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Inter-subject and ethnic differences in paracetamol metabolism

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1 The 24 h urinary excretion of paracetamol and its metabolites following a single oral dose of 1.5 g was compared in 111 Caucasians (Scotland), 67 West Africans (Ghana) and 20 East Africans (Kenya).

2 The fractional recovery of the mercapturic acid and cysteine conjugates of paracetamol was 9.3% in the Caucasians compared with only 5.2% and 4.4% in the Ghanaians and Kenyans respectively ($P = < 0.0005$). This probably indicates markedly reduced metabolic activation of paracetamol in the Africans.

3 There were no ethnic differences in the sulphate conjugation of paracetamol, but the mean fractional recovery of the glucuronide conjugate in Caucasians (54%) was significantly less than in the Africans (58%).

4 The sulphate conjugation of paracetamol was increased and glucuronide conjugation reduced in Caucasian females compared with males. A similar trend was seen in the Ghanaians but there were no other significant sex differences.

5 The range of intersubject variation in the metabolic activation of paracetamol was sixty fold compared with only a three fold variation in glucuronide and sulphate conjugation. This has important implications for susceptibility to paracetamol hepatotoxicity following overdosage especially in a small subgroup showing extensive metabolic activation.

6 These ethnic differences in paracetamol metabolism may be related to genetic or environmental factors including differences in diet and protein intake.

Keywords paracetamol ethnic differences metabolic activation conjugation diet

Introduction

Paracetamol is a widely used and normally very safe analgesic which is metabolised by parallel pathways of oxidation and conjugation (Prescott, 1980). It is thus an ideal drug for the study of factors influencing the different routes of drug metabolism in man. The microsomal oxidation of paracetamol yields a highly reactive intermediate metabolite (Mitchell *et al.*, 1974) which is normally preferentially conjugated with glutathione and eventually excreted as mercapturic acid and cysteine conjugates. With overdosage, glutathione is depleted and hepatic and renal damage may occur (Prescott & Critchley, 1983). Hepatotoxicity following paracetamol overdosage is dose related but there are considerable individual differences in susceptibility depending on the extent of metabolic activation and capacity for glutathione synthesis (Mitchell & Jollow, 1975). Thus inter-subject and ethnic differences in paracetamol metabolism have toxicological as well as pharmacogenetic relevance.

Although ethnic differences in drug metabolism are well established (Kalow 1982), there is little information in this regard concerning paracetamol. Mucklow *et al.* (1980) found a lower paracetamol clearance and longer half-life in Asian immigrants compared with white Caucasians working in London. However, Sommers *et al.* (1985) found higher paracetamol clearances in black villagers from Southern Africa than had

been reported in Caucasians. Ghanaians have a reduced capacity for the hydroxylation of debrisoquine (Woolhouse *et al.*, 1979) and possibly phenytoin (Andoh *et al.*, 1980) but not nortriptyline (Woolhouse *et al.*, 1984).

Paracetamol metabolism in man is influenced by a number of factors including age, dose (Prescott, 1980), tobacco (Mucklow *et al.*, 1980) and other drug therapy such as the contraceptive pill (Critchley *et al.*, 1983b; Miners *et al.*, 1983), anticonvulsants and anti-tuberculous chemotherapy (Prescott *et al.*, 1981). Conflicting results have been reported in respect of alcohol consumption (Mucklow *et al.*, 1980; Critchley *et al.*, 1983a). In our experience, the pattern of urinary paracetamol metabolite excretion varies considerably between individuals but is remarkably constant within individuals.

In order to study inter-subject and ethnic differences in paracetamol disposition in more detail we compared its metabolism in healthy young Caucasians, West Africans and East Africans.

Methods

Subjects

Three groups of subjects were studied: (a) 111 white Caucasians (medical students and other hospital staff) residing in Edinburgh, (b) 67 West African medical students living in Accra, Ghana and (c) 20 East African students from a well populated rural area in West Kenya. The Kenyans were reasonably well nourished on a diet of maize and vegetables with occasional meat. The Ghanaians had a slightly more westernised diet, although rice and maize predominated. Details of the subjects are given in Table 1. None was taking regular medications or drank more than the equivalent of 2 pints of beer daily (i.e. 4 units (32 g) of ethanol). Ethanol consumption was graded as light (up to 7 units weekly) and moderate (8–28 units weekly).

An additional three Caucasians, six Ghanaian and twelve Kenyan Africans were excluded from the study because the total urinary recovery of paracetamol and metabolites was less than 500 mg or there was evidence of significant hydrolysis of the glucuronide conjugate with more than 20% of the total excreted as unchanged paracetamol.

Drug administration and urine collection

Following an overnight fast, the subjects provided a blank urine specimen and then took 1.5 g of paracetamol (Panadol, Sterling-Winthrop) with water. Urine was collected for the next 24 h and during this period the subjects took no other drugs or ethanol. The Ghanaian urine samples were transported airfreight in dry ice to the U.K. but the Kenyan samples were kept at 0–4 °C and not frozen until arrival in Edinburgh. Otherwise, all samples were stored deep frozen until the time of analysis.

Table 1 Details of subjects

	Ethnic origin		
	Caucasian	*Ghanaian*	*Kenyan*
Total (% males)	111 (59%)	67 (76%)	20 (90%)
Age (years)	23 ± 5	23 ± 5	18 ± 2
Weight (kg)	65 ± 11	—	61 ± 5
Tobacco smokers	10	6	0
Ethanol—none	15	60	20
—light	92	5	0
—moderate	4	2	0

Assay

Paracetamol and its sulphate, glucuronide and mercapturic acid and cysteine conjugates in urine were estimated by high performance liquid chromatography using a modification of the method of Howie *et al.* (1977). An Altex pump (model 110A) was used with a Waters Associates 441 UV detector set to 254 nm and peak areas were measured with a Hewlett-Packard 339A integrator. Radial compression columns (Waters Associates, Model RCM100) packed with 10μ octadecyl-bonded silica were used with a 330:1 mixture of 1% acetic acid and ethyl acetate at a flow rate of 3 ml min^{-1}. *N*-acetylpropionyl-4-aminophenol was added to the urine as the internal standard and the mixture injected directly onto the column. Samples with very low concentrations of the mercapturic acid and cysteine conjugates were assayed using an electrochemical detector (Model LC-4A, Bioanalytical Systems Inc.) with a LC-17 flow cell and a TL-5 glassy carbon electrode set at a potential of +0.8 volts. The concentrations of the conjugates were calculated as paracetamol equivalents.

Urinary creatinine was measured by the alkaline picrate (Jaffe) method and used as an index of the completeness of the 24 h urine collections.

Data analysis

The proportions of paracetamol and its conjugates excreted are expressed as percentages of the total recovered in the urine in 24 h. Results are given as means ± s.d. and probabilities calculated using both the unpaired Student's *t*-test and the Wilcoxon (Mann–Whitney) rank sum test. For probit plots of cumulative probability, values were ranked in ascending order and the probabilities calculated as percentages using the formula: 100 x (rank of individual value $-0.5)/n$ (Documenta Geigy, 1975). The cumulative percentages were converted to their respective probit numbers using the probit transformation tables of Fisher and Yates.

Results

The subjects were reasonably well matched for age but the proportions of females and individuals consuming alcohol were lower in the Africans than in the Caucasians (Table 1). The mean total 24 h recovery of the administered dose as unchanged paracetamol and its conjugates combined in the three groups is shown in Table 2 and was significantly lower in the Ghanaians and Kenyans than in the Caucasians. The 21 subjects excluded due to their 24 h total recovery being less than 500 mg also had very low creatinine recoveries (< 0.5 g 24 h^{-1}) indicating incomplete urine collections.

Glucuronide and sulphate conjugation

The Africans excreted a significantly greater proportion as the glucuronide conjugate (58%) than did the Caucasians (54%, Table 2). However, there were no significant ethnic differences in the proportions recovered as the sulphate conjugate (29–32%, Table 2). The inter-subject variation in the extent of glucuronide and sulphate conjugation in the three ethnic groups varied over a range of less than three-fold (Figure 1).

The frequency distributions of the fractional excretion of the glucuronide and sulphate conjugates in the three groups are shown in Figure 1 and are approximately symmetrical. Within each group the fractional recovery as sulphate and glucuronide conjugates showed the expected inverse relationship. In each case, the regression line of this relationship had a slope of -1 and the correlation coefficient was highly significant ($r = -0.73$ to -0.92, $P = < 0.001$, Table 3). The mean sulphate to glucuronide ratios ranged from 0.50–0.60 (Table 3). Probit plots of the ratios are shown in Figure 2. The Kenyan values lie on a straight line consistent with a normal distribution. However, in the Caucasian and Ghanaian groups the probit plots show a change in gradient at the probit value of approximately 5, indicating an asymmetrical distribution.

Mercapturic acid and cysteine conjugates

There were highly significant ethnic differences in the extent of metabolic activation of paracetamol as shown by the fractional recoveries of its mercapturic acid and cysteine conjugates. The mean recoveries of these metabolites combined in the Ghanaians and Kenyans were only 56 and 47% of that in the Edinburgh subjects ($P = < 0.0005$). Furthermore, the Kenyans excreted significantly less cysteine conjugate than the Ghanaians ($P = < 0.05$, Table 2).

The ethnic differences in the frequency distributions of recovery of these two conjugates are shown in Figure 3. The probit plot (Figure 4)

Table 2 Percentage 24 h urinary recovery of paracetamol and its conjugates

	Sulphate	*Glucuronide*	*Mercapturic acid*	*Cysteine*	*Unconjugated paracetamol*	*Total (% of dose)*
Caucasians (111)						
Mean	31.2	53.8	5.0	4.3	5.7	86
s.d.	4.7	6.2	1.9	1.6	2.6	15
Ghanaians (67)						
Mean	32.0	58.2**	2.3**	2.9**	4.6*	76*
s.d.	7.7	8.7	1.3	1.5	2.0	13
Kenyans (20)						
Mean	28.9	57.9*	2.3**	2.1**	8.8**	63*
s.d.	3.8	4.0	1.0	1.3	3.5	17

Significantly different from Caucasians (*$P < 0.01$, **$P < 0.0005$).

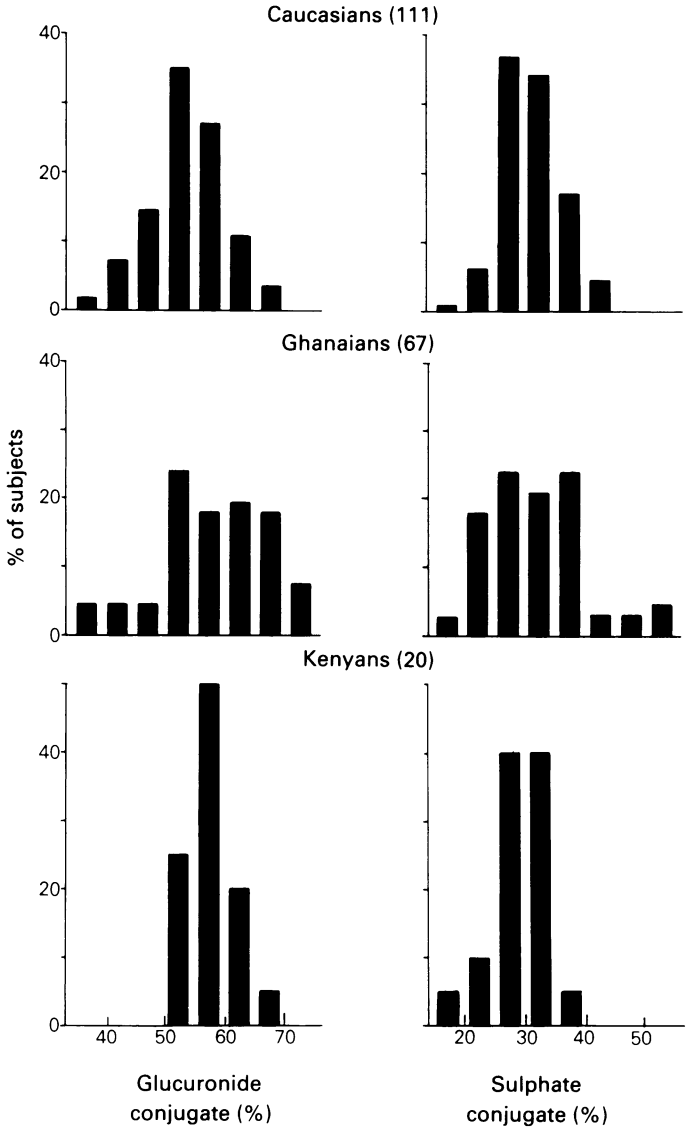

Figure 1 Frequency distribution (in 5% increments) of percentage of total excreted as paracetamol glucuronide and sulphate conjugate in Caucasians, Ghanaians and Kenyans.

Table 3 Urinary paracetamol sulphate to glucuronide conjugate ratios (S/G) and coefficients of correlation between the two conjugates.

	Mean S/G	*Range S/G*	*S to G correlation coefficient* (r)
Caucasians (111)	0.60	0.29–1.20	−0.73*
Ghanaians (67)	0.58	0.25–1.41	−0.92*
Kenyans (20)	0.50	0.35–0.69	−0.76*

* $P = < 0.001$

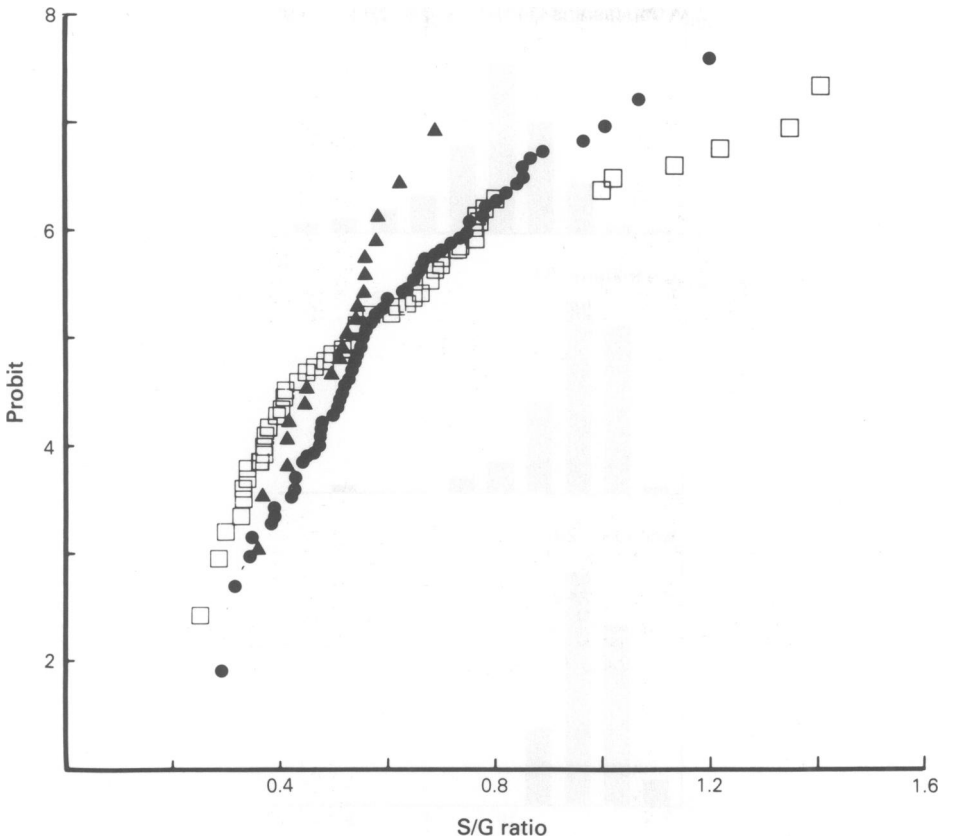

Figure 2 Probit plots of paracetamol sulphate to glucuronide conjugate (S/G) ratios in Caucasians ●, Ghanaians □ and Kenyans ▲.

further emphasises these differences and the deviation of the maximum values to the right in the Caucasians and Ghanaians reveals a skewed distribution with unexpectedly extensive metabolic activation of paracetamol in a few individuals. There was very wide inter-subject variation and the fractional recovery of the mercapturic acid plus cysteine conjugates varied over a 60 fold range in the three groups.

There was a significant inverse relationship between the fractional recovery of the mercapturic acid plus cysteine conjugates and the glucuronide conjugate in the Caucasians and the Ghanaians with correlation coefficients of −0.52 and −0.44 respectively ($P = < 0.01$). There was no such correlation with the sulphate conjugate, unchanged paracetamol or the total amount recovered.

In the Caucasians and Ghanaians there were highly significant correlations between the fractional urinary recovery of the mercapturic acid and cysteine conjugates (correlation coefficients = 0.70 and 0.74 respectively, $P = < 0.01$).

Sex differences and the effects of ethanol and tobacco

Comparison of the effects of these factors was not always possible because their distribution between the groups was unequal and the numbers too small. However, Caucasian females excreted significantly less glucuronide and more sulphate conjugate than males. A similar trend in Ghanaians failed to reach statistical significance (Table 4).

In the Caucasians and Ghanaians, the pattern of urinary metabolite excretion was essentially the same in smokers and those who drank ethanol as in those who did not. There was no evidence of any relationship between ethanol consumption and the extent of metabolic activation of paracetamol.

Discussion

The most important finding in this study was the highly significant ethnic difference in the

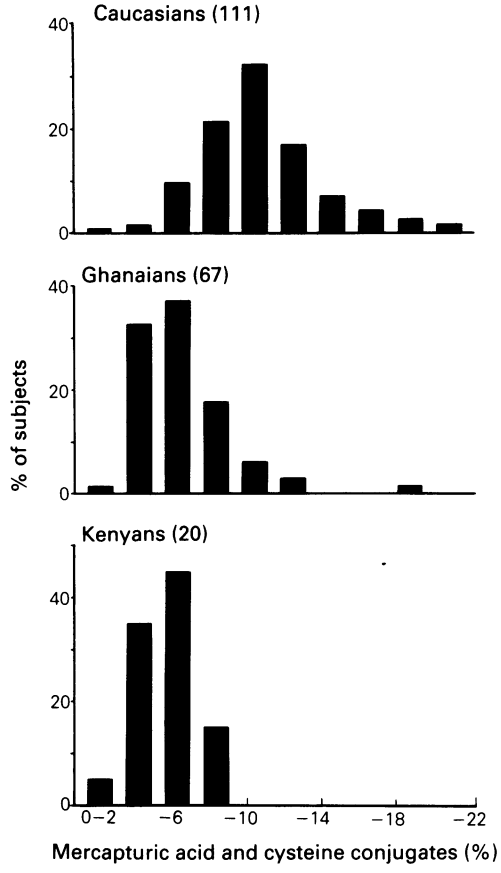

Figure 3 Frequency distribution (in 2% increments) of percentage of total excreted as the combined mercapturic acid and cysteine conjugates of paracetamol in Caucasians, Ghanaians and Kenyans.

fractional recovery of the mercapturic acid and cysteine conjugates of paracetamol. The mean combined recovery of these conjugates of 9.3% in the Caucasians was about twice that observed in the Ghanaian and Kenyan Africans (5.2% and 4.4%, respectively). An even lower recovery of these conjugates (3.3%) was recently reported in Chinese and Indian Asians living in Singapore (Koh Yoke Khim, 1985). These findings are consistent with reduced capacity for microsomal oxidation in these Africans and Asians, but it is not known whether genetic factors or environmental effects such as differences in diet or exposure to inducing agents are responsible. Alternatively, an increased capacity for glucuronide conjugation might be responsible for the reduced formation of mercapturic acid and cysteine conjugates in the Africans. However, patients taking hepatic microsomal enzyme inducing drugs excrete normal amounts of the glutathione-derived conjugates despite enhanced glucuronide conjugation (Prescott *et al.*, 1981).

The overall range of intersubject variation in the metabolic activation of paracetamol throughout the three ethnic groups was sixty-fold compared with only three-fold in respect of glucuronide and sulphate conjugation. This extreme variation has important implications for the susceptibility to paracetamol hepatotoxicity following over dosage and it must be presumed that the few individuals with very extensive metabolic activation are particularly vulnerable. It probably has much wider significance for the toxicity of other drugs and chemicals dependent on oxidative metabolic activation.

There were no ethnic differences in the extent of sulphate conjugation of paracetamol but there was a small but significant sex difference. The increased sulphate conjugation in women was reflected in a reduction in glucuronide conjugation.

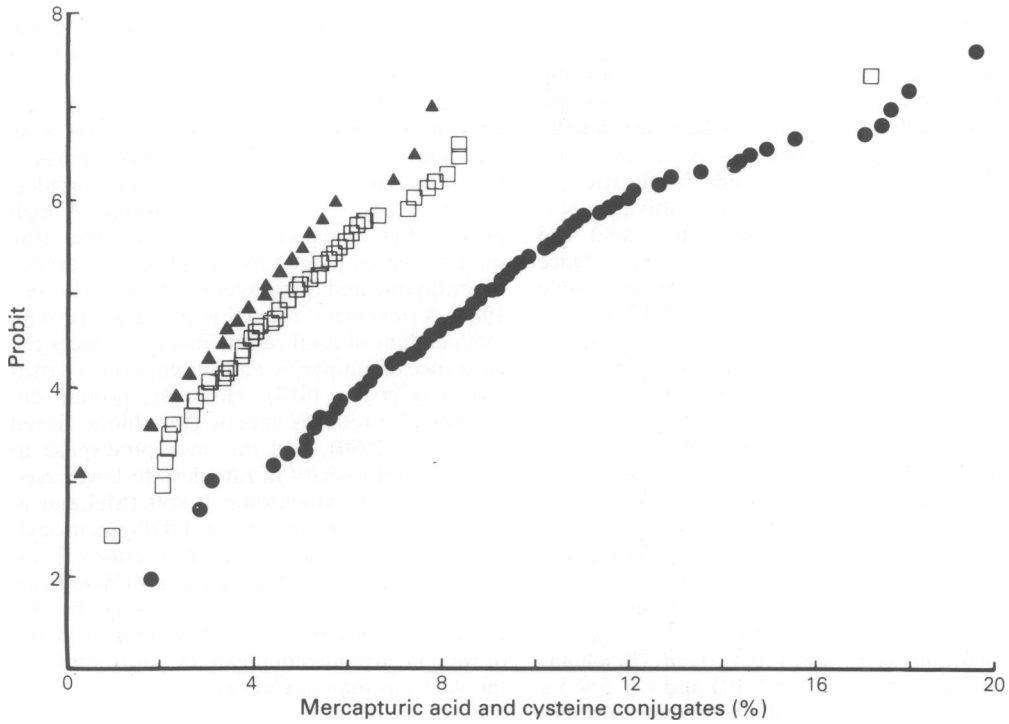

Figure 4 Probit plots of percentage of total excreted as the combined mercapturic acid and cysteine conjugates of paracetamol in Caucasians ●, Ghanaians □ and Kenyans ▲.

Table 4 Sex differences in the percentage 24 h urinary recovery of paracetamol and its conjugates.

	Sulphate	*Glucuronide*	*Mercapturic acid*	*Cysteine*	*Unconjugated paracetamol*
Caucasian males (65)					
Mean	30.4	55.4	5.0	4.0	5.2
s.d.	4.9	6.1	2.0	1.6	2.1
Caucasian females (46)					
Mean	32.3*	51.5**	5.0	4.7*	6.5*
s.d.	4.2	5.7	1.7	1.5	3.0
Ghanaian males (51)					
Mean	31.4	58.8	2.4	3.0	4.4
s.d.	7.5	8.7	1.4	1.6	1.9
Ghanaian females (16)					
Mean	33.8	56.3	2.1	2.6	5.2
s.d.	8.1	8.7	1.0	1.1	2.2

* Significantly different from males of same ethnic group (*$P < 0.05$, **$P < 0.001$)

The latter was also reported by Miners *et al.* (1983). However, these workers only studied eight males and eight females and found no corresponding sex difference in the fractional recovery of the sulphate conjugate.

Some subjects were excluded from the study because their urine contained large amounts of unchanged paracetamol with little or no glucuronide conjugate. It was assumed that these samples had been contaminated by β-glucuronidase producing bacteria because only the glucuronide conjugate was affected and the relative proportions of the sulphate, mercapturic acid and cysteine conjugates were not reduced. Once frozen to −20 °C all four conjugates are stable for at least 3 years (Adriaenssens, 1980) and our stability studies also show that the mercapturic acid and cysteine conjugates are resistant to incubation with β-glucuronidase (Type H-2 extract from *Helix pomatia*, Sigma Chemical Company Ltd.) for over 48 h at pH 5 and 37 °C.

Minor hydrolysis of the glucuronide conjugate in other samples cannot be excluded and may explain the slightly smaller fractional recovery of paracetamol glucuronide in females compared with males. The mean total 24 h recovery of unchanged paracetamol and its conjugates was less in the Africans than the Caucasians and the mean urinary creatinine recoveries in Ghanaians and Caucasians were 1.0 ± 0.3 and 1.3 ± 0.5 g 24 h^{-1}, respectively. A similar reduced urinary recovery of debrisoquine has also been reported in Ghanaians and Egyptians (Woolhouse *et al.*, 1979; Mahgoub *et al.*, 1979).

Very few subjects smoked or drank alcohol in quantity but these factors did not appear to influence the pattern of paracetamol metabolism. A positive correlation between cigarette smoking and paracetamol clearance was reported by Mucklow *et al.* (1980) but they only compared the overall rate of paracetamol elimination and no information was provided concerning the relative activity of the different pathways of its metabolism as in the present study.

Diet can have important effects on drug metabolism (Guengerich, 1984). The Africans were well nourished but their protein intake, particularly with regard to meat, was probably considerably less than that of the Caucasians. A high protein diet appears to enhance oxidative drug metabolism as judged by the plasma clearance of antipyrine and theophylline (Anderson *et al.*, 1982). Conversely, reduction in dietary protein independent of total caloric intake, reduces the clearance of antipyrine and aminopyrine (Krishnaswamy *et al.*, 1984). However, protein deficiency also reduces hepatic glutathione (Reed & Beatty, 1980), and this may predispose to paracetamol toxicity in rats despite lower concentrations of cytochrome P-450 (McLean & Day, 1975). The activity of UDP-glucuronyltransferase (but not sulphotransferase) is increased in rats by protein deficiency (Woodcock & Wood, 1971), and it is interesting that we found a significantly higher fractional recovery of the glucuronide conjugate of paracetamol in the Africans than in Caucasians.

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