$) phenotypes. The 4-hydroxylation of debrisoquine by PM subjects following a 10 mg oral dose was capacity-limited and displayed significant dose-dependency over a range of 1–20 mg. In contrast, the EM subjects' ability to perform this metabolic oxidation did not deviate from first-order kinetics over a dose range of 10–40 mg.

2 The disposition of debrisoquine in plasma following a 10 mg oral dose has been studied in EM ($n = 4$) and PM ($n = 3$) subjects. Whilst PM subjects displayed significantly higher plasma levels of debrisoquine at all time points following 1 h post-dosing, and higher values for areas under the plasma concentration-time curve (EM: 105.6 ± 7.0 ng ml⁻¹ h; PM: 371.4 ± 22.4 ng ml⁻¹ h, $2P < 0.0001$), neither debrisoquine plasma half-life (EM: 3.0 ± 0.5 h; PM: 3.3 ± 0.4 h) nor renal clearance of the drug (EM: 152.8 ± 30.3 ml min⁻¹; PM: 137 ± 4.5 ml min⁻¹) displayed significant inter-phenotype differences.

3 The results of these investigations show that the phenotyping of individuals for debrisoquine oxidation status by means of a 'metabolic ratio' derived from a single 0–8 h urine sample has a sound kinetic basis. The kinetic differences between the two phenotypes would strongly suggest that the metabolic defect manifested in PM subjects is one of pre-systemic elimination capacity.

Introduction

The metabolic transformation of drugs in man is a major determinant of drug plasma levels and subsequent responsiveness. The rate at which metabolism of many drugs proceeds shows marked inter-individual variability, sometimes sufficient at the extremes to precipitate either failure of therapy or an exaggerated response, or even an adverse drug reaction. A variety of attempts have been made to develop indices of drug metabolizing capacity, both with a view to predicting the rate of metabolism of given drugs by reference to the rate of metabolism of a standard probe, and also with a view to predicting the effect of various disease states or exposure to environmental chemicals on the metabolism of commonly used therapeutic agents. To this end, compounds such as aminopyrine and antipyrine have been extensively investigated as suitable probes. It has become increasingly apparent from recent reports that drugs such as these, which are metabolized by a multiplicity of competing pathways, are only useful in this context when partial clearances down these pathways can be determined (Danhof, 1980).

The antihypertensive agent debrisoquine (Declinax®), is metabolized in man predominantly

to 4-hydroxydebrisoquine, but the ability to perform this reaction shows pronounced inter-individual variation. The index which has been employed in the study of debrisoquine metabolism is the 'metabolic ratio', this being the quotient: (% dose as debrisoquine/% dose as 4-hydroxydebrisoquine) eliminated in the 0–8 h urine following a 10 mg oral dose of debrisoquine. This index is bimodally distributed in the general population (Mahgoub *et al.*, 1977). Family studies have shown that this bimodality originates in a single gene locus control of the metabolic reaction (Evans *et al.*, 1980). Two debrisoquine oxidation phenotypes are discernible in the population, the 'extensive metabolizer' (EM) and 'poor metabolizer' (PM). The poor metabolizing trait is autosomal recessive, arising from a pair of variant alleles ($D^L D^L$), and is seen in 9% of the British white caucasian population. Characteristically, a PM subject will excrete less than 2% of an oral dose of debrisoquine in the metabolized form, and display a metabolic ratio of 20 or greater.

EM subjects, both homozygous ($D^H D^H$) and heterozygous ($D^H D^L$), may metabolize 10–80% of an oral dose of debrisoquine. The antimode separating

the two phenotypes has been determined as a metabolic ratio of 12.6 (Evans *et al.*, 1980). It has been ascertained that PM subjects have a greater sensitivity to the hypotensive effects of debrisoquine (Idle *et al.*, 1978) and a variety of toxicological implications have been associated with both oxidation phenotypes (Idle *et al.*, 1981; Ritchie *et al.*, 1980).

The purpose of this report is to present the pharmacokinetic consequences of this oxidation defect in terms of a comparison of the disposition of debrisoquine in the two oxidation phenotypes following the standard 10 mg oral dose used in the phenotype assessment procedure. Evidence will be presented to show that the metabolism of debrisoquine by subjects of the PM phenotype is capacity-limited, conforming to Michaelis-Menten rather than first-order kinetics.

In addition, a study of the effect of dose on the ability of both phenotypes to metabolize debrisoquine is described. The EM subjects studied displayed no evidence of deviation from first-order kinetics over a four-fold dose range; in contrast, the ability of each PM subject studied to metabolize debrisoquine was significantly reduced by each dosage increment.

Methods

Plan of investigation and analytical techniques

Five normal, apparently healthy subjects (5M; age range 22–30 years; body weights 65–92 kg) previously identified as EM, and five (4M, 1F; age range 20–45 years; body weights 63–90 kg) previously identified as PM participated in these investigations. All gave full informed consent and appropriate approval for the study was granted by the Ethics Committee of St Mary's Hospital.

Each subject voided his or her bladder immediately before commencement of the study, and then received the free-base equivalent of 10 mg debrisoquine, given orally as Declinax® (Roche Products) tablets. All subjects provided serial urine samples at 1, 2, 3, 4, 6, 8 and 12 h post-dosing; three of the PM subjects studied also provided urine samples *ad libitum* over the period 12–48 h post-dosing. A 20 ml aliquot of each measured urine sample was stored at –20°C prior to its analysis for debrisoquine and 4-hydroxydebrisoquine by electron-capture detection gas-liquid chromatography (Erdtmansky & Goehl, 1975; Idle *et al.*, 1979). Appropriate aliquots of each serial urine sample were pooled to yield a 0–12 h urine sample which was analysed for the phenolic metabolites of debrisoquine 5-, 6-, 7- and 8-hydroxydebrisoquine by electron-capture gas-liquid chromatography (Idle *et al.*, 1979).

Blood (10 ml) was sampled in four of the EM and three of the PM subjects by venepuncture at 0, 0.5, 1,

1.5, 2, 3, 4, 6 and 8 h post-dosing; the blood was immediately centrifuged (1000 g for 10 min) in lithium heparin containers. Plasma samples were stored at –20°C prior to analysis for debrisoquine content as described below.

To plasma (0.4 ml) was added 7-methoxyguanosine (gift of Pfizer Ltd, Sandwich, U.K.; 25 ng in 50 µl water) as internal standard in screw-top septum vials (15 ml). The mixture was then buffered with 1M sodium bicarbonate (0.6 ml), and benzene (3 ml) and hexafluoroacetylacetone (Koch-Light Ltd., U.K.; 0.3 ml) were added. The vials were heated in an aluminium heating block at 100°C for 1 h, then allowed to cool to room temperature, whereupon 3M sodium hydroxide (10 ml) was added and the mixture vortexed for 10 s. Phase separation was achieved by centrifugation for 30 min at 1000 g; 1–5 µl of the organic phase were taken for gas-liquid chromatographic analysis. The method employed a Packard Model 417 gas-liquid chromatograph, equipped with a tritium-scandium electron-capture detector (75 mCi). The 1.8 m glass column (i.d. 3 mm) was packed with 3% OV-1 on Chromosorb WHP (80–100 mesh).

The carrier gas (5% methane in argon) flow rate was 60 ml min⁻¹. The oven, detector and injection port temperatures were 195°, 235° and 250° respectively. The retention times of debrisoquine and 4-hydroxydebrisoquine derivatives relative to the internal standard were 0.29 and 0.44 respectively. Calibration standards in the range 5–200 ng ml⁻¹ were prepared from blank plasma and passed through the assay procedure with test samples. The limit of detection of the assay was 5 ng ml⁻¹, and the coefficient of variation did not exceed 10% for any standard in the above concentration range.

Analysis of data

Urinary excretion of debrisoquine and 4-hydroxydebrisoquine The overall dispositional rate constant (β) for debrisoquine elimination was calculated from the terminal linear portion of semilogarithmic plots of the rate of urinary excretion of the drug by the method of Cummings *et al.* (1967). Ultimate recoveries of drug and metabolite in urine were calculated from equation (1):

$$(1) \text{ Ultimate recovery} = \frac{\text{Observed recovery (0–12 h)} + \text{Excretion rate at 12 h}}{\beta}$$

where the excretion rate at 12 h was obtained by extrapolation of the terminal linear portions of the semilogarithmic plots of urinary excretion rate of drug or metabolite against time.

Rates of urinary excretion of 4-hydroxydebrisoquine were tested for deviation from first-order kinetics by a statistical analysis based on the approach of Levy *et*

al. (1972). The observed urinary excretion rate of 4-hydroxydebrisoquine (V) and the calculated 'body load' of debrisoquine available for metabolism (D_B ; Cummings *et al.*, 1967) were tested for fit to a standard linearized form of the Michaelis-Menten equation (the Hanes plot, equation (2)):

$$(2) \quad \frac{D_B}{V} = \frac{D_B}{V_{\max}} + \frac{D_M}{V_{\max}}$$

where $V_{\max}$ is the maximal metabolite excretion rate, and D_M the body load of debrisoquine giving rise to a half-maximal metabolite excretion rate. If, for any individual of either phenotype, a plot of D_B/V (ordinate) against D_B yielded a slope significantly greater than zero, it was assumed that appreciable saturation of the 4-hydroxylation pathway had occurred, and the Michaelis-Menten parameters $V_{\max}$ and D_M were calculated from slope and intercept values. These parameters in turn were employed to obtain an estimate of the apparent formation rate constant for 4-hydroxydebrisoquine (K_{4-OH}), since:

$$(3) \quad K_{4-OH} = \frac{V_{\max}}{D_M}$$

Where a Hanes plot did not yield a slope significantly greater than zero an independent estimate of K_{4-OH} was obtained from the mean value of D_B/V for the subject in question. The reciprocal of this mean value gives a direct estimate of K_{4-OH} , since under first-order conditions, equation (2) contracts thus:

$$(4) \quad \frac{D_B}{V} = \frac{D_M}{V_{\max}} = \frac{1}{K_{4-OH}}$$

Profiles of the decline in debrisoquine plasma concentration with time were assessed for each subject in terms of the peak debrisoquine plasma concentration, and also the area under the debrisoquine plasma concentration-time curve. The area under the plasma concentration-time curve for the duration of the investigation (AUC_{0-8}) was estimated by the trapezoidal rule. The total area under the plasma concentration-time curve ($AUC_{0-\infty}$) was calculated from equation (5)

$$(5) \quad AUC_{0-\infty} = AUC_{0-8} + \frac{C_8 \cdot t_{1/2}}{0.693}$$

where C_8 = the observed plasma debrisoquine concentration at 8 h post-dosing, and $t_{1/2}$ = the half-life of the terminal log-linear phase of the decline in plasma concentration. The renal clearance (CL_R) of debrisoquine over the study period was calculated from equation (6):

$$CL_R = \frac{\text{Urinary recovery of debrisoquine 0-8 h}}{AUC_{0-8}}$$

Any comparison of kinetic or metabolic parameters

between the two oxidation phenotypes was performed by unpaired Student's *t*-test.

The kinetic model employed assumes that metabolite elimination is very rapid in relation to the rate of metabolite formation, and the method of estimating ultimate recoveries of metabolites assumes that elimination is terminal and linear in the final period of the study; the use of ultimate recoveries of drug related material does, however, preclude the necessity of assuming quantitative absorption of the oral dose. It should be stressed that the kinetic variables quoted are employed as relative rather than absolute measures of oxidation capacity in the two debrisoquine oxidation phenotypes, and that any assumptions required by the kinetic analysis were applied equally to both groups of subjects. Similarly, the treatment of plasma elimination data assumed that elimination of the drug had become terminal and linear within the period of the investigation, but again this assumption was applied equally to the two phenotypes. The data to be presented demonstrate that the two oxidation phenotypes are kinetically distinct, the PM group displaying non-first-order and the EM group first-order patterns with respect to debrisoquine 4-hydroxylation, and there is thus no generally applicable pharmacokinetic model which can be confidently applied to both phenotypes to estimate more absolute measures of 4-hydroxylation capacity.

Results

The urinary excretion of debrisoquine and 4-hydroxydebrisoquine in EM and PM subjects

The patterns of urinary excretion of debrisoquine and 4-hydroxydebrisoquine observed in five EM and five PM subjects following a 10 mg oral dose of debrisoquine are shown in Tables 1 and 2. The mean ($\pm$ s.d.) excretion rate profiles of drug and metabolite in the two phenotypes are shown in Figure 1. Urinary excretion rates of debrisoquine and 4-hydroxydebrisoquine in the EM group displayed a log-linear decline after a brief absorption phase. In contrast, the PM group displayed a log-linear decline in the rate of debrisoquine excretion, combined with rates of metabolite excretion which remained approximately constant throughout the study period. In view of this apparent departure from first-order kinetics displayed by the PM group with respect to 4-hydroxydebrisoquine excretion, these data were tested for fit to a linearized form of the Michaelis-Menten equation as described in the **Methods** section. The Hanes plot derived from the PM subjects' excretion pattern yielded a slope of 51.67 ± 13.00 ($P < 0.001$). In contrast, the Hanes plot derived from EM subjects yielded a slope of 0.17 ± 0.66 ($P > 0.5$), indicating no

Table 1 Urinary excretion of debrisoquine (D) and 4-hydroxydebrisoquine (4OH) in serial urine samples 0–48 h following a 10 mg oral dose. All values are expressed as percentage of the administered dose.

Time (h)	EM subject					PM subject				
	1	2	3	4	5	1	2	3	4	5
	D	4OH	D	4OH	D	D	4OH	D	4OH	D
0–1	n.d.*	0.19	1.61	2.00	n.d.	0.95	3.49	0.22	0.12	0.12
1–2	1.69	4.10	1.85	2.95	1.60	2.44	4.93	3.21	2.52	7.38
2–3	1.35	4.32	1.27	2.41	2.60	4.81	5.20	4.18	1.18	4.75
3–4	1.36	4.01	1.17	2.25	1.32	3.15	3.60	3.29	1.00	4.84
4–6	1.48	4.08	1.40	3.50	1.90	4.48	3.38	4.94	0.98	3.94
6–8	1.00	2.30	1.26	2.36	1.22	2.36	2.74	3.32	0.78	3.82
8–12	1.44	3.60	1.80	3.32	3.00	5.00	4.04	3.68	1.12	3.28
12–18	—	—	—	—	—	—	—	—	—	—
18–24	—	—	—	—	—	—	—	—	—	—
18–28	—	—	—	—	—	—	—	—	—	—
18–30	—	—	—	—	—	—	—	—	—	—
28–48	—	—	—	—	—	—	—	—	—	—
30–48	—	—	—	—	—	—	—	—	—	—

*Not detected

Table 2 Urinary recovery of drug-related material 0–12 h following a 10 mg oral dose of debrisoquine p.o. in EM ($n = 5$) and PM ($n = 5$) volunteers.

	EM subjects					PM subjects				
	1	2	3	4	5	1	2	3	4	5
	Mean $\pm$ s.d.					Mean $\pm$ s.d.				
Debrisoquine (% dose)	8.3	10.4	11.6	27.4	7.7	13.1 $\pm$ 8.2	49.5	34.3	33.0	27.8
4-OH-debrisoquine (% dose)	22.6	18.8	23.2	22.8	27.8	23.0 $\pm$ 3.2	2.3	1.4	2.3	2.2
Total phenolic metabolites (% dose)	5.2	8.6	7.3	2.9	4.9	5.8 $\pm$ 2.2	2.8	2.5	2.8	5.6
Total (% dose)	36.1	37.8	42.1	53.1	40.4	41.9 $\pm$ 6.7	54.6	38.2	38.3	35.6

Debrisoquine (% dose) 35.8 $\pm$ 8.1
 4-OH-debrisoquine (% dose) 2.1 $\pm$ 0.4
 Total phenolic metabolites (% dose) 3.6 $\pm$ 1.3
 Total (% dose) 41.6 $\pm$ 7.5

Figure 1 Urinary excretion rates (V) of debrisoquine (●) and 4-hydroxydebrisoquine (○) in EM ($n = 5$) and PM ($n = 5$) subjects following a 10 mg oral dose of debrisoquine (mean $\pm$ s.d.)

significant departure from first-order expectations. This graphical analysis and the parameters derived from it are shown in Figure 2 and Table 3 respectively. The apparent first-order rate constant describing the formation of 4-hydroxydebrisoquine (K_{4-OH}) was lower in PM subjects than EM subjects by a factor of 3.5–4.5.

The elimination half-life of debrisoquine, as judged from the slopes of semilogarithmic urinary excretion rate plots was essentially the same in both groups (Table 3), and similarly the overall recovery of drug related material (Table 2) showed no significant interphenotype variation. An extended study (12–48 h post-dosing) in three of the PM subjects indicated that rates of 4-hydroxydebrisoquine excretion became log-linear, paralleling those of debrisoquine, within 12–15 h post-dosing.

The plasma disposition of debrisoquine in EM and PM subjects

The decline in plasma concentration of debrisoquine in EM ($n = 4$) and PM ($n = 3$) subjects following a 10 mg oral dose of the drug is shown in Figure 3. The PM subjects attained significantly higher ($2P < 0.001$) plasma levels at all time points following 1 h post-dosing. The parameters derived from these data are shown in Table 4. It is evident that the three PM subjects studied were exposed to 3–4 times the amount of debrisoquine than were the EM subjects,

Figure 2 Hanes plot relating urinary excretion rate of 4-hydroxydebrisoquine (V) and calculated body-load of debrisoquine (D_B) for EM (○; $n = 5$) and PM (●; $n = 5$) subjects following a 10 mg oral dose of debrisoquine.

Table 3 Kinetic parameters describing the overall disposition and 4-hydroxylation of debrisoquine by EM ($n = 5$) and PM ($n = 5$) subjects following a 10 mg oral dose of debrisoquine (mean $\pm$ s.d.)

	EM	PM	
β (h^{-1})	0.19 ± 0.03	0.16 ± 0.02	(NS)
$K_{4\text{-OH}}$ (h^{-1})	0.060 ± 0.15	0.017 ± 0.005	($2P < 0.005$)
V_{max} (mg h^{-1})	—*	0.019 ± 0.004	
D_{M} (mg)	—*	1.11 ± 0.28	

β : Apparent overall disposition rate constant

$K_{4\text{-OH}}$: Apparent formation rate constant for 4-hydroxydebrisoquine

V_{max} : Maximal rate of formation of 4-hydroxydebrisoquine

D_{M} : Body load of debrisoquine give rise to 50% V_{max}

* No deviation from first-order kinetics

irrespective of whether the phenotypes are compared in terms of peak plasma concentration or area under the plasma concentration-time curve. In marked contrast, the parameters describing the elimination of debrisoquine which has reached the systemic circulation (elimination $t_{1/2}$ and renal clearance) show no significant interphenotype differences.

Dose-dependency of debrisoquine metabolism in EM and PM subjects

The effects of increasing dose on the metabolic ratio as studied in four EM and three PM subjects are summarized in Table 5. In the PM group the mean metabolic ratio showed a statistically significant in-

crease with each dosage increment, whereas the metabolic ratios displayed by the EM subjects studied did not alter significantly over the four-fold dose range employed.

Discussion

These investigations show that major differences exist in debrisoquine 4-hydroxylation capacity between individuals of the extensive and poor metabolizing phenotypes. This metabolic reaction is clearly capacity-limited in the PM group following a 10 mg dose of debrisoquine, whereas EM subjects dosed over a range extending to 40 mg displayed no evidence of departure from first-order kinetics.

The validity of the kinetic analysis of urinary excretion data employed here depends on two critical assumptions. The first is that the analytical procedures used account for all debrisoquine-related material. Whilst the 0–12 h urinary recoveries quoted here are incomplete it has been demonstrated in a previous study that, following oral administration of [^{14}C]-debrisoquine, all urinary radioactivity could be accounted for as the compounds measured here, namely debrisoquine, 4-hydroxydebrisoquine and the 5-, 6-, 7- and 8-hydroxy phenolic metabolites. This study also showed that the faecal route of elimination was of minor importance (Idle *et al.*, 1979). The second assumption is that formation of 4-hydroxydebrisoquine rather than its renal clearance is rate-limiting for the appearance of this metabolite in urine. Whilst this assumption has not been directly tested here, the excretion of 4-hydroxy-debrisoquine paralleled that of debrisoquine over the whole study period in the case of EM subjects, and over the period 12–48 h post-dosing in the case of PM subjects. Such parallel excretion rates would only be seen if metabolite formation rather than excretion were rate-limiting for appearance in urine (Cummings *et al.*, 1967).

A second striking feature of this study was the

Figure 3 Debrisoquine plasma concentration (C ; mean $\pm$ s.d.) in EM (O; $n = 4$) and PM ($\bullet$; $n = 3$) subjects following a 10 mg oral dose of debrisoquine.

Table 4 Kinetic parameters derived from plasma levels of debrisoquine in EM ($n = 4$) and PM ($n = 3$) subjects following a 10 mg oral dose of debrisoquine (mean $\pm$ s.d.)

	EM	PM	
Peak plasma concentration (ng ml ⁻¹)	21.9 $\pm$ 2.9	72.7 $\pm$ 11.8	(2P < 0.0001)
AUC _{0-∞} (ng ml ⁻¹ h)	105.6 $\pm$ 7.0	371.4 $\pm$ 22.4	(2P < 0.0001)
Plasma $t_{1/2}$ (h)	3.0 $\pm$ 0.5	3.3 $\pm$ 0.4	(NS)
Renal clearance (ml min ⁻¹)	152.8 $\pm$ 30.3	137.0 $\pm$ 4.5	(NS)

failure of the metabolic defect to prolong the overall elimination of debrisoquine in the PM group. Whilst these subjects displayed higher levels of debrisoquine in both plasma and urine, neither plasma $t_{1/2}$ or renal clearance showed significant interphenotype differences. These observations would strongly suggest that where an individual has extensive 4-hydroxylation capacity, the metabolic reaction occurs pre-systemically. This interpretation is substantiated by

Table 5 The effect of dose on debrisoquine metabolic ratio in EM ($n = 4$) and PM ($n = 3$) subjects

Dose (mg)	Metabolic ratio	
	EM	PM
1	—	11.5 $\pm$ 5.3
10	0.8 $\pm$ 0.4	20.7 $\pm$ 9.7
20	0.6 $\pm$ 0.3	47.7 $\pm$ 11.9
40	0.8 $\pm$ 0.7	—

the coincidence of peak excretion rates for drug and metabolite seen in EM subjects, and is in agreement with the observations of Silas *et al.* (1978). If this is indeed the case, the assessment of the metabolic capacity of the EM group in terms of a formation rate constant probably represents a considerable underestimate. A formation rate constant strictly refers to systemic elimination events, and it is now well established that the rate of metabolism of drugs under-

going extensive pre-systemic elimination is perfusion-limited, and is thus more dependent on liver blood flow than metabolic capacity *per se* (Wilkinson & Shand, 1975). By implication, the 3–4 fold differences found between apparent formation rate constants are also a considerable underestimate of the true inter-phenotype difference in metabolic capacity.

In conclusion, the two debrisoquine oxidation phenotypes previously described in terms of a urinary 'metabolic ratio' have proved to be kinetically distinct. In the EM phenotype, debrisoquine 4-hydroxylation proceeds by first-order kinetics over a four-fold dose range, apparently on the 'first passage' of the drug through the gut wall or liver. The ability of the PM subject to perform this reaction is markedly dose-dependent, and proceeds by capacity limited kinetics.

In our hands the debrisoquine phenotyping procedure has proved both reproducible and predictive with respect to the metabolism of a variety of other drugs. PM subjects have been shown to have relatively impaired abilities to metabolize phenacetin and guanoxan (Sloan *et al.*, 1978), phenytoin (Sloan *et al.*, 1981), sparteine (Bertilsson *et al.*, 1980) and phenformin (Oates *et al.*, 1982). We would advocate the use of the oxidation phenotyping procedure in the safety evaluation of both new and existing drugs where metabolic oxidation is a major determinant of the duration and intensity of a compounds pharmacological effects.

References

- BERTILSSON, L., DENGLER, J.H., EICHELBAUM, M. & SCHULZ, H.U. (1980). Pharmacogenetic covariance of defective *N*-oxidation of sparteine and 4-hydroxylation of debrisoquine. *Eur. J. clin. Pharmacol.*, **17**, 153–155.
- CUMMINGS, A.J., MARTIN, B.P. & PARK, G.S. (1967). Kinetic considerations relating to the accrual and elimination of drug metabolites. *Br. J. Pharmacol. Chemother.*, **29**, 136–149.

- DANHOF, M. (1980). *Antipyrine metabolite profile as a tool in the assessment of the activity of different drug oxidising enzymes in man*. Ph.D. Thesis, University of Leiden.
- ERDTMANSKY, P. & GOEHL, T.J. (1975). Gas liquid chromatographic electron capture determination of some mono-substituted guanidino-containing drugs. *Analyt. Chem.*, **47**, 750-752.
- EVANS, D.A.P., MAHGOUB, A., SLOAN, T.P., IDLE, J.R. & SMITH, R.L. (1980). A family and population study of the genetic polymorphism of debrisoquine oxidation in a British white population. *J. med. Genet.*, **17**, 102-105.
- IDLE, J.R., MAHGOUB, A., ANGELO, M.M., DRING, L.G., LANCASTER, R. & SMITH, R.L. (1979). The metabolism of [^{14}C]-debrisoquine in man. *Br. J. clin. Pharmacol.*, **7**, 257-266.
- IDLE, J.R., MAHGOUB, A., LANCASTER, R. & SMITH, R.L. (1978). Hypotensive response to debrisoquine and hydroxylation phenotype. *Life Sci.*, **22**, 979-984.
- IDLE, J.R., OATES, N.S., SHAH, R.R. & SMITH, R.L. (1981). Is there a genetic predisposition to phenformin-induced lactic acidosis? *Br. J. clin. Pharmacol.*, **11**, 418-419P.
- LEVY, G., TSUCHIYA, T. & AMSEL, L.P. (1972). Limited capacity for salicyl phenolic glucuronide formation and its effects on the kinetics of salicylate elimination in man. *Clin. Pharmacol. Ther.*, **13**, 258-268.
- MAHGOUB, A., IDLE, J.R., DRING, L.G., LANCASTER, R. & SMITH, R.L. (1977). Polymorphic hydroxylation of debrisoquine in man. *Lancet*, **ii**, 584-586.
- OATES, N.S., SHAH, R.R., IDLE, J.R. & SMITH, R.L. (1982). Genetic polymorphism of phenformin 4-hydroxylation. *Clin. Pharmacol. Ther.*, **32**, 81-89.
- RITCHIE, J.C., SLOAN, T.P., IDLE, J.R. & SMITH, R.L. (1980). Toxicological implications of polymorphic drug metabolism. In *Environmental chemicals, enzyme function and human disease*. Ciba Foundation Symposium, **76**, 219-244.
- SILAS, J.M., LENNARD, M.S., TUCKER, G.T., SMITH, A.J., MALCOLM, S.L. & MARTEN, T.R. (1978). The disposition of debrisoquine in hypertensive patients. *Br. J. clin. Pharmacol.*, **5**, 27-34.
- SLOAN, T.P., IDLE, J.R. & SMITH, R.L. (1981). Influence of the $\text{D}^{\text{H}}/\text{D}^{\text{L}}$ alleles regulating debrisoquine oxidation on phenytoin hydroxylation. *Clin. Pharmacol. Ther.*, **29**, 493-497.
- SLOAN, T.P., MAHGOUB, A., LANCASTER, R., IDLE, J.R. & SMITH, R.L. (1978). Polymorphism of carbon oxidation of drugs and clinical implications. *Br. med. J.*, **2**, 655-657.
- WILKINSON, G.R. & SHAND, D.G. (1975). A physiological approach to hepatic drug clearance. *Clin. Pharmacol. Ther.*, **18**, 377-390.

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