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COMPARISON OF THE *IN VIVO* AND *IN VITRO* RATES OF FORMATION OF THE THREE MAIN OXIDATIVE METABOLITES OF ANTIPYRINE IN MAN

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1 The metabolism of antipyrine to its three main oxidative metabolites, 4-hydroxyantipyrine, 3-hydroxymethylantipyrine and norphenazone was investigated *in vivo* and *in vitro* in separate groups of subjects with normal hepatic function and in the same group of patients with suspected liver disease.

2 The rank order for the rate of formation of the three metabolites of antipyrine was similar *in vivo* and *in vitro*.

3 There was no significant correlation between the rates of formation of any pair of antipyrine metabolites either *in vivo* or *in vitro*.

4 Despite this there was a significant correlation between the *in vivo* and *in vitro* rates for formation of each of the three metabolites in the same group of patients.

5 It is concluded that determination of rates of formation of antipyrine metabolites from their excretion in urine provides an indication of the activity of the enzymes involved in their formation.

Introduction

Antipyrine has been widely used since 1968 as a substrate in studies of hepatic drug oxidation in man (reviewed in Vesell, 1979). The plasma half-life of the parent compound has been used as an index of oxidation rate (Vesell & Page, 1968) and later the concept of total body clearance of antipyrine was introduced as providing a more accurate reflection of enzyme activity (Vesell & Page, 1969). Antipyrine is a particularly suitable substrate for studies of drug oxidation. It depends almost entirely upon metabolism for its elimination (Brodie *et al.*, 1949), absorption after an oral dose is 100%, there is no presystemic elimination (Andreasen & Vesell, 1974), protein binding is minimal (Brodie *et al.*, 1949) the drug distributes rapidly into total body water (Brodie *et al.*, 1949) and elimination is from a single compartment by first order kinetics (Huffman, Shoeman & Azarnoff, 1974).

With the realisation that there are multiple forms of hepatic cytochrome P-450, both in animals and in man (reviewed in Lu & West, 1980), it became apparent that the three main oxidative metabolites of antipyrine could be produced by different forms of cytochrome P-450 (Huffman *et al.*, 1973; Danhof, Krom & Breimer, 1979). Thus, the overall rate of metabolism of antipyrine will provide an index only of the net activity of all those forms of cytochrome P-450 involved in its metabolism. Antipyrine could not, therefore, be used as a general substrate for all oxidative activities.

Several groups have recently measured the main oxidative metabolites of antipyrine in an attempt to assess the activities of individual forms of hepatic cytochrome P-450 (Danhof, de Groot-van der Vis & Breimer, 1979; Kellerman & Luyten-Kellerman, 1979). Although it now seems likely that each metabolite is the product of more than one form of cytochrome P-450 (Kahn *et al.*, 1980), measurement of the individual metabolites should enable a degree of specificity to be introduced into such studies. For example, rifampicin differentially induces the formation of norphenazone from antipyrine (Toverud *et al.*, 1980) and also the hydroxylation of hexobarbitone (Breimer, Zilly & Richter, 1977). It is thus tempting to conclude that the form of cytochrome P-450 induced by rifampicin is particularly effective in catalysing these two reactions.

Although measurement of antipyrine metabolites is becoming increasingly widespread in studies of drug oxidation in man, it has yet to be demonstrated that the urinary excretion of these metabolites accurately reflects the activity of those enzymes responsible for their production. We have therefore

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Table 1 Clinical details of subjects participating in study comparing *in vivo* and *in vitro* rates of antipyrine oxidation

Subject	*Sex*	*Age (years)*	*Histological appearance of liver biopsy*	*Cigarettes smoked/day*	*Alcohol ingestion* (Units/week)*
1	M	73	Normal	0	4
2	F	40	Normal	15	0
3**	M	58	Normal	50	0
4	F	56	Hepatitis	0	0
5	F	32	Hepatitis	10	120
6†	F	55	Hepatitis	0	0
7	M	45	Cirrhosis	20	110
8	M	39	Steatosis	10	300
9	F	46	Steatosis	20	140
Mean ± s.d.		50 ± 12			

*1 unit = 1/2 pt beer = 1 glass of wine = 1 glass of spirits.
**Patient 3 received diazepam, prednisolone and diamorphine for the 4 weeks prior to study.
†Patient 6 received azathiaprine, prednisolone, atenolol, hydrallazine and glibencamide for 12–36 months prior to study.
No other patient was taking regular medication for at least 4 weeks prior to study.

compared the rates of formation of the three main oxidative metabolites of antipyrine, 3-hydroxymethylantipyrine (3-OHMeAP), 4-hydroxyantipyrine (4-OHAP) and norphenazone (NP), *in vivo* and *in vitro* both in different groups of subjects and in the same subjects.

Methods

Hepatic tissue

Wedge biopsies of liver, taken by the Department of Surgery, were obtained for diagnostic purposes during laparotomy in patients with abnormal liver function tests, lymphoma, suspected secondary tumour or macroscopic areas of abnormality in the liver. Part of the biopsy surplus to histological requirement was made available to us for studies of antipyrine oxidation *in vitro*. Only those biopsies that were histologically normal or that showed minor abnormalities such as non-specific infiltrates were included in this study.

Normal subjects

Antipyrine, 600 mg, was administered orally to ten healthy volunteers (age 24–36 years) after an overnight fast. None of the subjects had any clinical evidence of hepatic, haematological or renal disease and baseline haemoglobin, urea and electrolytes, and liver function tests were all normal. All subjects were non-smokers and refrained from any other drug ingestion for the 7 days prior to and for the period of the study. Blood samples of 10 ml were collected by venipuncture at 0, 3, 5, 8, 24 and 48 h after administration of the drug. Plasma was separated by centrifugation of the samples at 1500 *g* for 15 min and

Table 2 Biochemical liver function tests in nine patients with suspected liver disease participating in study comparing *in vivo* and *in vitro* rates of antipyrine oxidation

Subject	*AST (IU/l)*	*Alkaline phosphatase (IU/l)*	*Bilirubin (μmol/l)*	*Albumin (g/l)*
1	17	242	12	45
2	34	82	42	44
3	28	182	10	41
4	45	87	13	41
5	142	106	16	40
6	142	65	11	42
7	80	168	6	43
8	33	71	7	51
9	97	60	17	49
Normal values	< 40	<130	<17	35–55

stored at −20°C until analysis. Subjects collected all urine for 48 h over sodium metabisulphite (1 g/12 h collection) in 4 × 12 h collections. The volume of each collection was noted and an aliquot frozen at −20°C until analysis.

In vivo *and* in vitro *metabolism in the same subjects*

A group of nine patients entering Hammersmith Hospital with suspected hepatic disease for diagnostic liver biopsy agreed to participate in the study. Clinical and other relevant details of these patients are shown in Tables 1 and 2. All patients were hepatitis B surface antigen negative. Three days prior to biopsy the patients received a single oral dose of 600 mg antipyrine. Blood and urine samples were collected as previously described for the normal subjects. Needle biopsies of liver were taken percutaneously using the Menghini technique and tissue surplus to histological requirement was made available for studies of antipyrine metabolism *in vitro*.

For all studies Local Research Ethics Committee permission was obtained and written informed consent was given by all subjects taking antipyrine for *in vivo* studies of drug oxidation.

Preparation of hepatic microsomal fractions

Biopsy material was immediately placed in ice cold 0.25M potassium phosphate buffer pH 7.25 containing 0.15M potassium chloride and 1 mM EDTA. The tissue was homogenised and microsomal fractions prepared by differential centrifugation as previously described (Boobis *et al.*, 1980). The microsomal pellets were resuspended in 0.25M potassium phosphate buffer pH 7.25 containing 30% (v/v) glycerol and the suspensions stored at −80°C until assayed. Under these conditions of preparation and storage human hepatic monooxygenase activity is stable for at least 12 months.

Analytical procedures

Antipyrine in plasma (Prescott, Adjepon-Yamoah & Roberts, 1973) and its metabolites in urine (Danhof, de Groot-van der Vis *et al.*, 1979; Murray, 1980; Toverud *et al.*, 1981) were determined by previously published procedures. Antipyrine oxidation *in vitro* with [3-^{14}C]-antipyrine as substrate was measured by a radiometric-HPLC method as previously described (Kahn *et al.*, 1981). Microsomal protein content was assayed by a modification (Boobis *et al.*, 1980) of the method of Lowry *et al.* (1951) with crystalline bovine serum albumin (Sigma fraction V) as standard. Standard biochemical liver function tests (aspartate transaminase, alkaline phosphatase, bilirubin and albumin) were measured using a Technicon Plus Autoanalyser.

Analysis of results

Half-life ($T_{½}$) and C_0, the concentration of antipyrine at zero time, were calculated from the least squares regression line fitted to a semilog plot of plasma concentration of antipyrine against time after administration of the drug. Volume of distribution (V) was calculated from Dose/C_0. Total body clearance (Cl) was determined from the relationship

$$Cl = \frac{Dose \cdot f}{AUC}$$

where f is the fraction of the dose available in the systemic circulation and assumed to be 1, and AUC is the area under the plasma concentration curve for antipyrine calculated by the trapezoidal rule with extrapolation to infinity.

In vivo clearance to a metabolite (Cl_M) was calculated from

$$Cl_M = \frac{M}{AUC_t}$$

where M is the amount of metabolite excreted in urine over the interval t and AUC_t is the area under the plasma concentration curve for antipyrine over the same interval.

Michaelis-Menten constants for antipyrine metabolite formation *in vitro* were calculated by an

Table 3 Pharmacokinetic parameters for antipyrine disposition in healthy volunteers and in patients with suspected liver disease

Subjects	n	$T_{½}$ *(h)*	*V (l)*	*Cl (ml/min)*	*Cl (3-OHMeAP) (ml/min)*	*Cl (4-OHAP) (ml/min)*	*Cl (NP)*
Normal subjects	10	12.0 ± 1.0	40.2 ± 1.4	56.1 ± 3.8	7.6 ± 0.9	14.1 ± 1.9	6.0 ± 0.5
Patients with suspected liver disease	9	13.0 ± 1.9	27.3 ± 3.3	28.3 ± 4.4	4.7 ± 1.1	11.7 ± 3.6	4.5 ± 0.7

Values are mean ± s.e. mean.

Table 4 Michaelis-Menten parameters for antipyrine oxidation *in vitro* by microsomal fractions of human liver

	Metabolite		
	3-Hydroxymethylantipyrine	*4-Hydroxyantipyrine*	*Norphenazone*
Vmax (nmol mg^{-1} min^{-1})	0.60 ± 0.23	0.57 ± 0.20	0.34 ± 0.18
Km (mM)	9.0 ± 1.3	7.3 ± 1.1	5.9 ± 0.6
$\frac{Vmax}{Km}$	0.062	0.074	0.054

Values are mean ± s.e. mean ($n = 3$).
Liver samples were histologically normal or showed minor non-specific changes.

iterative programme based on non-linear least squares regression analysis to fit the equation

$$v = \frac{Vmax.s}{Km + s}$$

where v is the velocity of the reaction
s is the substrate concentration
Vmax is the maximum velocity of the reaction
and Km is the apparent affinity constant (Michaelis-Menten constant).

Results

The kinetics of antipyrine disposition in normal volunteers are shown in Table 3. The major metabolite was 4-OHAP, followed by 3-OHMeAP with smaller amounts of NP being produced. The Michaelis-Menten parameters for antipyrine oxidation *in vitro* are shown in Table 4. Km values for all three metabolites were high, 7.7 mM for 3-OHMeAP, 6.9 mM for 4-OHAP and 5.3 mM for NP. It has been shown that for compounds eliminated by first order processes the intrinsic clearance closely approximates

$$\frac{Vmax}{Km}$$

(Rane, Wilkinson & Shand, 1977). The values of this parameter for the three metabolites of antipyrine are also shown in Table 4.

When the intrinsic clearance to each metabolite was normalised to that of norphenazone (Table 5) the relative *in vivo* clearance to 4-OHAP:3-OHMeAP:NP was 2.35:1.27:1 which correlated with the relative *in vitro* clearance values which were 1.37:1.15:1. A similar correlation between relative *in vivo* and *in vitro* rates of antipyrine metabolite formation was found in patients with suspected liver disease (Table 5).

In the nine subjects studied both *in vivo* and *in vitro* there were no significant correlations between the clearance *in vivo* to any pair of metabolites (Figure 1) or between the *in vitro* rates of formation of any pair of metabolites (Figure 2). In the latter case only Vmax was determined because of the small amount of tissue available.

When the *in vivo* clearance to any metabolite was correlated with its corresponding *in vitro* rate of formation in the same subject there were significant correlations for all three metabolites (Figure 3), the most significant correlation being that for 4-OHAP.

Table 5 Comparison of relative rates of formation of antipyrine metabolites *in vivo* and *in vitro* in normal subjects and in patients with suspected liver disease.

	Relative clearance[1] *to*		
Normal subjects	*4-OHAP*	*3-OHMeAP*	*NP*
in vivo[2] ($n = 10$)	2.35	1.27	1
in vitro[3] ($n = 3$)	1.37	1.15	1
Patients with suspected liver disease			
in vivo[2] ($n = 9$)	2.39	1.06	1
in vitro[4] ($n = 9$)	1.74	1.37	1

[1]Clearance values have been normalised to those of norphenazone which has been given a value of unity
[2]*In vivo* clearance was expressed in terms of clearance to a metabolite
[3]*In vitro* clearance was calculated from $\frac{Vmax}{Km}$
[4]*In vitro* rates of formation of antipyrine metabolism were determined using Vmax only.

Discussion

Antipyrine elimination in man depends upon three major oxidative routes of metabolism and there is increasing evidence that these reactions are catalysed primarily by different forms of cytochrome P-450 (Huffman *et al.*, 1973; Kahn *et al.*, 1980). In fact, there was no correlation between rates of formation of any pair of metabolites observed in the present study although this could have been due to the limited

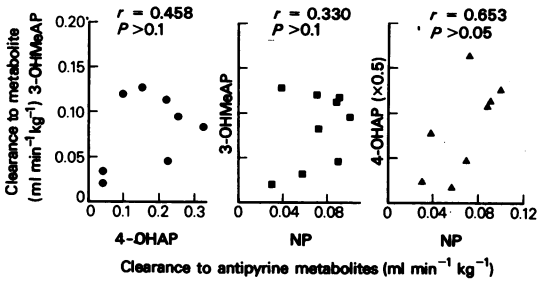

Figure 1 Correlations between *in vivo* rates of formation of antipyrine metabolites in nine patients with suspected liver disease. Clearance to a metabolite was calculated as described in the text.

number of patients studied. However, Danhof (1980) found only relatively weak correlations in a larger group of subjects. Thus, it seems likely that there are different forms of cytochrome P-450 involved in the formation of the metabolites of antipyrine, but there may well be overlap in their specificities.

The involvement of several forms of cytochrome P-450 in antipyrine metabolism means that measurement of total body clearance of antipyrine can provide, at best, only an aggregate of their activity. Thus, several groups have measured antipyrine metabolites in urine, to overcome this problem (Danhof, de Groot-van der Vis *et al.*, 1979; Kellerman & Luyten-Kellerman, 1979), but it has yet to be demonstrated that these accurately reflect the activities of those enzymes producing them.

In order that urinary metabolite excretion reflects enzyme activity several criteria must be met. (1). The fraction of the drug absorbed into the systemic circulation must be known. For antipyrine all of an oral dose is absorbed (Andreasen & Vesell, 1974). (2). Elimination should be first order, which is true for antipyrine (Huffman *et al.*, 1974). (3). The rate of

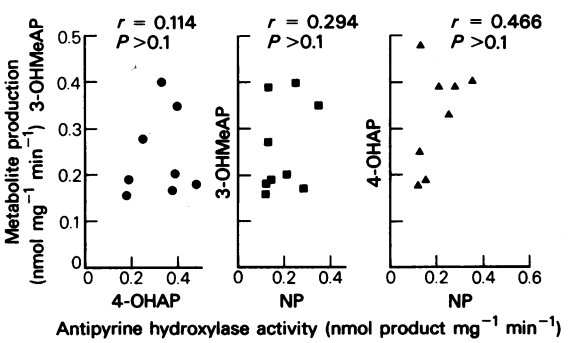

Figure 2 Correlations between *in vitro* rates of formation of antipyrine metabolites in nine patients with suspected liver disease. Enzyme activity was determined in hepatic microsomal fractions with saturating concentrations of antipyrine as described under Methods.

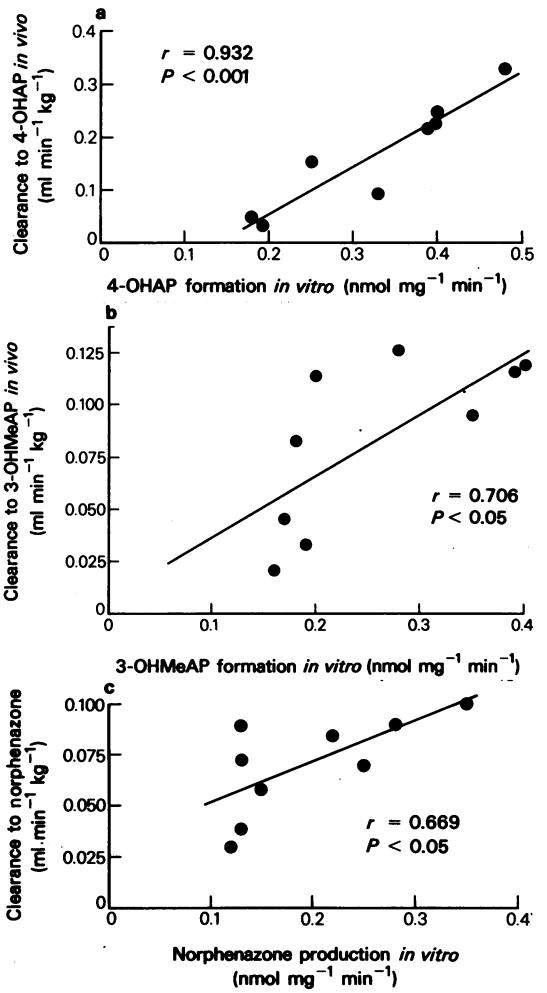

Figure 3 Correlations between *in vivo* and *in vitro* rates of formation of (a) 4-hydroxyantipyrine, (b) 3-hydroxymethylantipyrine and (c) norphenazone in nine patients with suspected liver disease. Each point represents the values for *in vivo* clearance (on the ordinate) and *in vitro* metabolite activity (on the abcissa) for an individual patient.

excretion of a metabolite should be rapid compared with its rate of formation. This appears to be true for the three main metabolites of antipyrine. The half-life of antipyrine derived from urinary rates of excretion of each of the three metabolites correlated significantly with the value determined from saliva or plasma concentrations of the parent compound (Huffman *et al.*, 1974; Danhof & Breimer, 1979) thus demonstrating that oxidation is the rate limiting step in the appearance of these metabolites in urine. (4). The metabolite should not be further metabolised, except to conjugates which can be hydrolysed thereby

permitting quantitative assessment of the amount of metabolite excreted to be made. The main oxidative metabolites of antipyrine were believed to be end products of phase 1 metabolism but several recent papers suggest that further oxidation can occur (Yoshimura, Shimeno & Tsukamoto, 1971; Stafford *et al.*, 1974; Bässmann, Böttcher & Schüppel, 1979). Although these appear to represent relatively minor routes of loss, their contribution to antipyrine metabolite disposition has yet to be fully assessed. (5). The metabolite should be excreted entirely via the kidneys. The physico-chemical properties of antipyrine metabolites and their conjugates are such that they should be excreted almost exclusively via the kidneys, and what evidence is available appears to support this suggestion (Danhof, de Groot-van der Vis *et al.*, 1979). However there are no definitive studies in man to show that renal excretion is the only route of elimination of these metabolites. (6). *In vivo* the metabolite should be produced only by hepatic monooxygenase activity. This is difficult, if not impossible, to prove in man. The demonstration of antipyrine metabolism by extrahepatic tissue *in vitro* does not mean that such metabolism occurs *in vivo*, though the reverse is probably true (lack of metabolism *in vitro* should imply no metabolism *in vivo*).

Only a few studies have been performed in which *in vivo* and *in vitro* rates of metabolism have been compared in the same subjects. The *in vivo* clearance of antipyrine has been compared with the *in vitro* metabolism of other substrates in the same subjects by a number of groups (e.g. Farrell, Cooksley & Powell, 1979; Pelkonen *et al.*, 1980). Such studies most probably reflect the overall pathology of the liver rather than the activity of any individual enzyme. Fraser (1976) has shown a significant correlation between the *in vivo* and *in vitro* 3-hydroxylation of amylobarbitone in man whereas Darby & Grundy (1973) found no correlation between the *in vivo* clearance of tolbutamide and its *in vitro* hydroxylation. McManus & Ilett (1979) demonstrated a correlation between overall elimination of antipyrine *in vivo* and *in vitro* in several species but not in man. In the present study a significant correlation was found for the rank order of antipyrine metabolite formation *in vivo* and *in vitro* in different groups of subjects and in the same group of patients. Further, there were significant correlations between the *in vivo* and *in vitro* rates of formation of each of the three main metabolites of antipyrine in the same subjects.

The correlations found in this study were not sufficiently good to be predictive and this may be because only Vmax values were used to assess *in vitro* enzyme activity. Although the Km values for the formation of the three metabolites were similar and inter-subject variation in the Km values was small (Table 4) any variation in Km would affect the correlation coefficients. In addition, *in vitro* activity was determined on needle biopsy specimens and although such samples appear to be reasonably representative of the whole liver (Boobis *et al.*, 1980) it is quite possible that in liver disease there is some regional variation in enzyme activity throughout the organ. Despite these possible sources of error significant correlations were found and it is concluded that measurement of antipyrine metabolites in urine can provide a useful measure of the activity of those enzymes involved in their production.

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References

ANDREASEN, P.B. & VESELL, E.S. (1974). Comparison of plasma levels of antipyrine, tolbutamide, and warfarin after oral and intravenous administration. *Clin. Pharmac. Ther.*, **16**, 1059–1065.

BÄSSMANN, H., BÖTTCHER, J. & SCHÜPPEL, R. (1979). Dihydroxyphenazone as a urinary metabolite of phenazone in different species including man. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **309**, 203–205.

BOOBIS, A.R., BRODIE, M.J., KAHN, G.C., FLETCHER, D.R., SAUNDERS, J.H. & DAVIES, D.S. (1980). Monooxygenase activity of human liver in microsomal fractions of needle biopsy specimens. *Br. J. clin. Pharmac.*, **9**, 11–19.

BREIMER, D.D., ZILLY, W. & RICHTER, E. (1977). Influence of rifampicin in drug metabolism: differences between hexobarbital and antipyrine. *Clin. Pharmac. Ther.*, **21**, 470–481.

BRODIE, B.B., AXELROD, J., SOBERMAN, R. & LEVY, B.B. (1949). The estimation of antipyrine in biological materials. *J. biol. Chem.*, **179**, 25–29.

DANHOF, M. (1980). *Antipyrine metabolite profile as a tool in the assessment of the activity of differing oxidising enzymes in man.* PhD Thesis, University of Leiden, The Netherlands.

DANHOF, M. & BREIMER, D.D. (1979). Studies of the different metabolic pathways of antipyrine in man. 1. Oral administration of 250, 500 and 1000 mg to healthy volunteers. *Br. J. clin. Pharmac.*, **8**, 529–537.

DANHOF, M., DE GROOT-VAN DER VIS, E. & BREIMER, D.D. (1979). Assay of antipyrine and its primary metabolites in plasma, saliva and urine by high-performance liquid chromatography and some preliminary results in man. *Pharmacology*, **18**, 210–223.

DANHOF, M., KROM, D.P. & BREIMER, D.D. (1979).

Studies on the different metabolic pathways of antipyrine in rats: influence of phenobarbital and 3-methylcholanthrene treatment. *Xenobiotica*, **9**, 695–702.

DARBY, F.J. & GRUNDY, R.K. (1973). The metabolism of [ureyl-^{14}C]tolbutamide *in vitro* and *in vivo* in man. *Life Sci.*, **13**, 97–105.

FARRELL, G.C., COOKSLEY, W.G.E. & POWELL, L.W. (1979). Drug metabolism in liver disease: activity of hepatic microsomal metabolising enzymes. *Clin. Pharmac. Ther.*, **26**, 483–492.

FRASER, H.F. (1976). *Effects of the environment and disease on drug metabolism in man.* Ph.D. Thesis, London.

HUFFMAN, D.H., SHOEMAN, D.W. & AZARNOFF, D.L. (1974). Correlation of the plasma elimination of antipyrine and the appearance of 4-hydroxyantipyrine in the urine of man. *Biochem. Pharmac.*, **23**, 197–201.

HUFFMAN, D.H., SHOEMAN, D.W., PENTIKÄINEN, P. & AZARNOFF, D.L. (1973). The effect of spironolactone on antipyrine metabolism in man. *Pharmacology*, **10**, 338–344.

KAHN, G.C., BOOBIS, A.R., BLAIR, I., BRODIE, M.J. & DAVIES, D.S. (1980). Antipyrine as an *in vitro* probe of mixed function oxidase activity. *Br. J. clin. Pharmac.*, **9**, 284P.

KAHN, G.C., BOOBIS, A.R., BLAIR, I.A., BRODIE, M.J. & DAVIES, D.S. (1981). A radiometric-HPLC assay for the simultaneous determination of the three main oxidative metabolites of antipyrine in studies *in vitro*. *Analyt. Biochem.* (in press).

KELLERMAN, G.H. & LUYTEN-KELLERMAN, M. (1979). Antipyrine metabolism in man. *Life Sci.*, **23**, 2485–2490.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.C. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

LU, A.Y.H. & WEST, S.B. (1980). Multiplicity of mammalian microsomal cytochromes P-450. *Pharmac. Rev.*, **31**, 277–295.

McMANUS, M.E. & ILETT, K.F. (1979). Comparison of rate of hepatic metabolism *in vitro* and half-life for antipyrine *in vivo* in three species. *Xenobiotica*, **9**, 107–118.

MURRAY, S. (1980). An improved gas chromatographic-mass spectrometric assay for the estimation of norphenazone in urine. *Biomed. Mass. Spec.*, **7**, 179–182.

PELKONEN, O., SONTANIEMI, E., TOKOLA, O. & AHOKAS, J.T. (1980). Correlation between cytochrome P-450 and oxidative metabolism of benzo[a]pyrene and 7-ethoxycoumarin in human liver *in vitro* and antipyrine elimination *in vivo*. *Drug Metab. Disposition*, **8**, 218–222.

PRESCOTT, L.F., ADJEPON-YAMOAH, K.K. & ROBERTS, E. (1973). Rapid gas-liquid chromatographic estimation of antipyrine in plasma. *J. Pharm. Pharmac.*, **25**, 205–207.

RANE, A., WILKINSON, G.R. & SHAND, D.G. (1977). Prediction of hepatic extraction ratio from *in vitro* measurement of intrinsic clearance. *J. Pharmac. exp. Ther.*, **200**, 420–424.

STAFFORD, M., KELLERMAN, G., STILLWELL, R.N. & HORNING, M.G. (1974). Metabolism of antipyrine by the epoxide-diol pathway in the rat, guinea pig and human. *Res. Comm. chem. Path. Pharmac.*, **8**, 593–605.

TOVERUD, E-L., BOOBIS, A.R., BENNETT, P.N., WHITMARSH, V., BRODIE, M.J., MURRAY, S. & DAVIES, D.S. (1980). Differential effects of rifampicin treatment on *in vivo* production of antipyrine metabolites in man. *First World Conference in Clinical Pharmacology and Therapeutics, London,* Abstract 0705.

TOVERUD, E-L., BOOBIS, A.R., BRODIE, M.J., MURRAY, S., BENNETT, P.N., WHITMARSH, V. & DAVIES, D.S. (1981). Differential induction of antipyrine metabolism by rifampacin. *Eur. J. clin. Pharmac.* (in press).

VESELL, E.S. (1979). The antipyrine test in clinical pharmacology. Conceptions and misconceptions. *Clin. Pharmac. Ther.*, **26**, 275–286.

VESELL, E.S. & PAGE, J.G. (1968). Genetic control of drug metabolism in man; antipyrine. *Science*, **16**, 72–73.

VESELL, E.S. & PAGE, J.G. (1969). Genetic control of the phenobarbital-induced shortening of plasma antipyrine half-lives in man. *J. clin. Invest.*, **48**, 2202–2209.

YOSHIMURA, H., SHIMENO, H. & TSUKAMOTO, H. (1971). Metabolism of drugs. LXX. Further study on antipyrine metabolism. *Chem. pharm. Bull.*, **19**, 41–45.

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